Preparation of Gelatin Blended Films for Drug Delivery

Suchipha Wannaphatchaiyong

A Thesis Submitted in Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Pharmaceutical Sciences
Prince of Songkla University
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Major Program	Pharmaceutical Sciences

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อเตรียมและประเมินแผ่นฟิล์มยาชาชนิดเมทริกซ์สำหรับใช้เป็นแผ่นฟิล์มเยื่อบุช่องปากหรือแผ่นแปะผิวหนัง โดยเจลาตินถูกใช้เป็นพอลิเมอร์หลัก แป้งมันสัมปะหลังพรีเจลาติไนซ์ (แอลฟาสตาร์ช) หรือแป้งสาคูเจลาติไนซ์ถูกใช้เป็นพอลิเมอร์ผสม กลีเซอรีน โพรพิลีนไกลคอล พอลิเอทิลีนไกลคอล 400 หรือน้ำยางโปรตีนต่ำ ถูกใช้เป็นพลาสติไซ-เซอร์ ยาลิโดเคนในรูปเบสหรือเกลือไฮโดรคลอไรด์ถูกใช้เป็นยาต้นแบบ แผ่นฟิล์มเตรียมได้จากวิธีการผสมและวิธีการผสมก่อนอบแห้งด้วยตู้อบลมร้อน ปริมาณของแป้งและพลาสติไซเซอร์มีผลต่อการดูดซับน้ำ การกร่อน และความยืดหยุ่นของแผ่นฟิล์ม แผ่นฟิล์มถูกประเมินลักษณะด้านความเข้ากันโดยการใช้เครื่องวิเคราะห์เนื้อสัมผัส กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด กล้องจุลทรรศน์แรงอะตอม ฟูเรียทรานส์ฟอร์มอินฟราเรดสเปกโตรสโคปี ดิฟเฟอเรนเชียลสแกนนิ่งแคลอริเมทรี และการประเมินความเข้ากันด้วยการเลี้ยวเบนรังสีเอกซ์ เจลาตินผสมกับ 5 ส่วนในร้อยส่วนของเจลาติน (phg) ของแอลฟาสตาร์ช และ 25 phg ของกลีเซอรีน (Gαgly) ทำให้ได้แผ่นฟิล์มที่เหมาะสมสำหรับการนำส่งยาทางเยื่อบุช่องปาก นอกจากนี้เจลาตินผสมกับ 5 phg ของแป้งสาคูเจลาติไนซ์ และ 50 phg ของน้ำยางโปรตีนต่ำ (GSNR) ทำให้ได้แผ่นแปะที่เหมาะสมสำหรับการนำส่งทางผิวหนัง ลิโดเคนเบสและลิโดเคนไฮโดรคลอไรด์ถูกผสมลงในแผ่นฟิล์ม Gαgly และแผ่นแปะ GSNR ใช้ฟรานซ์ดิฟฟิวชันเซลล์ดัดแปรสำหรับการศึกษาการปลดปล่อยยาและการซึมผ่านของยาในหลอดทดลอง ใช้เยื่อหุ้มตัวอ่อนของไข่ไก่ (CAM) และหนังหมูแรกเกิดเป็นโมเดลสำหรับการประเมินผลการซึมผ่านของยาลิโดเคนผ่านเยื่อบุในช่องปากและผิวหนังตามลำดับ ทั้งยาลิโดเคนเบสและลิโดเคนไฮโดรคลอไรด์สามารถปลดปล่อยออกจากแผ่นฟิล์ม Gαgly และแผ่นแปะ GSNR การปลดปล่อยยาของลิโดเคนไฮโดรคลอไรด์สูงกว่าลิโดเคนเบสทั้งในแผ่นฟิล์ม Gαgly และแผ่นแปะ GSNR นอกจากนี้ตัวยาเหล่านี้ยังสามารถซึมผ่านเยื่อหุ้มตัวอ่อนของไข่ไก่และหนังหมูแรกเกิด สำหรับแผ่นฟิล์ม Gαgly ลิโดเคนไฮโดรคลอไรด์สามารถซึมผ่านเยื่อหุ้มตัวอ่อนของไข่ไก่ได้มากกว่าลิโดเคนเบส สำหรับแผ่นแปะ GSNR ลิโดเคนไฮโดรคลอไรด์ซึมผ่านผิวหนังหมูแรกเกิดต่ำกว่าลิโดเคนเบส แสดงให้เห็นว่าการปลดปล่อยหรือซึมผ่านของลิโดเคน

ขึ้นอยู่กับคุณสมบัติของยา แผ่นฟิล์มผสมยา Gαgly และแผ่นแปะผสมยา GSNR แสดงรูปแบบจลนศาสตร์ของการปลดปล่อยยาและการซึมผ่านของยาที่แตกต่างกัน จลนศาสตร์การปลดปล่อยยาของแผ่นฟิล์ม Gαgly ในระยะสั้นและ 8 ชั่วโมงเหมาะสมกับจลนศาสตร์อันดับศูนย์และจลนศาสตร์อันดับหนึ่งตามลำดับ จลนศาสตร์การซึมผ่านของยาส่วนใหญ่เหมาะสมกับจลนศาสตร์อันดับหนึ่ง สำหรับแผ่นแปะ GSNR จลนศาสตร์การปลดปล่อยยาเหมาะสมกับจลนศาสตร์อันดับหนึ่งหรือจลนศาสตร์ของฮิกูชิ จลนศาสตร์การซึมผ่านของยาของแผ่นแปะ LB-GSNR เป็นจลนศาสตร์อันดับศูนย์หรือจลนศาสตร์อันดับหนึ่ง ในขณะที่แผ่นแปะ LH-GSNR ไม่มีความแตกต่างทางสถิติของจลนศาสตร์ทั้ง 3 ชนิด ผลจากการศึกษาความคงตัวเป็นระยะเวลา 3 เดือน แสดงให้เห็นว่าแผ่นฟิล์มหรือแผ่นแปะเหล่านี้ควรแนะนำให้เก็บที่อุณหภูมิต่ำ นอกจากนี้ การประเมินกระดายเคืองอยู่ในระดับต่ำของแผ่นฟิล์มผสมยา Gαgly โดยใช้เยื่อหุ้มตัวอ่อนของไข่ไก่ แสดงให้เห็นว่าฟิล์มมีความปลอดภัยต่อเยื่อบุในช่องปาก โดยสรุปเจลาตินและแป้งสามารถผสมกับพลาสติไซเซอร์และยาชา เช่น ลิโดเคนเบสและลิโดเคนไฮโดรคลอไรด์ แล้วให้แผ่นฟิล์มที่เหมาะสำหรับเป็นฟิล์มนำส่งยาทางเยื่อบุช่องปากหรือแผ่นแปะผิวหนัง

Thesis Title Preparation of Gelatin Blended Films for Drug Delivery

Author Miss Suchipha Wannaphatchaiyong

Major Program Pharmaceutical Sciences

Academic Year 2018

ABSTRACT

This study aimed to prepare and evaluate the matrix type anesthetic films for using as oral transmucosal (OTD) or transdermal drug delivery systems (TDDS). Gelatin was chosen as the main polymer. Pregelatinized tapioca starch (alpha starch) or gelatinized sago starch was used as a blended polymer. Glycerin (GLY), propylene glycol (PG), polyethylene glycol 400 (PEG400), or deproteinized natural rubber latex (DNRL) was selected as a plasticizer. Either lidocaine base (LB) or its hydrochloride salt (LH) was used as a model drug. The films were obtained by mixing and casting methods before being dried in hot air oven. The amounts of starch and plasticizer affected the water uptake, erosion, and elasticity of films. The films were characterized for their compatibility by using texture analyzer, scanning electron microscopy (SEM), atomic force microscopy (AFM), fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), and X-ray diffractometry (XRD). Gelatin blended with 5 part per hundred of gelatin (phg) alpha starch and 25 phg GLY (Gαgly) presented as the appropriate film for OTD. In addition, gelatin blended with 5 phg gelatinized sago starch and 50 phg DNRL (GSNR) gave the suitable patch for TDDS. LB or LH was incorporated into both Gαgly film and GSNR patch. The modified Franz diffusion cells were applied for *in vitro* drug release and permeation studies. Chick chorioallantoic membrane (CAM) and newborn pig skin were used to evaluate the permeation of lidocaine as buccal and skin models, respectively. Both LB and LH could release from Gαgly films and GSNR patches. The release of LH was higher than LB in both Gαgly films and GSNR patches. Moreover, the drugs could permeate through both CAM and newborn pig skin. For Gαgly films, LH could permeate through CAM higher than LB. For GSNR patches, LH could permeate through newborn pig skin lower than LB. This indicated that the lidocaine release or permeation from these films depended on drug property. Medicated Gαgly

films and GSNR patches exhibited different kinetics of drug release and permeation. Most drug release kinetics of Gαgly films in short time and 8 h drug release were fitted to zero order kinetic and first order kinetics, respectively. Most drug permeation kinetics were fitted to first order kinetics. For GSNR patches, most drug release kinetics were fitted to first order or Higuchi's kinetic model. The permeation kinetics of LB-GSNR patches were zero order kinetics or first order kinetics, while that of LH-GSNR patches was not statistically different for three types of kinetics. The results from the stability test for 3 months indicated that these films or patches were recommended to be stored at low temperature. Moreover, the low irritation with CAM test of medicated Gαgly films signified their safety for buccal delivery. In conclusion, gelatin and starch could be blended with plasticizer and anesthetic drug such as LB and LH to obtain the suitable film for use as oral transmucosal films or transdermal patches.

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Suchipha Wannaphatchaiyong

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
°C	degree Celsius
µm	micrometer(s)
µl	microliter(s)
α st, αSt	alpha starch or gelatinized tapioca starch
θ	theta, angle of incidence
AFM	atomic force microscopy
ANOVA	analysis of variance
CAM	chorioallantoic membrane
cm	centimeter(s)
cm^2	square centimeter(s)
DNRL	deproteinized natural rubber latex
DSC	Differential scanning calorimetry
EA	embryo age
EAB	elongation at break
EE	entrapment efficacy
e.g.	exampli gratia, for example
Eq	equation
et al.	et alii, and others
etc.	et cetera, and the other things
Fig.	figure
FT-IR	fourier transform infrared spectroscopy
g	grams
Gαgly	gelatin/alpha starch/glycerin
Ggly	gelatin/glycerin
GEL	gelatin
GLY	glycerin
GNR	gelatin/deproteinized natural rubber latex biomembrane(s)

LIST OF ABBREVIATIONS AND SYMBOLS (cont.)

GRAS	generally regarded as safe
GSNR	gelatin/gelatinized sago starch/deproteinized natural rubber latex biomembrane(s)
HET-CAM	hen's egg-chorioallantoic membrane test
HPLC	high performance liquid chromatography
Hev	hevein alpha-globulin
h, hrs	hour(s)
ICH	The International Conference on Harmonisation
i.e.	id est, that is
IUIS	International Union of Immunological Societies
K	keratinized tissue
KBr	potassium bromide
KHz	kilohertz
LB	lidocaine base
LH	lidocaine hydrochloride
log P	partition coefficient
M	molar
m^2	square meter(s)
mm	millimeter(s)
mg	milligram (s)
min	minute(s)
MW	molecular weight
mL	milliliter(s)
Mpa	megapascal
N	newton
N.D.	not determined
N/m	newton per meter
No.	number
NK	nonkeratinized tissue

LIST OF ABBREVIATIONS AND SYMBOLS (cont.)

NRL	natural rubber latex
OECD	Organisation for Economic Co-operation and Development
OTD	oral transmucosal drug delivery
p	probability value, p-value or significance
PBS	phosphate buffer solution
PEG400	polyethylene glycol 400
PG	propylene glycol
pH	the negative logarithm of the hydrogen ion concentration
Ph. Eur.	European Pharmacopoeia
phg	part per hundred of gelatin
pKa	the negative base-10 logarithm of the acid dissociation constant
r	correlation coefficient
R^2	coefficient of determination
rpm	revolution(s) per minute
RT	room temperature
S.D.	standard deviation
sec	second(s)
SEM	scanning electron microscopy
Si	silicon
Si_3N_4	silicon nitride
SPF	specific pathogen free
TDDS	transdermal drug delivery system
Tg	glass transition temperature
TGA	thermogravimetric analysis
USP	the United States Pharmacopoeia
UTS	ultimate tensile strength
w/v	weight by volume
w/w	weight by weight
WHO	World Health Organization

LIST OF ABBREVIATIONS AND SYMBOLS (cont.)

X	magnification(s) microscope
XRD	X-ray diffraction

LIST OF PAPERS AND PROCEEDINGS

The content of this thesis is based on the following papers and referred to their sequence of experimental design in the text. The publications are attached as chapters 3, 4, and the appendices of the thesis.

Paper 1 Wannaphatchaiyong, S., Boonme, P., Pichayakorn, W. 2017. Gelatin films and its pregelatinized starch blends: Effect of plasticizers. *Key Engineering Materials*. 751: 230-235.

Paper 2 Wannaphatchaiyong, S., Suksaeree, J., Waiprib, R., Kaewpuang, A., Saelee, W., Pichayakorn W. 2019. Gelatin/gelatinized sago starch biomembranes as a drug delivery system using rubber latex as plasticizer. *Journal of Polymers and the Environment*. https://doi.org/10.1007/s10924-019-01510-2.

Proceeding 1 Wannaphatchaiyong, S., Boonme, P., Taweepreda, W., Suksaeree, J., Pichayakorn, W. 2016. Rubber latex as plasticizer for gelatin/gelatinized sago starch biomembranes, The 3rd International Congress on Advanced Materials (AM2016) November 27-30, 2016, Centara Grand at Central Plaza Ladprao, Bangkok, Thailand, The 3rd International Congress on Advanced Materials (AM2016), Programs and abstract book, p. 230.

Proceeding 2 Wannaphatchaiyong, S., Boonme, P., Pichayakorn, W. Gelatin films and its pregelatinized starch blends: Effect of plasticizers. 2016. The 9th International Conference on Materials Science and Technology (MSAT9) December 14-15, 2016, Swissôtel Le Concorde, Bangkok, Thailand, The 9th International Conference on Materials Science and Technology (MSAT9), Abstract book, p. 285.

Petty patent 1 วิวัฒน์ พิชญากร, ประภาพร บุญมี, **ศุจิภา วรรณภาสชัยยง**, วิรัช ทวีปรีดา. 2561. อนุสิทธิบัตรไทย *เลขที่ 13750*. แผ่นแปะผิวหนังสูตรผสมเจลาตินและยางพารา. กรุงเทพฯ: กรมทรัพย์สินทางปัญญา กระทรวงพาณิชย์.

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

There are various routes of drug delivery systems to administer drugs for patients such as oral, rectal, parenteral, topical, etc., which must be examined, and guidelines provided for the recommended dosage for various ages, weights, and states of health to facilitate drug administration by the selected routes of the appropriate dosage forms. The patch or film is one of the dosage forms that can be developed for transdermal or oral drug delivery.

A transdermal patch or film is a medicated patch applied to the surface of the skin to deliver a specific dose of drug through the skin into the bloodstream. Transdermal drug delivery system (TDDS) can improve patient compliance because it is non-invasive, painless, and simple to apply. It also avoids first pass metabolism through the liver, so this can increase its therapeutic efficacy and reduce any side effects of the drug (Delgado-Charro and Guy, 2001; Keleb, *et al.*, 2010; Arunachalam, *et al.*, 2010). This delivery system can regulate the rate of drug release and permeation over a period of several hours.

The oral route is also one attractive mode of drug administration for patch or film formulations. Oral transmucosal drug delivery (OTD) can bypass the hepatic first pass metabolism and avoid drug degradation in the gastrointestinal tract. It is useful for pediatric or dysphagia patients. Oral drug delivery is simple and non-invasive when compared with parenteral administration because of the abundant blood flow and high permeability rate of the oral mucosa (Lam, *et al.*, 2014). Therefore, this delivery system provides for a fast release and permeation of a drug.

Polymers are important not only as TDDS that could control the drug release rate but also OTD that could dissolve and deliver the drug to the oral cavity (Nagaraju, *et al.*, 2013). They should be both biocompatible and chemically compatible with the adding drug and other components of the systems (Mujoriya and Dhamande, 2011). In this study, gelatin and starch have been chosen as polymers to formulate both the TDDS and OTD films. Gelatin and starch are natural polymers that are commonly

used in food, pharmaceutical, and medical applications because they are non-toxic, biocompatible, biodegradable, economical, and easily available (Tabata and Ikada, 1998; Yadav, *et al.*, 2013).

Gelatin is a protein obtained from collagen found in skins, bones, and connective tissues of humans and animals. Gelatin can be divided into two types, depending on the source of materials and the preparation methods, type A and B. Gelatin is slightly soluble in water and is completely soluble above 40°C. It is translucent, colorless or yellowish, brittle, and tasteless (Yadav, *et al.*, 2013). It is compatible with polyhydric alcohols such as sorbitol, propylene glycol, and glycerin which can modify the stiffness of gelatin films (Kim, 2004). It has good film-forming properties (Arvanitoyannis, 2002) and has been used in many formulations of pharmaceuticals such as pastes, suppositories, coating of tablets, capsules and films or patches. Nowadays, new gelatin types can be manufactured from the collagen of fish. The problems of fish gelatin from cold water species are its low gel modulus, low gelling and melting temperature (Leuenberger, 1991). This makes fish gelatin unsuited as mammalian gelatin replacements. Moreover, the use of fish gelatin in commercial products has a new problem in that collagen from fish has recently been declared a potential allergen (Sakaguchi, *et al.*, 1999; Hamada, *et al.*, 2001). In this study, the 160 bloom gelatin B is chosen to use because the results of a preliminary study showed that this gelatin can make good films, and the charge of the gelatin can change by itself depending on the environmental pH.

Starch is obtained from various fruits and vegetables such as corn, potato, rice, sago, banana, etc. It is a polysaccharide carbohydrate mainly made up of two types; amylose and amylopectin. Generally, starch has 20-25% amylose and 75-80% amylopectin (Brown and Poon, 2005). Amylose is a linear polymer of α-1,4 anhydroglucose that has good film-forming properties (Myllärinen, *et al.*, 2002). Amylose is water soluble but this solution can be unstable and tend to precipitate spontaneously. Amylopectin is a highly branched polymer that consists of α-1,4 chains linked by α-1,6 glucosidic branching points in every 25-30 glucose units (Durrani and Donald, 1995). Amylose and amylopectin are structured by hydrogen bonding in starch granules and include crystalline and non-crystalline regions. The ratio of amylose/amylopectin depends on the type and age of the source of starch. Starch

granules are not soluble in cold water because of strong hydrogen bonds. Heating starch in water disrupts the crystalline structure, and the hydroxyl groups of amylose and amylopectin interact with water molecules, thus some parts of the starch can be solubilized (Hoover, 2001). Depending on the type of starch, at temperatures between approximately 65 and 100°C create an irreversible gelatinization process that results in changes of the starch granules, i.e. loss of crystallinity, an increase of the water absorption and swelling of granules (Zhong, *et al.*, 2009) each requiring two steps hydration and diffusion of the solvent in the starch granules and the melting of the crystals of starch (Liu, *et al.*, 1991; Jenkins and Donald, 1998). Starch is most commonly used in daily life as a food ingredient. Moreover, it is used as a pharmaceutical excipient for example as a suspending agent, a disintegrating agent, a binder, for microspheres and as a film forming agent because it is one of the safest excipients from GRAS (Generally Regarded as Safe) that is listed by the WHO (World Health Organization) (Satyam, *et al.*, 2010). In this study, rice starch, glutinous starch and sago starch are used because they have different amounts of amylose. Rice starch has a low amount of amylose, sago starch has a medium amount of amylose, and glutinous starch has no amylose (Oates, 1996). Moreover, the commercially modified starches such as starch 1500® (pregelatinized corn starch) and alpha® starch (pregelatinized tapioca starch) have also been chosen for use in this study to compare with the native starches.

The gelatin/starch films are stiff or hard and brittle. Thus, in this study, water soluble plasticizers i.e., glycerin (GLY), polyethylene glycol 400 (PEG400), propylene glycol (PG) are used for improving the properties of the oral transmucosal films to give flexibility and elongation (Gontard, *et al.*, 1992) because they might be compatible with gelatin and starch, and produce more flexible films. These plasticizers are in the list 35 edition of the United States Pharmacopoeia (USP 35) which are appropriate to select and use in formulation (Snejdrova and Dittrich, 2012).

Natural rubber latex (NRL) is produced from *Hevea brasiliensis* which is a white or yellowish milky liquid (Boonrasri, *et al.*, 2018; Jayadevan and Unnikrishnan, 2018). It contains isoprene monomers which is the cis-1,4- polyisoprene polymer. It has good properties such as its ease to form films or patches, it is biocompatible and has high tensile strength and elasticity (Kawahara, *et al.*, 2004;

Suksaeree, *et al.*, 2014). NRL is suitable for use in TDDS but it contains some proteins which can cause allergic reactions (Perrella and Gaspari, 2002). The proteins content in NRL is a concerned for its use for medical treatments and the proteins should be first removed by several methods such as an alcalase enzyme and/or other chemicals treatments before using as materials in pharmaceutical and cosmetics (Suksaeree, *et al.*, 2012). In this study, thus, deproteinized NRL (DNRL) has also been chosen to mix in the gelatin/starch films as a new plasticizer because it has good properties which might improve the tensile strength and elasticity of the transdermal films.

Lidocaine base (LB) and its hydrochloride salt (LH) are local anesthetics with amide groups and are antiarrhythmic drug which were used as model drugs in this study. LB known as lignocaine and LH, also known as xylocaine hydrochloride or lignocaine hydrochloride, are widely applied as topical and local anesthesia because of moderate action and fast onset. LH can be soluble in an aqueous solution. They are used topically to relieve pain by affecting nerves and muscles and act as a voltage-gate sodium channel blocker which can inhibit the passage of sodium into the nerve cells and not send painful impulses to the brain (Brayfield, 2014). LH is available as an oral topical solution in viscous and in solution for use as a local anesthetic (Malamed, 2013). Nowadays, the commercial products of lidocaine transdermal patch and lidocaine oral transmucosal film still are not available in Thailand, then, they are beneficial to develop lidocaine transdermal patch and lidocaine oral transmucosal film for many patients who would necessary to use them in Thailand.

Therefore, the aims of this study are to formulate the transdermal patch and oral transmucosal film by using natural polymers blends; gelatin and starch, to study their physicochemical properties and *in vitro* drug release, and to evaluate the stability of the films or patches. Lidocaine and its hydrochloride salt form are used as the model drugs. Thus, the patches and films were expected to relieve pain.

1.2 The objectives of this study were as follows;

1. To study the effects of plasticizers and polymer blending on the properties of gelatin/starch blended films.
2. To formulate and evaluate the LB or LH gelatin/starch blended film for use as a transdermal patch.
3. To formulate and evaluate the LB or LH gelatin/starch blended film for use as an oral transmucosal film.
4. To evaluate the stability of LB and LH in both transdermal patch and oral transmucosal film.

CHAPTER 2

LITERATURE REVIEWS

2.1 Human skin

There are many dosage forms to use in topical or transdermal treatment, which skin surface is the site to deliver the drug, such as ointments, creams, gels, pastes, solutions, powders and patches. These dosage forms can be designed for local or systemic effects. For local effects, the drug is directly applied on the specific site which drug is desired to affect (Medical dictionary, 2009). For Systemic effects, the drug can be transported to whole body or many organ systems by blood circulation (Wikipedia contributors, 2018). The skin is the largest organ of human body which is more than 10-15% of body weight. The skin of an average adult body covers a surface area of approximately 2 m^2 and receives about one third of the blood circulating chemical and biological agents. The functions of the skin are protection of the major or vital internal organs from external influences, temperature regulations, control of water output and sensation (Walters and Roberts, 2002; Kanitakis, 2002). The skin is important to deliver, penetrate, or retain the drug because it acts as barrier which is effective and selective to chemical permeation (Barry, 2001). Then, the conditions of skin are morphology, biophysical and physicochemical which should be considered delivering the drug (Patel and Kavitha, 2011).

2.1.1 Structure and physiology of the skin

There are three major layers of the skin: epidermis, dermis and subcutaneous tissue (hypodermis) (Bohjanen, 2013; Gilaberte, *et al.*, 2016) as presented in Figure 1.

The epidermis is the outermost layer which has multiple functions. One of the important functions is controlling small molecules diffuse into systemic blood circulation. Its thickness is approximately 100-150 μm (Sheth and Mistry, 2011). The epidermis is a stratified squamous epithelium. Most of the cells in the epidermis are keratinocyte that originate from cells in the basal layer and change their shape, size, and physical properties when migrating to the skin surface. Cells move from the lower

layers up to the surface. It can be divided into five layers. The sort of epidermis layer from lower to upper is stratum basale, stratum spinosum, stratum granulosum, and stratum corneum for thin skin. The last layer is stratum lucidum which can be found only in thick skin, i.e., palm and sole of feet (Menon, 2002; Gaikwad, 2013), and it locates between stratum granulosum and stratum corneum.

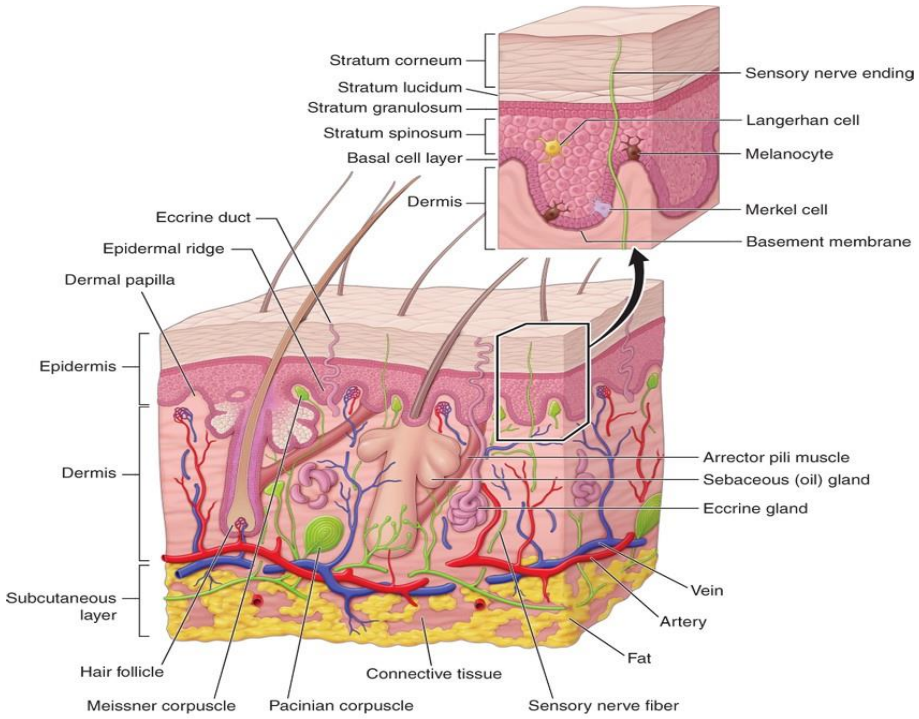

Figure 1 Schematic of a skin cross section (Bohjanen, 2013)

1) Stratum basale is a single layer of cells which is made of basal cells. A basal cell is cuboidal or columnar cell which is a precursor of the keratinocytes. All of keratinocytes are created from this single layer of cells as new keratinocytes and the existing cells is pushed towards the surface.

2) Stratum spinosum is spiny in appearance. It is composed of 8-10 layers of keratinocytes which result from cell division in stratum basale. The keratinocytes in this layer initiate the synthesis of keratin and release a water-repelling glycolipids which prevent water loss from body.

3) Stratum granulosum is more flattened keratinocytes with the thicken cell membranes and has 3-5 layers deep. The cells create the protein keratin and keratohyalin. These proteins give the grainy appearance in this layer.

4) Stratum lucidum is a smooth and seems to be translucent layer. This thin layer of cells is found only in the thick skin. These cells are densely packed with eleidin which derived from keratohyalin.

5) Stratum corneum is the most superficial layer of the epidermis. There are 15-30 layers of terminally differentiated keratinocytes (corneocytes) which are surrounded by long chain lipids. The tight packing of stratum corneum structure results in the obstacle of drug transportation. Therefore, this layer plays an important role in skin barrier function.

The dermis consists of collagenous fiber and elastic connective tissue which produced by fibroblasts. It locates between the epidermis and the hypodermis. It contains blood and lymphatic vessels, nerve endings, and other structures such as sweat glands and hair follicles. The drug can be absorbed by blood vessels into the circulation or penetrated from the junctions between epidermis and dermis. This is the one of the important channel for drug delivery via the skin passes through the epidermis into the dermis (Barry, 1988; Gaikwad, 2013).

The hypodermis or subcutaneous tissue is the layer that acts as the supporting for the epidermis and dermis. This layer is a network of fat cells which arranged in lobules and linked to the dermis by collagen and elastin fiber. Moreover, it binds the skin to underlying muscle. Its functions are energy storage region, provide nutrition support, regulate temperature and mechanic protection (Walters and Roberts, 2002; Tortora and Grabowski, 2006).

2.1.2 Drug transport pathways across the skin

The skin penetration pathways consist of an appendageal pathway and a transepidermal pathway as displayed in Figure 2 (Heisig, *et al.*, 1996; Barry, 2001). Normally, there is no specific pathway for transportation drugs. Most of the drugs are penetrated by a combination of both pathways which depend on the physicochemical properties (Roberts, 1997).

2.1.2.1 The appendageal pathway

The skin appendages include hair follicles, sweat glands, and associated sebaceous glands. This pathway is also called as the shunt pathway (Boddé, *et al.*, 1991; Heisig, *et al.*, 1996). The major penetration is through the hair follicles. However, skin

appendages are only 0.1% of the total surface of the human skin (Saini and Bajaj, 2014). This pathway is important for charged molecules and large polar compounds which remain on the intact stratum corneum. They could be transported through this route (Barry, 2001).

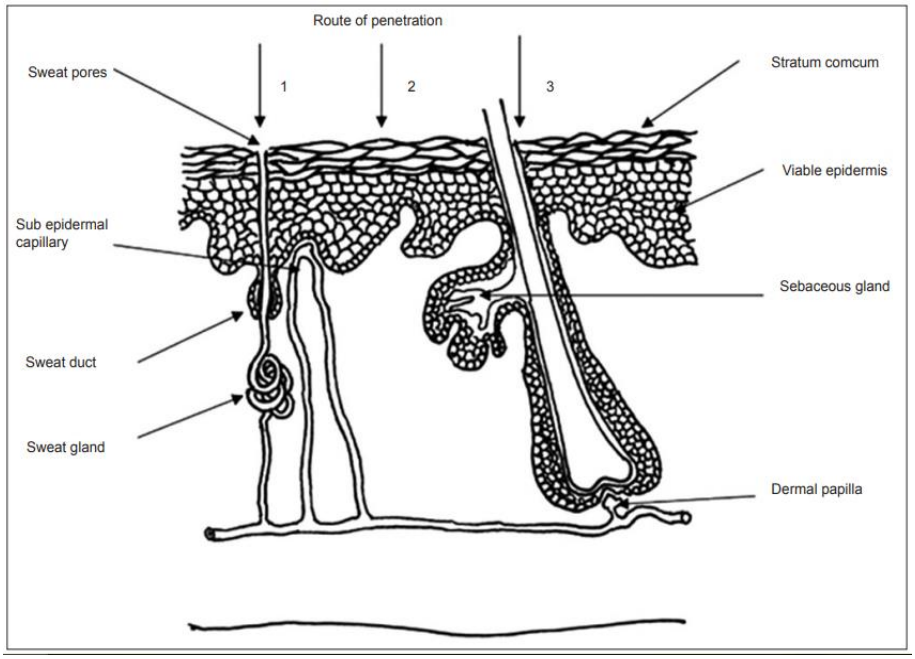

Figure 2 Schematic diagram of drug penetration pathway: (1) through the sweat ducts; (2) directly across the stratum corneum; (3) via the hair follicles with their associated sebaceous glands (Mathur, *et al.*, 2010)

2.1.2.2 The transepidermal pathway

The major route of skin penetration is through the intact epidermis which has two main pathways as shown in Figure 3. The stratum corneum structure has been described as “brick and mortar structure” (Benson, 2005). The bricks are referred to corneocytes, and the mortar is created by the intercellular lipids which arrange molecules surround corneocytes. The intercellular lipid composes of a mixture of ceramides, cholesterol, cholesterol ester, and fatty acids. The first pathway of this route is the transcellular route, the transportation of drugs starts with partition into the lipid bilayer after that across the lipid bilayer to the next keratinocytes. This route is suitable for lipophilic drugs. The other one is the intercellular route. The drugs stay in the lipid bilayer and move around the keratinocytes. The small-unchaged molecules and most of molecules are permeated by this pathway (Gandhi, *et al.*, 2012; Gaikwad, 2013).

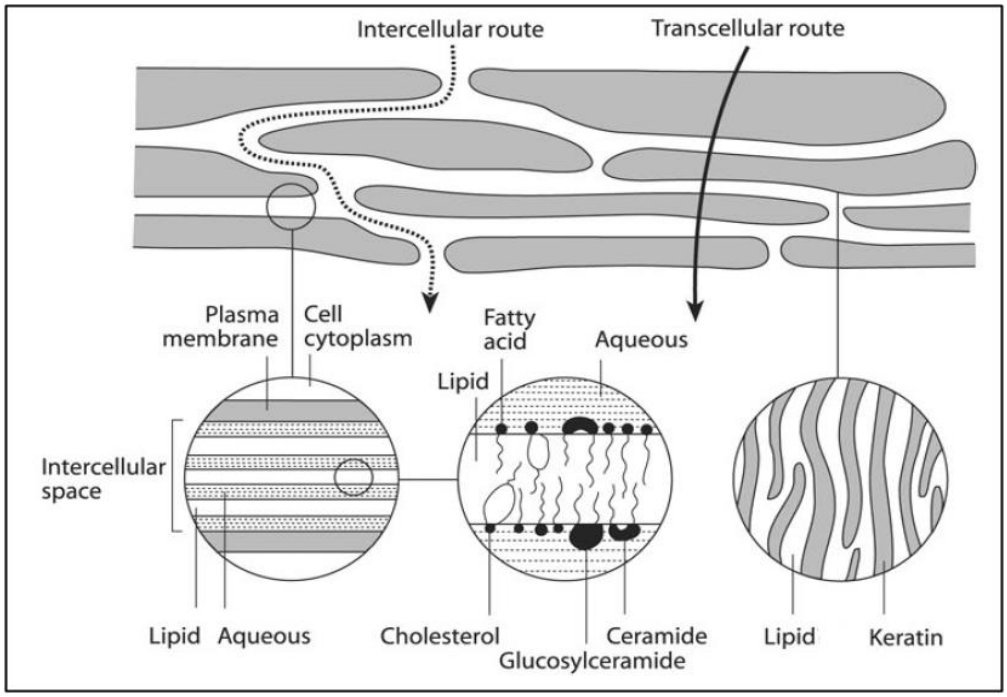

Figure 3 Schematic of stratum corneum and transepidermal routes of drug penetration (Barry, 2001)

In this route, the drug molecules can pass through the skin in various pathways depending on the physiochemical properties of drug and biological properties of skin (Wolff, 2000; Gaikwad, 2013).

2.2 Transdermal drug delivery

Transdermal drug delivery systems (TDDS) is defined as a formulation which is applied to the skin and is designed to deliver the active drug through the skin into the systemic circulation (Barry, 2001; Allen, *et al.*, 2011) and subsequently to receptor sites remote from the area of application (Walters, 1990). Films or patches is one of pharmaceutical dosage forms for using in TDDS.

2.2.1 Advantages and disadvantages of TDDs

There are many advantages of TDDS over the conventional injection and oral routes including improved bioavailability, improved patient compliance, reduced side effects and the maintenance of a stable or constant and controlled drug release (Mujoriya and Dhamande, 2011; Patel, *et al.*, 2012; Sharma, *et al.*, 2013). However, it still has some limitations or disadvantages such as irritated skin (erythema, itching, and local edema), and cannot allow ionic drugs or large sized molecule (should be below

800-1000 Daltons) to be absorbed through the skin, The skin itself has a major barrier function for the body against unwelcome molecules that differs between different persons and at different ages (Patel, *et al.*, 2012; Sharma, *et al.*, 2013).

2.2.2 Types of transdermal patches

Transdermal patches can be categorized by three basic design principles: (1) drugs in adhesive, (2) drugs in matrix, and (3) drugs in reservoir. Moreover, the reservoir type is later separated as peripheral adhesive type by a rate controlling membrane. There are many differences in design of patches as shown in Figure 4.

1) Drug in adhesive layer: The adhesive layer is between a liner and backing. It contains the drug which can adhere to the various layers together and release the drug to the skin (Williams and Barry, 2004). This design can be very thin film because the drug can be dissolved or dispersed within the ingredients of formulation.

2) Drug in matrix layer: This design contains the drug which be dispersed or dissolved in the hydrophilic or lipophilic polymer matrix. This polymeric matrix is fixed the surface area and thickness as a disk. Then, the polymeric disk is fixed onto an occlusive baseplate. The adhesive layer spreads along the patch and surrounds the disk (Saini and Bajaj, 2014).

3) Drug in reservoir: The drug can be in the form of solution, gel or simple dispersed in a solid polymer matrix. The drug reservoir is between a backing layer and a rate controlling membrane. The rate controlling membrane can be non-porous or microporous. Its external surface attaches an adhesive layer. The drug molecules can be delivered to skin by passing through the rate controlling membrane and adhesive layer (Ghosh, *et al.*, 2004).

The common components of patches are release liner, adhesive and backing layers. The release liner protects the patch during storage. It is removed before applying the patch on the skin. The adhesive layer adheres the patch to the skin. The backing layer protects the patch from external factors (Suksaeree, *et al.*, 2014; Saini and Bajaj, 2014). Besides, the polymers play an important role in TDDS because they control drug release from patch. The polymer should be chemically non-toxic, compatible, stable with the ingredients in formulation. The drug selection should also be concerned. The important drug properties for TDDS which able to penetrate the skin

are low molecular weight (<500 Daltons), adequate solubility in oil and water (log P = 1-3), low melting point (<150°C), and high potency (total daily dose<10 mg) (Barry, 2001). Moreover, it should have short half-life and must not irritate or allergic to the skin.

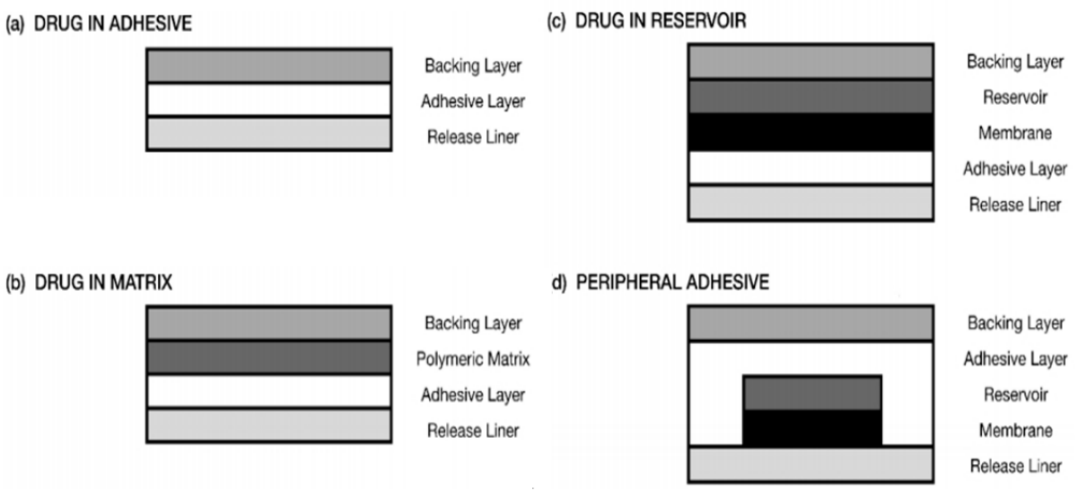

Figure 4 Typical transdermal drug delivery system designs (Walters and Roberts, 2002)

2.3 Oral cavity

The oral cavity is composed of the lips, cheek, tongue, hard palate, soft palate, and floor of mouth as shown in Figure 5 (Squier and Kremer, 2001; Patel, *et al.*, 2011). The oral mucosa lines inside of the cheek. It is composed of an outermost layer of stratified squamous epithelium, a lamina propia, followed by the submucosa as the innermost layer as shown in Figure 6. This oral mucosa structure is quite similar to the skin structure. The oral mucosal thickness varies depending on the site in the oral cavity as shown in Table 1. The function of the oral epithelium is to protect the tissue against harmful agents in the oral environment and from fluid loss (Dowty, *et al.*, 1992). There are three types of oral mucosa in the oral cavity: the lining mucosa is in the outer oral vestibule (the buccal mucosa) and the sublingual region (floor or the mouth), the specialized mucosa is in the hard palate (the upper surface of the mouth) and the gingiva, and the masticatory mucosa is located in the regions which have masticatory activity. The oral cavity presents physiological barriers for oral transmucosal drug delivery (OTD) including pH, fluid volumes, enzyme activity and the permeability of oral mucosa. The saliva is a weak buffer with a pH of around 5.5-7.0 and it provides a

water rich environment in the oral cavity that is appropriate for hydrophilic polymers to release the drug. The enzymes in buccal mucosa include aminopeptidases, carboxypeptidases, dehydrogenases, and esterases which are also barriers for delivery of drugs.

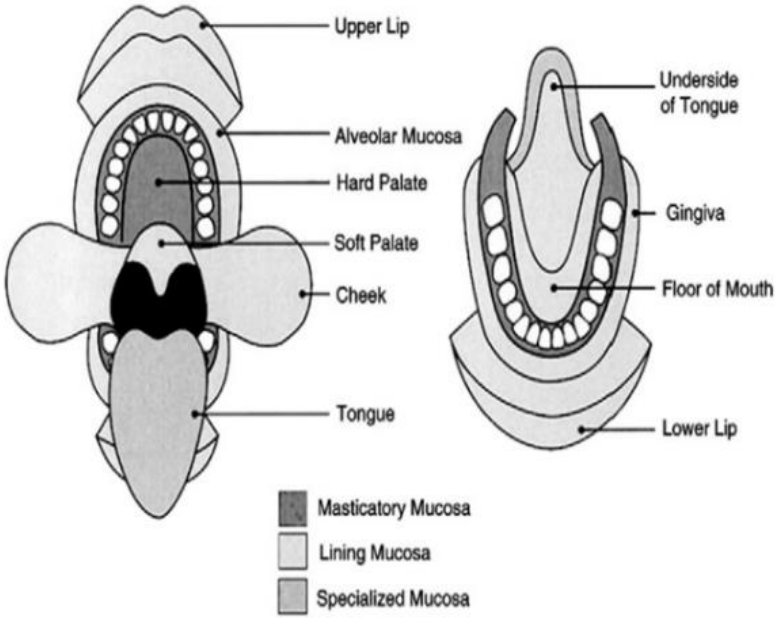

Figure 5 Schematic of the lining of mucosa in the mouth (Squier and Kremer, 2001)

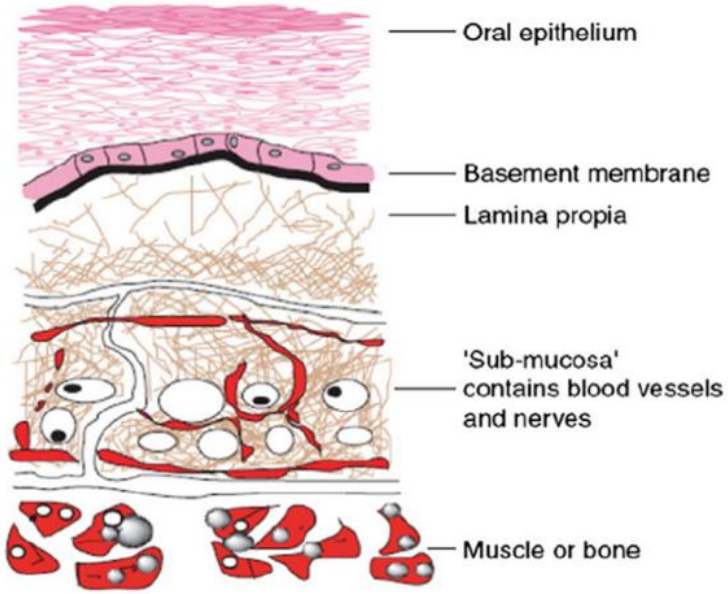

Figure 6 Structural of buccal mucosa (Smart, 2005)

Table 1 Characteristics of oral mucosa (Patel, *et al.*, 2011)

Tissue	Structure	Thickness (μm)	Turn-over time (days)	Surface area (cm^2)	Permeability	Blood flow*
Buccal	NK	500-600	5-7	50.2 ± 2.9	Intermediate	20.3
Sublingual	NK	100-200	20	26.5 ± 4.2	Very good	12.2
Gingival	K	200	-	-	Poor	19.5
Palatal	K	250	24	20.1 ± 1.9	Poor	7.0

Note: NK is nonkeratinized tissue, K is keratinized tissue and *In rhesus monkey (mL/min/100g tissue)

2.3.1 Drug transport routes across the oral mucosa

The drugs transportation across oral mucosa is included with passive diffusion, carrier-medicated active transport or other specialized mechanisms. There are two main routes for drug transportation through epithelial barrier. The paracellular route or intercellular route is transportation the drugs between adjacent epithelial cells, and the transcellular is transportation the drugs across epithelial cells. These routes are similar to drug transportation across the skin as the transepidermal pathway.

2.3.1.1 Paracellular route or intercellular route

The paracellular route is passive diffusion which is occurred by low molecular weight, hydrophilic molecules. The molecules move between the junctions of the epithelial cells. The tight junction in oral epithelia is rare. Then, epidermis of the skin occurs in this transportation more than oral epithelial. However, the intracellular space of oral epithelial cells is more lipophilic environment. Therefore, the lipophilic drugs may also be absorbed via the paracellular route.

2.3.1.2 Transcellular route

The transcellular route is a pathway for low molecular weight, lipophilic molecules. The molecules can be penetrated through several layers of cells until reach the blood capillaries.

2.4 oral transmucosal drug delivery

The oral route is the most common and preferred route for drug administration because of ease of administration and high level of patient compliance.

The drug delivery in the oral mucosal cavity is divided into two categories which are local delivery and systemic delivery via the buccal or sublingual mucosa.

2.4.1 Oral mucosal sites

2.4.1.1 Local delivery: The drugs are applied on the sites which can be retained a delivery system for a desired treatment and length of time.

2.4.1.2 Buccal delivery: The drugs are administrated on the lining of the cheek to the systemic circulation.

2.4.1.3 Sublingual delivery: The drugs are administrated on the membrane of ventral surface of the tongue and the floor of the mouth to the systemic circulation.

2.4.2 Advantages and disadvantages of OTD

OTD is similar to TDDS. It mainly delivers a drug through the oropharynx (Fukuda, 2015). However, the permeability of oral mucosa is about 4-4000 times more than the permeation of the skin (Patel, *et al.*, 2011). Therefore, there are several advantages and limitation on OTD. The advantages of OTD are the ease of administration, rich blood supply, can be used in unconscious or trauma patients, avoid first pass metabolism in the liver and increase bioavailability, etc. However, the OTD has limitations such as irritation of the mucosa or having an unpleasant taste, can use only a small drug dose, provides restriction with eating and drinking, etc. (Sravanthi, *et al.*, 2014).

2.5 Alternative tissue in scientific research

The permeation study has been required to evaluate the effectiveness of drug delivery from drug formulations. The drug should be diffused or absorbed into the membrane in a sufficient amount of therapeutic level. To predict the permeation of drug, a membrane that is similar to human membrane in histology or permeation profile is considered to use.

In TDDS, the human skin is replaced with the various animal skin models such as rat skin, pig skin, snake skin, rabbit skin or cadaver skin. The histological and biochemical properties in pig skin have been studied and shown the similarity in human skin. It provided enough area to determine percutaneous absorption

(Wester, *et al.*, 1998; Alkilani, *et al.*, 2015). Moreover, pig skin is easily to handle and can be received from slaughterhouse. Thus, the pig skin was considered to apply instead of human skin model in this study.

In OTD, many oral mucosal of rats, hamsters, dogs, and monkeys have been studied (Nicolazzo and Finnin, 2008). However, the mucosa in these animals cannot simulate the function of human buccal membrane. Recently, there is a report of chorioallantoic membrane (CAM) from chick embryo which be studied as the buccal membrane (Tay, *et al.*, 2011). The fertilization egg starts to fuse chorion and allantois together and become CAM about 4 days after the egg is laid. CAM plays an important role in embryo respiratory, ion exchange and controls the embryo temperature because a lot of blood vessels pass through this membrane. CAM composes of three layers as presented in Figure 7. First layer is the ectoderm. It includes with cuboidal cells and some capillaries. Second is the mesoderm, it consists of a matrix which composes of blood vessels and connective tissues. The last layer is the endoderm, it is made of squamous and cuboidal cells (Chutoprapat, *et al.*, 2014).

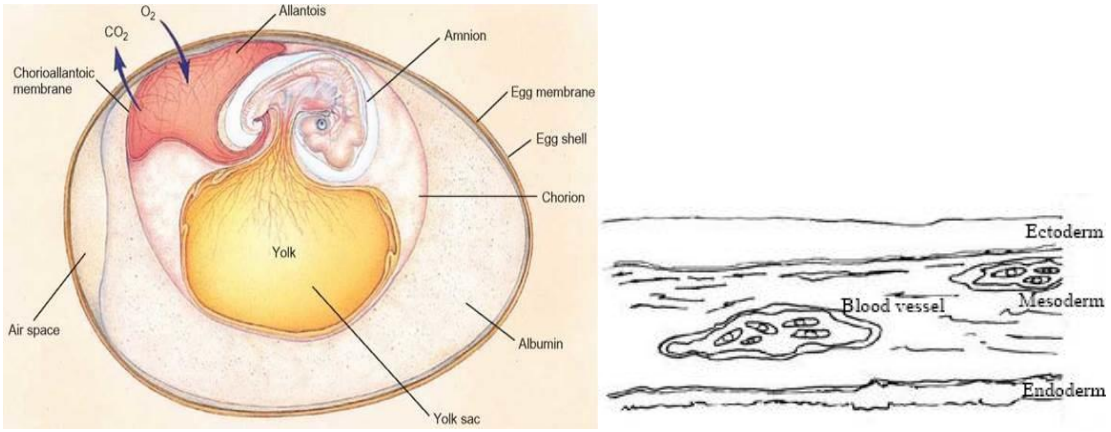

Figure 7 Schematic of chicken embryo (left) and the structure of CAM (right) (Source: https://schoolbag.info/biology/concepts/163.html and http://www.hindawi.com/journals/bmri/2010/940741/fig1/. Access May 5, 2019)

As CAM properties and structure is quite similar to retina, buccal mucosa, lungs, placenta and blood brain barrier tissues in human tissues (Tay, *et al.*, 2011), thus, CAM is the alternative of human tissue models for study. In this study, CAM was chosen to determine the permeability of drug as the human buccal tissue.

2.6 Polymers

Polymers are important in TDDS and OTD because they can control the drug release. The polymer can be used alone or combine to get the desired films or patches. Moreover, the polymer used in transdermal or oral film preparations should be stable, non-toxic, non-irritant and inexpensive (Nagar, *et al.*, 2011; Premjeet, *et al.*, 2011).

Polymers are broadly classified into 2 groups which are natural and synthetic polymers. Both natural and synthetic polymers are used in film preparations such as fast dissolving oral films (Nagar, *et al.*, 2011), buccal patches (Sravanthi, *et al.*, 2014) and transdermal patches (Premjeet, *et al.*, 2011).

- Natural polymers : Tragacanth, Sodium alginate, Guar gum, Xanthan gum, Starch, Gelatin, Chitosan, Natural rubber, Agarose, Pectin, Pullulan
- Synthetic polymers : Cellulose derivatives (Methylcellulose, Ethyl cellulose, Hydroxy ethyl cellulose, etc.), Poly (Acrylic acid) polymers (Carbomers, Polycarbophil), Poly hydroxyl ethyl methylacrylate, Polyethylene oxide, Poly vinyl pyrrolidone, Poly vinyl alcohol, Polyurea, Epoxy

In this study, gelatin, starches and natural rubber as natural polymers were chosen for oral film and transdermal patch preparations.

2.6.1 Gelatin

Gelatin is widely used in photographic, cosmetics, biomedical, pharmaceutical and food industries (Kumar, *et al.*, 2017). Its characteristics are translucent, colorless or yellowish, brittle and tasteless (Yadav, *et al.*, 2013). The major sources of gelatin are from skin and bone of bovine and porcine. It is prepared by partial acid hydrolysis which is gelatin type A, or partial alkaline hydrolysis which is gelatin type B. Due to religious and culture, gelatin is also isolated from fish. Nowadays, insects are also an alternative source of gelatin (Mariod and Adam, 2013). Gelatin can be soluble in water at temperature above 40°C. Various types of gelatin present different components depending on the source of the collagen and preparation method. It is a high molecular weight polypeptide which is between 15,000-40,000 Daltons (Foox and Zilberman, 2015). It contains about 20 amino acids which are connected by peptide bonds. All the essential amino acids can be found in gelatin except tryptophan (Mariod

and Adam, 2013). The gelatin properties, i.e. gel strength, viscosity, setting behavior, and melting point, are depended on molecular weight and the amino composition. The chemical structure of gelatin is shown in Figure 8. Gel strength or bloom is a function of the molecular weight of gelatin. The bloom strength of gelatin is related to its viscosity. In commercial, gelatin has 50-300 bloom. The different applications require the gelatin with different gel strength. In food industry, gelatin can be used as gel former, whipping agent, binding agent, film former, thickener, emulsifier, stabilizer, and adhesive agent. In pharmaceutical industry, gelatin is used for hard and soft capsules, tablet coating, granulation, encapsulation, and microencapsulation. It is also used as excipient in pharmaceutical formulations and film formulations. In this study, gelatin 160 bloom was chosen as the main polymer for film preparations because of its biocompatibility, biodegradability and good film forming properties (Ktari, *et al.*, 2014).

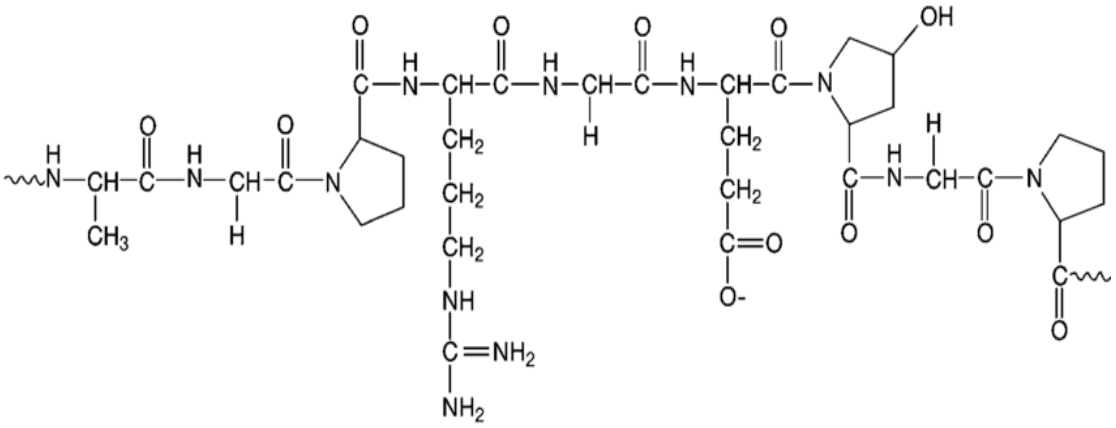

Figure 8 Chemical structure of gelatin (Kommareddy, *et al.*, 2007)

2.6.2 Starch

Starch is a polysaccharide (Hoseney, 1994). It is an energy storage material in plants. Starch granules are produced by photosynthesis and stored in chloroplasts in different parts such as tubers of potato plant, roots of tapioca plant, stem pith of sago tree, and seeds of corn and rice. The characteristics of pure starch powder are white, flavorless and odorless. Starch is composed of lots of glucose units and connected by glycosidic bonds which is polyglucans. The major polyglucans in starch are amylopectin and amylose which show different proportions in various sources (Tester, *et al.*, 2004; Plackett and Vázquez, 2004). Amylose is a linear or slightly

branched polymer which is soluble in water. It contains numerous of glucose units and linked by α-(1,4) glycosidic linkage in the chain (Figure 9(a)) (Mua and Jackson, 1997). Amylopectin is a highly branched polymer which is insoluble in water. It is made of numerous of glucose units and linked by α-1,4 glycosidic linkage in the chain and interlinked the chain by α-1,6 glycosidic linkage at the branch point (Figure 9(b)) (Smith and Martin, 1993).

In general, 20-25% of amylose and 75-80% of amylopectin are contained in starches (Brown and Poon, 2005). The proportion between amylose and amylopectin affects the properties of starch, i.e., viscosity, gel formation, gelatinization temperature, solubility and retrogradation properties (Herrero-Martínez, *et al.*, 2004; Schirmer, *et al.*, 2013). The native starches have limitation such as producing weak structures and breaking down when reheated or in acid environments. Then, starches can be modified by physical, chemical, enzymatical or genetical method to improve or change their properties. They are called modified starches. Physical modification is done to change the granular structure and makes native starch into cold water soluble such as annealing, retrogradation, and gelatinization. Chemical modification uses the reaction condition, substitution, and distribution of the substituents in the starch molecules. This modification includes etherification, esterification, cross linking, acid treatment, and oxidation. Genetic modification uses biotechnology to involve in starch biosynthesis such as amylose-free starch, high-amylose starch, and altered amylopectin structure. Starch and its derivatives are commonly used in daily life as a food ingredient. Furthermore, they have been used in many industries such as foods, plastics, cosmetics pharmaceutical, and biomedical applications (Neelam, *et al.*, 2012).

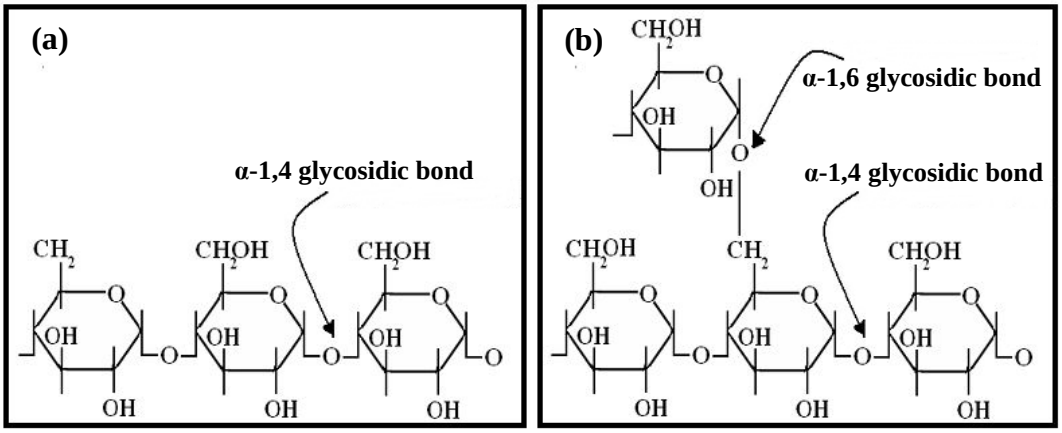

Figure 9 Structure of (a) amylose and (b) amylopectin (Chhabra, 2014)

2.6.3 Natural rubber

The rubber tree (*Hevea brasiliensis*) or para rubber tree has turned to the commercial source of natural rubber. Natural rubber is a native plant of Brazil. It is grown in tropical and subtropical environments (Onokpise, 2004). Nowadays, most of natural rubber is produced from Southeast Asia including Philippines, Indonesia, Malaysia, Thailand, and India.

Natural rubber latex (NRL) is achieved from a fluid in latex vessels which is in the bark of the tree. NRL is a white or yellowish milky-like fluid with negatively charged which has pH in the range of 6.5-7.0 (Subramaniam, 1999). NRL is composed of rubber particles and water which contains lipids, proteins, carbohydrates, amines, and some inorganic substances (Cabrera, *et al.*, 2013; Faita, *et al.*, 2014). The major component of rubber particle in NRL is poly (cis-1,4-isoprene) (Nishiyama, *et al.*, 1996; Roberts, 1988) which the structure is presented in Figure 10. After centrifugation, there are 4 main fractions which are rubber cream (white layer of rubber particles), Frey-Wyssling particles (orange or yellow layer), C-serum, and lutoids (bottom fraction) (Ferreira, *et al.*, 2009). Rubber particles have a diameter range in 0.05-3 μm and molecular weight approximately 50,000-3,000,000 Daltons. Two possible models of rubber particle have been presented arrangement of proteins and phospholipids on the rubber particle surface. First model is a double layer which an outer layer of rubber particle is covered with protein layer and phospholipid layer, respectively, as shown in Figure 11(a), and the other model is a monolayer which is mixed between protein and phospholipid as show in Figure 11(b) (Nawamawat, *et al.*, 2011).

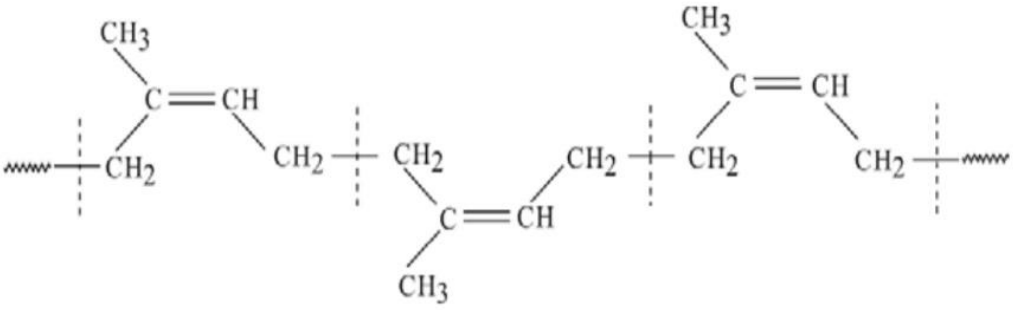

Figure 10 Molecular structure of cis-1,4-polyisoprene (Agostini, *et al.*, 2008)

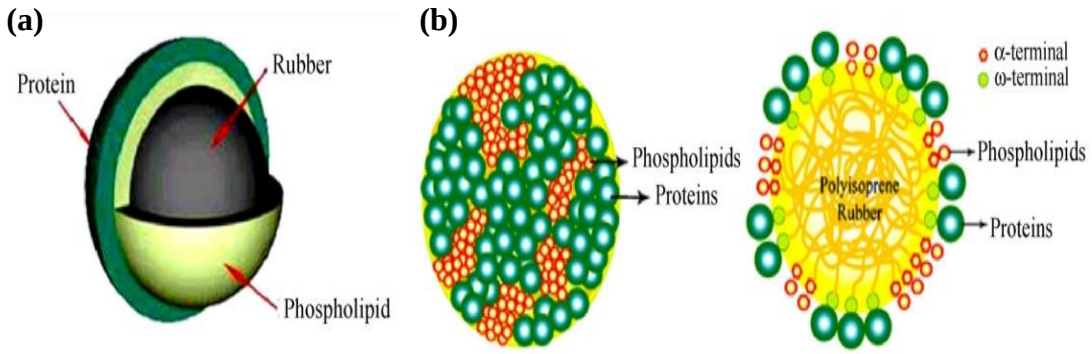

Figure 11 Models of the rubber particle (a) a double layer of proteins and phospholipids and (b) a mixed layer of proteins and phospholipids around the rubber particle (Nawamawat, *et al.*, 2011)

NRL is used in various applications such as tires, gloves, condoms, latex foams, etc. In addition, NRL is applied in medicals and pharmaceuticals as scaffolds, tablets, and transdermal patches (Herculano, *et al.*, 2009; Pichayakorn, *et al.*, 2012b; Panrat, *et al.*, 2013) due to its good properties such as high elasticity and tensile strength, abrasion resistance, biocompatibility, film forming and impermeability to gases and liquids (Mooibroek and Cornish, 2000; Pichayakorn, *et al.*, 2012a).

On the other hand, NRL allergy has been reported as protein retained in latex products caused an IgE-mediated hypersensitivity reaction or other severe anaphylaxis (Kelly, *et al.*, 1994). Allergens in NRL are proteins or chemical additives (Wakelin and White, 1999). At least 14 proteins of NRL are recognized by the International Union of Immunological Societies (IUIS) as allergenic. There are many Hevea latex allergens (Hev b1-14) (Raulf-Heimsoth, *et al.*, 2007; von der Gathen, *et al.*, 2017). Hev b1 and Hev b3 are the major allergenic proteins which located on surface of the large and small rubber particles, respectively (Yeang, *et al.*, 2002). Thus, these proteins are necessary removed. Deproteinization natural rubber latex (DNRL) has been prepared successfully in our research group by enzymatic method which can remove protein for more than 90% (Suksaeree, *et al.*, 2012; Pichayakorn, *et al.*, 2012a). In-house DNRL has been used to prepare transdermal patches which delivered nicotine, meloxicam or lidocaine (Pichayakorn, *et al.*, 2012a; 2012b; Waiprib, *et al.*, 2017) as well as tablets coated with NRL which delivered propranolol (Panrat, *et al.*, 2013).

2.7 Plasticizers

A plasticizer is an additive which incorporated in another material to increase distensibility, workability, or pliability (Godwin, 2011). Normally, plasticizers are high boiling point liquids with low molecular weight. The criteria for plasticizer selection in medicine and pharmacy are low toxicity, biocompatibility, compatibility with polymer, and affect on the drug release and mechanical properties (Snejdrova and Dittrich, 2012). Using plasticizers in various polymeric dosage forms such as microparticles, matrices, membranes, and implants have been studied. For TDDS or OTD, plasticizers are added into polymer to improve film forming properties and the appearance of films, decrease the glass transition temperature (Tg), prevent film cracking, increase film flexibility and receive desirable films.

Most of polymers used in pharmaceutical formulations are brittle and require the addition of plasticizer. Plasticizers act by penetrating between the polymer chains and interaction with the specific functional groups of polymer. It can reduce the interactions between polymer chain and form bonding with the polymer chains instead (Gal and Nussinovitch, 2009). The weaken interaction between polymer chains decreases tensile strength and Tg which increase the flexibility of films (Rahman and Brazel, 2004). The plasticizer is choosing by compatibility with the film forming polymer, solvent system, and ingredients in formulation. Thus, glycerin (GLY), propylene glycol (PG), and polyethylene glycol 400 (PEG400) have been used in this study as their structures are shown in Figure 12. These plasticizers are declared in USP 35-NF 30 as suitable plasticizer for selection in dosage forms formulations (Snejdrova and Dittrich, 2012).

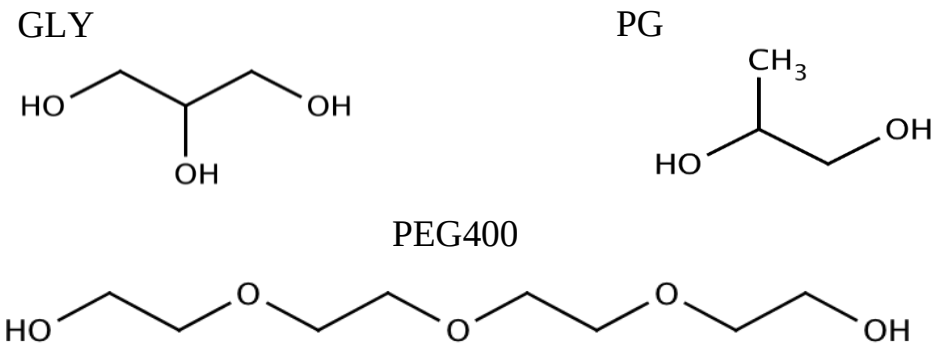

Figure 12 Chemical structure of plasticizers

GLY is also called glycerol or glycerin. It is colorless, odorless, sweet tasting and viscous liquid. GLY composes of three hydroxyl groups which is soluble in water and hygroscopic. It is widely used in pharmaceutical formulations. Moreover, it is orally taken for decrease eye pressure or suppository for laxative effect (Wikipedia contributors, 2004a).

PG is a clear, colorless and viscous liquid. It is a biodiesel byproduct that has two hydroxyl groups in chemical structure. It can be produced from propylene oxide or converted from GLY (Wikipedia contributors, 2004b). It is low toxicity and non-irritating to the skin.

PEGs are biocompatible polymer and soluble in water or organic solvents (Ivanova, *et al.*, 2014). They are synthesized by polymerization of ethylene oxide. PEGs have a broad range of molecular weights. PEG400 has molecular weight 400 Daltons which is colorless, clear and viscous liquid.

These plasticizers are polyols which are hydrophilic polymers. All of them have been used as sweetener, humectant, lubricant, surfactant, or solvent in pharmaceutical formulations. They are low toxicity and considered to be safe ingredients (Vieira, *et al.*, 2011; Snejdrova and Dittrich, 2012). Then, they are suitable to use film formulations as plasticizer.

2.8 Lidocaine

Lidocaine, also named as lignocaine or xylocaine, is an amide type local anesthetic and also used for treatment in the ventricular tachycardia (a cardiac arrhythmia) as an intravenous injection solution. In the European Pharmacopoeia, lidocaine has two forms as the free base and the hydrochloride salt. The structural formula of lidocaine base (LB) and lidocaine hydrochloride (LH) are 2-(diethylamino)-*N*-(2,6-dimethylaphenyl) acetamide and 2-diethylamino-*N*-(2,6-dimethylphenyl)-acetamide hydrochloride monohydrate as shown in Figure 13(a) and 13(b), respectively.

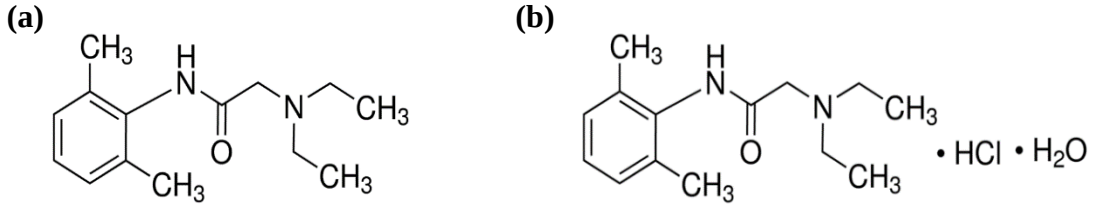

Figure 13 Chemical structures of (a) lidocaine base and (b) its hydrochloride salt

For local anesthetic, both LB and LH act as a voltage-gated sodium channel blocker which inhibit nerves transmission for sending painful impulses to the brain. They are widely used as local anesthetics (Liu, *et al.*, 2018). They are used in minor surgery and dental anesthetic by injection, topical applications to relief burning, itching and pain from skin (Dogrul, *et al.*, 2004; Kadioglu, *et al.*, 2013). Both LB and LH are white odorless substances. LB crystalline powder is fine needles, practically insoluble in water, very soluble in alcohol and methylene chloride, whereas LH is microcrystalline powder and very soluble in water, freely soluble in ethanol (Gröningsson, *et al.*, 1985; British Pharmacopoeia Commission., 2018).

A drug to be considered for TDDS must include good lipophilicity and water solubility at physiological pH, and high potency. The physicochemical properties of a selected drug are melting point (<150°C), high lipophilicity (log P = 1-3), good water solubility, and a small molecule (<500 Daltons). The physical properties of drugs include with melting point, log P and molecular weight are described. Those of LB properties are 68-69°C, 2.44, and 234.34 Daltons, respectively. While those of LH properties are 76-79°C, 2.84, and 288.82 Daltons, respectively. There are many dosage forms of LB and LH such as solutions, ointments, gels, buccal tablets, lozenges, and films or patches (Repka, *et al.*, 2005; Abu-Huwaij, *et al.*, 2007). In commercial, Lidoderm® and lignopad® are available as lidocaine transdermal patch to relief of neuropathic pain associated with herpes zoster and other pains. Besides, a drug for OTD selection criteria is small or moderate molecular weight, good solubility, and good stability in fast dissolving films (Mandeep, *et al.*, 2013) and biological half life between 2-8 h, passive absorption, and high therapeutic effect when given orally in buccal films (Raghavendra Roa, *et al.*, 2013). LB and LH have been studied as active ingredients in both fast dissolving and buccal film formulations (Abu-Huwaij, *et al.*, 2007; Xu, *et al.*, 2017). Then, both LB and LH are suitable for delivering in TDDS and OTD films.

2.9 *In vitro* drug release and permeation studies

In vitro studies also called test-tube experiments. These studies are employed by using biological molecules, cells, or microorganisms outside their normal biological context. The advantages of in vitro experiments are species-specific, easy controlling, convenience, and lower cost than in vivo experiments. The well-designed

in vitro release and permeation studies could predict the results as *in vivo* studies. However, *in vitro* experimental design must be carefully considered to avoid the erroneous results (Gummer, 1989; Smith and Haigh, 1992).

In vitro drug release can be evaluated by using standard or modified dissolution apparatus for film formulations. The various dissolution apparatus (Figure 14) have been used in transdermal patches such as (i) the paddle over disk/disk assembly method (USP apparatus 5/ Ph. Eur. 2.9.4.1) are made of paddle and vessel assembly from apparatus 2 with addition of disk assembly. The disk assembly is design to reduce the dead volume between the disk assembly and the bottom of the vessel which can hold the product, (ii) the rotating cylinder (USP apparatus 6/ Ph. Eur. 2.9.4.3) is modified from basket apparatus which the basket and shaft are replaced with hollow cylinder. The dosage unit is placed on the cylinder and immersed in medium fluid, (iii) reciprocating disk (USP apparatus 7) has a specifically designed disk sample holder inside the solution container and it is used for controlled release formulation and applied to small dosages, (iv) horizontal diffusion cell i.e. side-by-side or using diffusion cell, and (v) vertical type such as Franz diffusion cell (Choudhary, 2008; Hoffmann, *et al.*, 2011; Nair, *et al.*, 2013). In diffusion cells (iv and v), a donor and a receptor compartment are separated by a membrane. The conditions of in vitro studies such as solution or media, temperature, cell dimensions, and hydrodynamic conditions are controlled in these studies (Morales and McConville, 2011).

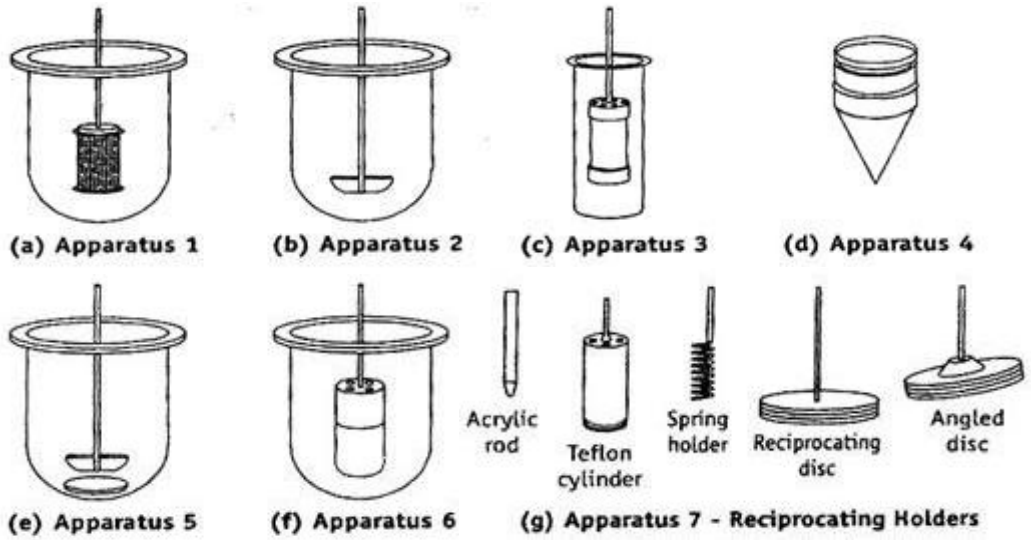

Figure 14 Types of dissolution USP apparatus (Bhowmick, *et al.*, 2014)

In vitro permeation studies can be evaluated by Franz-type diffusion cells which presented the quantitative assessment of rate and mechanism of drug permeation (Keshary and Chien, 1984). These Franz-type studies aim to improve the understanding of the processes, pathways and measure penetration of active ingredients through the membrane into a fluid reservoir (Godin and Touitou, 2007; Bhowmick, *et al.*, 2014). The advantages of *in vitro* permeation study are cost efficient and short time preparations for many samples. One of the most important components in permeation study is the membrane which may come from synthetic or biological sources as described above.

Franz-type diffusion cells are the most commonly used for *in vitro* release and permeation studies. The system has the donor and receptor chambers which are divided by the membrane. The membrane is contacted with the receptor fluid below. The receptor chamber is controlled temperature between 32-37°C to mimic the skin surface or body temperature by water jacket and kept homogenous in concentration and temperature of receptor fluid by a magnetic stirrer (Figure 15) (Bhowmick, *et al.*, 2014).

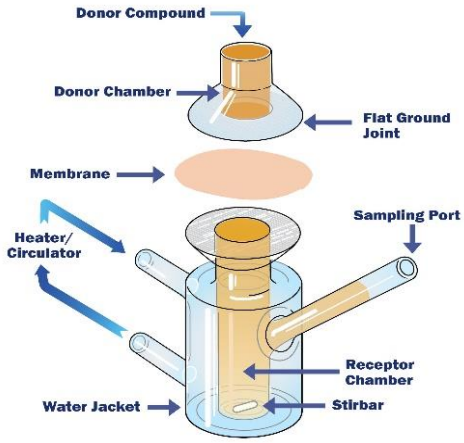

Figure 15 Modified Franz diffusion cell (PermeGear Incorporation, 2019)

2.10 Kinetics of drug release and permeation profiles

The *in vitro* drug release and permeation profiles are significant for their kinetic behavior which can analyze the kind of mechanism in drug release and permeation from formulation (Habib, *et al.*, 2010). The mathematical equations are used to describe the dependence of release in function of time and these can predict the release and permeation kinetics (Costa and Lobo, 2001; Bruschi, 2015). Thus, the

simple kinetics models such as zero order, first order and Higuchi's are useful to describe the release or permeation profile in this study.

Zero-order kinetics: The rate of release or permeation of zero order kinetics does not vary with increasing or decreasing concentrations that is constant until the activity has been exhausted. In this kinetics, a plot between the drug release or permeation (concentration) and time shows a linear response with the rate constant that have the slope of graph equal to k_0 (mg/h). The following relation expressed this model by Eq. 1.

$$Q_t = Q_0 + k_0 t \quad (1)$$

Where Q_t was the amount of drug release or permeation in time (mg)

Q_0 was the initial amount of drug in the formulation (mg)

First order kinetics: This is used to describe the absorption or elimination. The drug release rate is concentration dependent. Plotting the natural logarithm of the drug release or the permeation concentration versus time and observe whether the graph is linear. If the graph is linear with the rate constant (k_1, 1/h) and has a negative slope, the reaction must be a first-order reaction. The following relation expressed this model by Eq. 2.

$$\ln Q_t = \ln Q_0 + k_1 t \quad (2)$$

Higuchi's model: This describes the release of drugs from matrix systems as a square root of time. The graph will be linear when the plot of the LH release or permeation versus time has a rate constant (k_H, mg/$\sqrt{t}$). The following relation expressed this model by Eq. 3.

$$\frac{Q_t}{Q_0} = k_H \sqrt{t} \quad (3)$$

2.11 Irritation assessment

There are several ways to examine irritation potential of substances in the Organisation for Economic Co-operation and Development (OECD) guidelines such as guinea pig test, Draize test, however, they are harmful for animal and also considerable ethical concerns. Then, the alternative methods have been develop for evaluate irritation potential (Gerner, *et al.*, 2005; El Ghalbzouri, *et al.*, 2008). The

reconstructed epidermis, bovine corneal opacity, cell toxicity assessment and permeability assay have been available and accepted by OECD regulations. However, the cost is very high for testing. CAM has been suggested as an alternative membrane to screen irritation potential (Luepke, 1985). The hen's egg test-chorioallantoic membrane (HET-CAM) is employed the CAM at embryo age (EA) 10 because the blood vessels are extensively formed and make CAM sensitive to chemical and biological substances. A test substance is applied to the CAM surface. The changes in morphology of CAM are inspected and scored by naked eye. The scores are evaluated the irritation potential which hemorrhage, blood clotting or hyperemia is occurred in the time. This method requires only 5 minutes for testing and the reaction could be observed. Evaluation the irritancy property by CAM method has been used in ophthalmic microemulsions (Alany, *et al.*, 2006), topical gels (Singh, *et al.*, 2016), buccal microemulsions (Kaewbanjong, *et al.*, 2017), and many substances (Vinardell and Mitjans, 2008). Even though it cannot replace the other models but it can reduce using animal. Thus, CAM is a model which is sensitive, inexpensive and capable of high throughput or handle the large number of samples associated with formulations.

CHAPTER 3

This chapter presents the effect of plasticizers, which were glycerin, propylene glycol, and polyethylene glycol 400, improved the gelatin and gelatin/Alpha starch dissolving films. The physicochemical properties of films were evaluated. Lidocaine base and lidocaine hydrochloride were incorporated in these films and examined the physiochemical properties for using as edible films. This article has been published in the title of “Gelatin films and its pregelatinized starch blends: Effect of plasticizers” in *Key Engineering Materials*. 751: 230-235, 2017.

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Gelatin Films and its Pregelatinized Starch Blends: Effect of Plasticizers

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Keywords: Gelatin, Pregelatinized starch, Plasticizer, Film

Abstract. This study aimed to develop gelatin (GEL) and gelatin/Alpha starch (GEL/αSt) dissolving films as drug delivery by casting method. Because these films were brittle and lack of elasticity, therefore, glycerin (GLY), propylene glycol (PG) or polyethylene glycol 400 (PEG) in various amounts (5-30 part per hundred of gelatin; phg) was used as plasticizer. It was found that all types and amounts of plasticizer could be blended into gelatin solution and the transparent GEL films were formed, except the GEL/PEG films presented in opaque characteristics. However, 30 phg GLY blended film was too softy. Increasing amount of plasticizer caused a decrease in tensile strength and increase in elongation at break (EAB) of films. These GEL films swelled, dissolved and eroded in 2 hours. The αSt was also blended, and the effects of αSt amounts (5-30 phg) and plasticizer types (at 25 phg) on GEL film properties were studied. The αSt dispersions mixed well in gelatin solution and gave homogenous films. The swelling and erosion of GEL/αSt films in water were faster than those of GEL films. Increasing αSt amount prolonged the swelling time and decreased the degradation rate of GEL/αSt films. The tensile strength of GEL/αSt/GLY films slightly increased when the αSt amount increased but those of PEG and PG blended films were not different. The EAB of all plasticizer blended films decreased when the amount of αSt increased. Either lidociane or lidociane hydrochloride was mixed in GEL/αSt/GLY dissolving films to use as local anesthetic. The morphology, Fourier Transform Infrared Spectroscopy confirmed their compatibilities in these films, but Differential Scanning Calorimeter showed some changes that should be further evaluated.

Introduction

Green biofilms are biodegradable materials which are derived from natural biopolymers such as proteins, polysaccharides, lipids or their blends [1]. They have been developed as edible films and coating on foods and packing materials [2]. Moreover, these biomaterials are also used in medical and pharmaceutical applications [3]. Gelatin (GEL) is one of the proteins obtained from the collagen of animal skins and bones. It is interesting as biofilms because it has excellent film forming property [4], biodegradability and biocompatibility [5]. It is normally applied in foods, medicals and pharmaceuticals such as gelling agent, coating and binder [6]. However, it can hydrate, swell, and dissolve in warmed or hot water. Therefore, its dissolution behavior is defined by thermal environment. Moreover, gelatin is also hygroscopic substance.

Another interesting biodegradable material is starch. It is widely used in daily life as food. Moreover, it has been used in pharmaceutical formulations as binder, disintegrant, diluent, emulsifying agent, etc. [7]. It is natural polysaccharide which consists of amylopectin and amylose. Starch also presents a good film-forming property and could produce the transparent films [8]. Amylose gives stronger films and amylopectin presents different mechanical properties such as decrease in tensile stress [9]. Alpha starch (αSt) is a commercially pregelatinized tapioca starch which its properties is modified by physically treating. It could be easy to mix in gelatin solution during film preparation.

Because GEL film is dissolved in water under warm environment, therefore, the gelatinized αSt blends might enhance the degradation rate of GEL films to be faster because this αSt could form gel

in cold water. Thus, the combination of GEL and αSt films was developed in this study. In preliminary study, however, it was found that the GEL/αSt films were still brittle. Glycerol (GLY), propylene glycol (PG) or polyethylene glycol 400 (PEG) are normally used as plasticizers in pharmaceutical film applications. They were chosen to improve the mechanical properties of GEL/αSt films. Lidocaine (LB) or lidocaine hydrochloride (LH) was used as model drug to load into these films because both drugs can use in fast dissolving buccal anesthetic films [10].

Materials and Methods

Materials

The 160 bloom gelatin was purchased from PB Gelatins (Tessenderlo, Belgium). Commercially pregelatinized tapioca starch (αSt) was obtained from Thaiwah (Bangkok, Thailand). GLY and PEG were from Sigma-Aldrich (Munich, German). PG was from P.C. Drug center Co., LTD (Bangkok, Thailand). LB and LH were purchased from Sigma-Aldrich (Shanghai, China). Distilled water was used throughout the experiments. All other solvents and chemicals used were pharmaceutical or analytical grade and used without further modification.

Preparations of films

The 15%w/w gelatin solution was prepared by dispersing the gelatin powder in distilled water, heating at 45°C until it was homogeneous and then cooling down at room temperature. The water soluble plasticizers (GLY, PG, PEG) was added into gelatin solution at 5-30 part per hundred of gelatin (phg), stirred, then poured this homogenous solution into Petri dish and dried at 50°C to form GEL films.

The αSt was slowly sprinkled in distilled water, stirred until it mixed well as 5%w/w αSt solution. GEL/αSt films were also prepared by casting method which the gelatin solution was mixed with the different amounts of αSt solution at 5-30 phg. The plasticizer was then added into this mixed solution, stirred and poured into Petri dish. The cast films were dried at 50°C.

For medicated films, the 5%w/v drug solution was firstly prepared. LB was dissolved in an equal of methanol:water mixture, while LH could be dissolved in water. The drug loading was previously calculated to form the approximately 5%w/w of drug in dried films. Therefore, the calculated volume of drug solution was slowly added and stirred to get homogenous solution after mixing each type of plasticizer. These mixtures were poured into Petri dish and dried at 50°C.

All dried films were peeled off and stored in desiccators at room temperature.

Characterizations of films

Film thickness and weight uniformity

The films were observed visually for homogeneity without phase separation. The thickness of films was measured by using vernier micrometer (Teclok Corporation, Japan) with an accuracy of 0.01 mm. The different 5 parts of films were cut into 2 × 2 cm^2 and weighed. The average weight of the films was calculated.

Swelling and erosion

The films were cut into 2 × 2 cm^2 and weighed (W_0), then immersed in distilled water at room temperature. The excess water at the surface of films was gently absorbed by blotting paper. The hydrated films were weighed at different time intervals (W_w) until 2 hours. Then, they were dried in hot air oven at 50°C overnight and weighed again (W_d). The percentages of swelling and erosion were calculated by Eq. 1 and Eq. 2, respectively.

$$\% \text{ Swelling} = ([W_w - W_0]/W_0) \times 100 \quad (1)$$

$$\% \text{ Erosion} = ([W_0 - W_d]/W_0) \times 100 \quad (2)$$

Mechanical properties

The films were cut into 1 × 4 cm^2. The mechanical properties were evaluated by using TA.XT plus texture analyzer (Stable Micro System, UK) with a 500 N loaded cell. The gauge length of the test area was 10 mm. The cross-head speed was controlled at 10 mm/min. The ultimate tensile strength (UTS) and elongation at break (EAB) were reported.

Fourier Transform Infrared Spectroscopy (FT-IR)

The powder samples were pulverized with dry potassium bromide (KBr) before compressing into a KBr disc. The film samples were examined directly using the Attenuated Total Reflection Fourier Transform Infrared. They were scanned over a wavenumber region of 400-4000 cm^{-1} by FT-IR spectrophotometer (Perkin Elmer, USA) at a resolution of 4 cm^{-1} with 16 scans. The IR transmission spectra were recorded.

Differential Scanning Calorimetry (DSC)

The 5-10 mg of film or raw material was placed into the DSC pan which was hermetically sealed. The sample was heated from 25-450°C at a specified heating rate (10°C/min) in an atmosphere of nitrogen gas by the DSC instrument (Model DSC7, Perkin Elmer, USA).

Results and Discussion

GEL films and GEL/αSt films with or without drug could be prepared by casting method which used GLY, PG or PEG as plasticizer. GEL/GLY and GEL/PG films were transparent but GEL/PEG films were opaque after storage. The opacity of PEG plasticized films might be due to the over compatibility concentration limit in polymer that made it to phase separation [11]. The 30 phg of GLY in GEL film could not be peeled off because it was too softy. Therefore, the maximum of 25 phg plasticizers were then added into GEL/αSt films. The thicknesses of GEL/plasticizer films, GEL/αSt/plasticizer films without drug, and with either LB or LH films were 0.316±0.010, 0.314±0.005, 0.294±0.023 and 0.294±0.037 mm, respectively. Their average weights were 0.1419±0.0174, 0.1356±0.0140, 0.1503±0.0163 and 0.1552±0.0235 mg/4 cm^2, respectively. All GEL/plasticizer films showed the highest swelling at 20-60 min, after that they eroded slowly as shown in Fig. 1(a,b,c). Their erosion was nearly completed in 2 hours. The swelling of GEL/αSt films which used plasticizer at 25 phg were less than non-starch films as shown in Fig. 1(d,e,f). But their swelling rates were faster than those of GEL/plasticizer films, after that they eroded completely. However, increasing the αSt amount tended to decrease the erosion of films due to the gel formation of αSt in swolen films. The swelling of LH films were higher than LB films as shown in Fig. 2, and the erosion of LB films were lower than non-medicated and LH films due to the soluble LH and insoluble LB properties in water. LH is soluble in water, thus, it could enhance the water diffusion into the matrix to form the swollen films and erode rapidly.

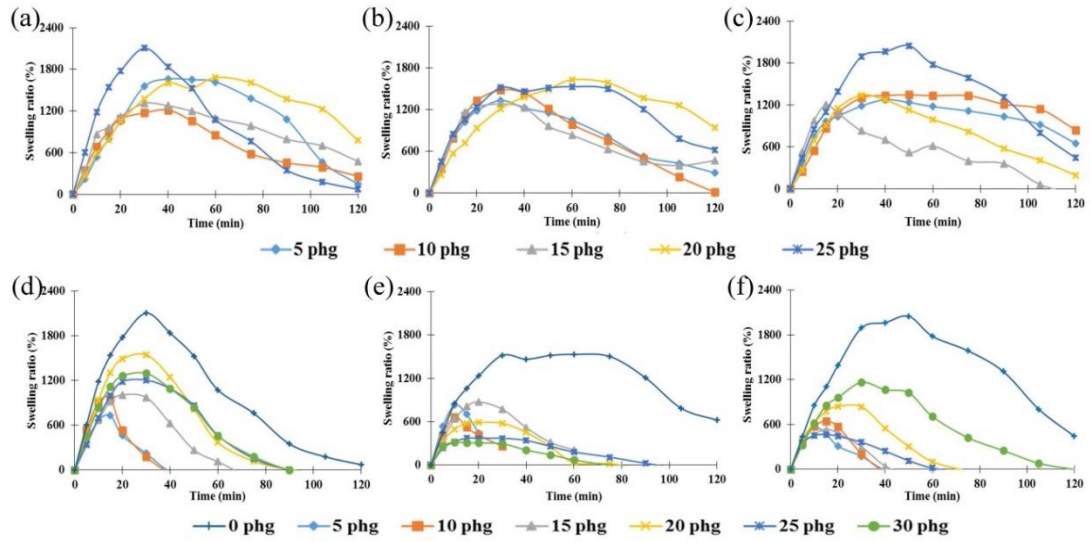

Figure 1 Swelling ratios of (a-c) GEL/plasticizer films in various amounts of plasticizer and (d-f) GEL/αSt films with 25 phg of plasticizer in various amounts of αSt [(a,d) GLY, (b,e) PG or (c,f) PEG]

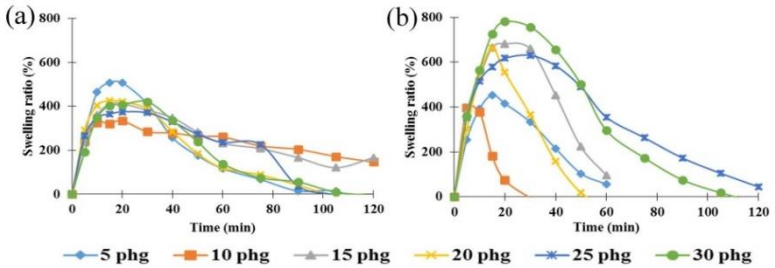

Figure 2 Swelling ratios of GEL/αSt films with 25 phg GLY in various amounts of αSt (a) LB, (b) LH

The UTS of GEL/GLY film was lower than those of GEL/PG and GEL/PEG films as shown in Fig. 3(a). Increasing the amounts of plasticizer could decrease the UTS. The EAB of films presented the different values at 15-25 phg of plasticizer which those of GEL/GLY films were higher than those of GEL/PG and GEL/PEG films as shown in Fig. 3(d). The EAB was increased by increasing the amounts of plasticizer. This indicated that these plasticizers could decrease the brittle and increase the elasticity. GLY was better plasticizer than PG and PEG for GEL film improvement because its UTS value was lower and its EAB value was higher than those of PG and PEG blended films. GLY is the smallest straight chain molecule and acts as the hygroscopic plasticizer which could be inserted between protein chains of gelatin and got more water absorption into the films structure [12]. The GEL/αSt/PG or GEL/αSt/PEG films showed no difference in UTS and EAB when the amount of αSt increased. However, increasing the amount of αSt could increase the UTS and decrease the EAB of GEL/αSt/GLY films in both non-medicated [Fig. 3(b,e)] and medicated films [Fig. 3(c,f)]. Blending of either LB or LH showed the slight decrease of UTS and the slight increase of EAB but not much difference. This might be due to the inserting of drug molecules in polymer films that resulting in the lower mechanical properties of blended films.

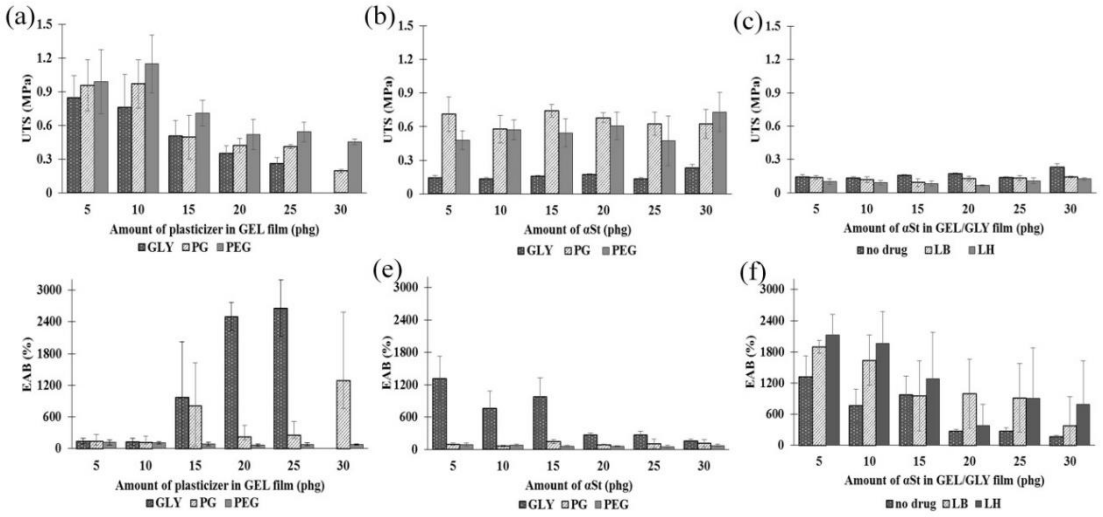

Figure 3 UTS (upper) and EAB (lower) of (a,d) GEL/plasticizer films, (b,e) GEL/αSt/plasticizer films and (c, f) GEL/αSt films with 25 phg GLY including either LB or LH

In FT-IR spectra (Fig. 4), most of them showed the broad OH and/or NH stretching in range of 3275-3300 cm^{-1}, C-H stretching at 2930 cm^{-1}, C=O stretching (amide I) at 1635-1660 and NH bending (amide II) at 1525-1550 cm^{-1}. The FT-IR spectrum of gelatin showed peaks at 1650 (amide I) and 1541 (amide II) cm^{-1} [13,14]. No any new peak was observed in the spectra of blended films indicating their compatibilities. Moreover, the DSC thermograms in Fig. 5 showed peak at 69, 80,

and 311°C which were LB, LH, GLY, respectively. The small peak of gelatin was also found at 61°C. However, GEL/GLY film and GEL/αSt/GLY film with and without drug revealed the new peaks between 125-177°C. This might be due to the plasticization effect of GLY in gelatin chain that could enhance the chain mobility. Therefore, a change in thermal property was observed. Moreover, endothermic peaks of either LB or LH disappeared from the GEL/αSt/GLY films. This might be due to several reasons, for example (1) the very small amount of drugs incorporated that could not be determined, (2) the change to amorphous form of drug after solubilization during film preparation, or (3) the disappearance of the endothermic peak of both drugs together with the appearance of new peaks at 125-177°C could indicate that drugs might undergo some chemical interaction with polymer during casting process. Therefore, the drug property after extraction from these films should be further evaluated.

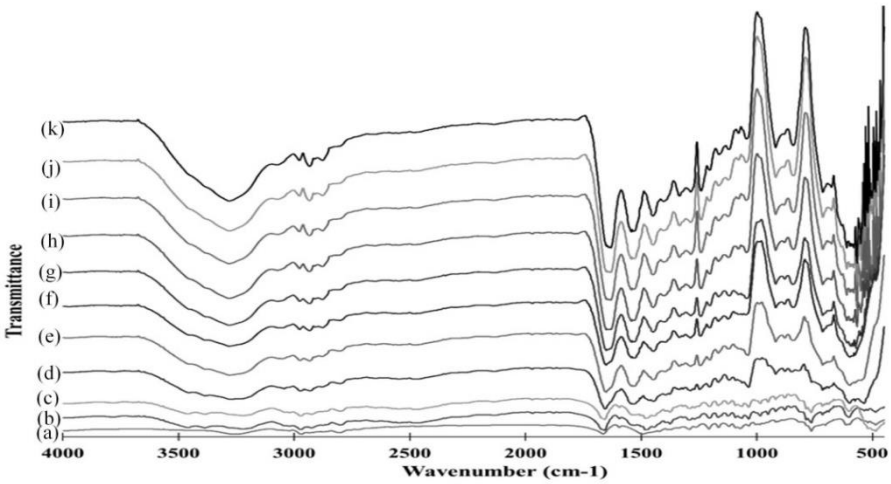

Figure 4 FT-IR spectra of (a) LB, (b) LH, (c) gelatin, (d) GEL/GLY film, (e) GEL/PG film, (f) GEL/PEG film, (g) GEL/αSt/GLY film, (h) GEL/αSt/PG film, (i) GEL/αSt/PEG film, (j) GEL/αSt/GLY/LB film, (k) GEL/αSt/GLY/LH film

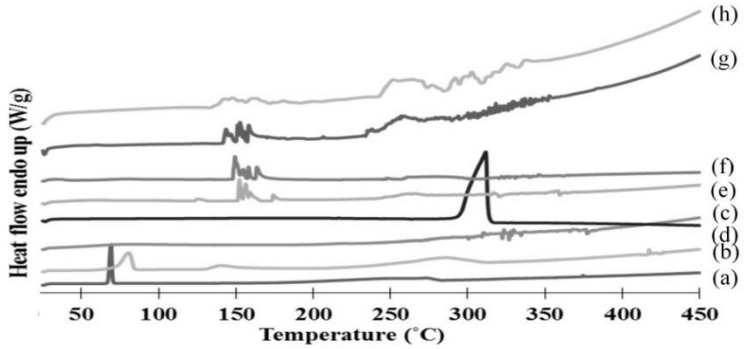

Figure 5 DSC thermograms of (a) LB, (b) LH, (c) gelatin, (d) GLY, (e) GEL/GLY film, (f) GEL/αSt/GLY film, (g) GEL/αSt/GLY/LB film, (h) GEL/αSt/GLY/LH film

Conclusions

The GEL films and GEL/αSt films could be prepared by casting method which GLY, PG or PEG could be added as plasticizer to improve the brittleness of films. Increasing the amount of plasticizer resulted in decreasing the UTS. However, it resulted in increasing the EAB of films only using GLY. The GLY was better plasticizer than PG and PEG for GEL and GEL/αSt film preparations which gave the transparent films without brittleness. The αSt blends showed the decrease in

swelling, but they were faster degradation than GEL film itself. However, increasing the amount of αSt tended to decrease the erosion rate of films. The 5%w/w of LB or LH could be mixed in the films and gave the good properties in flexibility and erosion of films. FT-IR spectra exhibited the compatibility of all ingredients, but DSC thermograms showed some changed between drugs, GLY, and gelatin that should be confirmed. LB and LH films would be further studied in drug release properties for drug delivery systems.

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CHAPTER 4

This chapter presents the preparation and characterization of biomembranes using deproteinized natural rubber latex (DNRL) as plasticizer. Lidocaine base and its hydrochloride salt were incorporated in these biomembrane and evaluated the physiochemical properties for using as transdermal patches. This article has been published in the title of “Gelatin/gelatinized sago starch biomembranes as a drug delivery system using rubber latex as plasticizer” in *Journal of Polymers and the Environment*., 2019. https://doi.org/10.1007/s10924-019-01510-2.

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ORIGINAL PAPER

Gelatin/Gelatinized Sago Starch Biomembranes as a Drug Delivery System Using Rubber Latex as Plasticizer

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Abstract
Preparation and properties of gelatin and gelatin/gelatinized sago starch biomembranes as a drug delivery system are presented. Deproteinized natural rubber latex (DNRL) was chosen to blend as a plasticizer in biomembranes due to its elasticity. The opaque biomembranes were formed, and the effect of DNRL amounts (10–50 part per hundred of gelatin; phg) on mechanical properties of biomembranes was investigated. It was discovered that increasing the amounts of DNRL could decrease the brittleness of biomembranes. However, these DNRL blended biomembranes still swelled, dissolved in water, and eroded within 2 h. The 50 phg DNRL was further chosen for biomembrane preparations. Then, the 5–30 phg gelatinized sago starch which was prepared in-house was added. These gelatin/gelatinized sago starch biomembranes were homogenous and opaque. They portrayed good water uptake properties, and the highest values occurred in 20–30 min, then these biomembranes eroded slowly. Increasing the amounts of sago starch in biomembranes raised the water uptake rates but reduced the erosion properties. Biomembranes with and without starch blends showed the same tensile strengths. Their elongations at break increased when the amounts of starch were raised but still were lower than that of gelatin/DNRL biomembranes. The morphology, scanning electron microscopy, atomic force electron microscopy, Fourier transform infrared spectroscopy, differential scanning calorimeter, and X-ray diffractometer confirmed the compatibility of ingredients in biomembranes. Lidocaine and its hydrochloride salt were successfully incorporated in these biomembranes with good properties. The drug release and permeation of biomembrane showed good drug controlled release and were able to permeate through the pig skin.

Keywords Gelatin · Sago starch · Natural rubber latex · Biomembranes · Drug delivery

Introduction

Nowadays, polymeric biomembranes are normally prepared from many natural biopolymers such as starch [1], gelatin [2], whey protein [3], chitosan [4], etc. These biopolymers can easily form films. Moreover, they are safe because of their biodegradability and biocompatibility [5]. Thus, they are of interest for film productions in several applications such as photography, foods, cosmetics and pharmaceuticals [6]. Therefore, these systems are noteworthy for drug delivery in transdermal, buccal and oral systems.

Gelatin is a natural polymer derived from collagen of animal skin and bones. It is widely used in medicine and pharmaceuticals [7, 8]. Moreover, gelatin has good film forming property [9] which might provide a controlled release system for drug delivery [10]. However, gelatin films are quickly soluble and erodible; therefore, they are difficult to use in long-acting drug delivery systems. Gelatin has been prepared as transdermal films by the casting method and the effects of various proportions and concentrations of gelatin on their physical properties have been studied. It was found that all films of gelatin and glycerin blends were suitable as they had flexibility, clarity and elasticity. In that study, diclofenac sodium was used for studying in vitro drug release and in vitro permeation through cellophane membrane [10]. Moreover, gelatin has also been combined with other polymers such as chitosan [11], pectin [12] and starch

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[13] for improving the films properties such as controlling drug release rate, physical property, etc.

Starch is also an interesting biodegradable material widely used in daily life as a food ingredient and pharmaceutical formulation as a binder, disintegrant, diluent, etc. [14]. Starch is a natural polymer which consists of amylose and amylopectin. Amylose is a linear molecule with a few branches which can give stronger films. Amylopectin is a highly branched molecule leading to different mechanical properties of films [15]. The starch can be suspended in water, and after being heated at or above gelatinization temperature, it is gelatinized, and starch granules swell to many times their original size and crystalize inside the granules [16]. Sago starch is the natural starch isolated from sago palms (*Metroxylon sagu* Rottb.) which are distributed in South Pacific islands and extend westward through Melanesia into Indonesia, Malaysia and Thailand [17]. Sago starch has been used for food preparation and in cosmetic formulations [18]. Due to the very high water uptake property of gelatinized sago starch, the combination of gelatin and gelatinized sago starch is interesting to study for drug delivery systems. However, in previous research, gelatin/gelatinized sago starch biomembranes were too brittle [19]. This mechanical problem could be improved with plasticizer addition. In film technology, there are many plasticizers such as glycerol, ethylene glycol, sorbitol, fatty acid, etc., which are used to increase the flexibility of the films [6, 20, 21].

The natural rubber latex (NRL) is natural polymer produced by *Hevea brasiliensis* or Para rubber trees. It is composed of cis-1,4-polyisoprene polymer. The properties of interest in NRL polymers are high elasticity and easy to prepare films [22]. NRL has been used in industrial products such as tires, gloves, balloons, tubes and condoms [23–25] and in developed pharmaceutical formulations such as film coated tablets [26] and transdermal patches [27]. Due to the high lipophilicity of NRL polymers, its property could be modified by blending NRL with several hydrophilic polymers [28]. Moreover, the protein allergens in NRL should also be removed due to their allergy causes [29]. There are several studies which use deproteinized NRL (DNRL) as a major polymer for transdermal drug delivery systems; for example, nicotine transdermal patches [30], lidocaine transdermal patches [31] and sulindac electrically controllable transdermal patches [32]. Thus, in this study, DNRL was chosen for use as a plasticizer to increase the flexibility of gelatin and gelatin/gelatinized sago starch blended biomembranes.

This study aimed to prepare gelatin/gelatinized sago starch biomembranes with DNRL as plasticizer. Lidocaine, the model drug in either base form (LB) or hydrochloride salt (LH), was loaded into the biomembranes, and the physicochemical properties of these biomembranes were evaluated.

Materials and Methods

Materials

The 160 bloom gelatin was purchased from PB Gelatins (Tessenderlo, Belgium). Sago starch was prepared in-house from sago trees in Nakorn-Srithammarat province, Thailand. DNRL was prepared in-house by our research group using the enzymatic method [33]. LB and LH were purchased from Sigma-Aldrich (Shanghai, China). Distilled water was used throughout the experiments. All other solvents and chemicals used were pharmaceutical or analytical grade and used without further modification. For drug permeation through pig skin, the pig skin was obtained from a newborn pig which had died naturally after birth. The weight of the newborn pig was between 1.4 and 1.8 kg. The dead newborn pigs were directly purchased from a local pig farm in Songkhla province which was regulated by the Department of Livestock Development, Thailand, and promptly prepared to surgically remove the skin. The hair, subcutaneous fat and other extraneous tissues were removed from the skin. The morphological integrity of this prepared skin was observed firstly. After that, the pig skin was stored at − 20 °C and used within 3 months [34–36].

Preparation of Biomembranes

Gelatin/DNRL biomembranes (GNR) were formed by a casting method in which 10, 20, 30, 40 or 50 phg of DNRL was used as the plasticizer. The gelatin was dissolved in distilled water, heated to 45 °C, cooled to room temperature and adjusted to the final concentration of 15% w/w gelatin solution. DNRL was added into the gelatin solution. The mixed solution was poured into the Petri dish and heated at 50 °C for 24 h until a dried film formed.

Gelatin/gelatinized sago starch biomembranes (GSNR) were also prepared by the casting method. The 15% w/w gelatin was prepared in the same method as described above. The gelatinized sago starch was prepared by heating 5% w/w sago dispersion until it boiled and cooled down. The gelatin solution was mixed with various amounts of gelatinized sago starch at 5, 10, 15, 20, 25 and 30 phg. The 50 phg DNRL was chosen to add in the mixed gelatin/gelatinized sago starch and stirred until mixed well. For medicated biomembranes, LB was dissolved in 1:1 mixture of methanol:water to get 4% w/v of drug solution, while LH was dissolved in water in the same concentration. Either LB or LH solution was slowly added and stirred to get homogenous dispersion after mixing DNRL in gelatin solution. These mixtures were poured into the

Petri dish and dried at 50 °C for 48 h until a dried film formed. The amount of either LB or LH loading was calculated in advance until there was a final concentration of 5% drug in dry basis biomembranes.

Characterizations of the Biomembranes

The physical appearance of biomembranes was optically observed for film homogeneity. Five pieces of each biomembrane were prepared, and their thicknesses were measured by using vernier micrometer (Teclok Corporation, Japan) with an accuracy of 0.01 mm. The microstructure observation of biomembranes was studied by using an atomic force microscope (AFM; model nanosurf easyscan2, Switzerland) with a non-contact mode using static mode etched silicon probes (ACLA). The roughness of each biomembrane was calculated in three regions from AFM results using easyscan2 control software and Gwyddion. Scanning electron microscope (SEM; model FEI: SEM-Quanta 400, USA) was used for investigating the top surface, the bottom surface and a cross-section of biomembranes.

The five dried GNR and GSNR biomembranes and three dried medicated biomembranes were cut into 2 cm × 2 cm squares, weighed (W_0), and then immersed in distilled water at room temperature. The excess water of hydrated biomembranes was moped by filtered paper and weighed at different intervals (W_w) for 2 h. They were then dried in a hot air oven at 50 °C overnight and weighed again (W_d). The water uptake and erosion studies of the biomembrane were evaluated. The percentages of water uptake and erosion were calculated by Eq. 1 and 2, respectively.

$$\% \text{ Water uptake } = ([W_w - W_0] / W_0) \times 100 \quad (1)$$

$$\% \text{ Erosion } = ([W_0 - W_d] / W_0) \times 100. \quad (2)$$

The five samples of dried biomembranes were cut into 1 cm × 4 cm. Their mechanical properties were determined by using TA.XT plus texture analyser (Stable Micro System, UK). The ultimate tensile strength (UTS) and elongation at break were evaluated.

The functional groups of each ingredient and their composite biomembranes were investigated by Fourier transform infrared spectroscopy (FT-IR; model Spectrum One, Perkin-Elmer, USA). The range of wavelength scanning, resolution, scan interval and number of scans of FT-IR conditions were 450–4000 cm^{-1}, 4 cm^{-1}, 1 cm^{-1} and 8 scanning times, respectively. The thermal analysis was evaluated by differential scanning calorimeter (DSC; model DSC7, Perkin-Elmer, USA) heating from room temperature to 450 °C with the scanning rate of 10 °C/min in an atmosphere of nitrogen gas. The compatibility of each ingredient was evaluated. The X-ray diffractometer (XRD; model X'Pert MPD, Philips, the Netherlands) was used to detect the compounds in materials and biomembranes. The running XRD parameter was 40 kV, 35 mA, scan range (2θ) 5–90°, step size (2θ) 0.05°, time/step 1 s and scan speed 3°/min. These evaluations were tested without any replication because these were only screening studies for the compatible evaluation, and each instrument was calibrated before determining each sample.

The drug extraction from biomembranes was evaluated by using methanol as a solvent because of its ability to dissolve both LB and LH. The 1 cm × 2 cm biomembrane was cut into small pieces and 10 mL of methanol was added. The biomembranes were extracted by sonicating for 15 min and resting for 24 h. After that, they were sonicated for 1 h, and diluted with phosphate buffer saline solution (PBS) to the suitable concentration for analysis by high performance liquid chromatography (HPLC, Agilent 1100 series, USA.) at 254 nm. The HPLC column was Themo scientific BDS HYPERSIL C18 Diameter (150 × 4.6 mm, particle size of 5 µm) The mobile phase was 50 mM ammonium acetate with 1% v/v acetic acid:methanol (60:40% v/v) and 0.1% v/v triethylamine of the total volume, and the flow rate was 0.8 mL/min. The injection volume of sample was 50 µL. This extraction was done in triplicate. The drug content (% w/w) was calculated as the ratio of drug extraction ($D_{analyze}$) and the accurate weight of dried biomembranes ($W_{accurate}$) following Eq. 3. The percentage of drug entrapment efficiency (% Drug EE) was calculated from the drug extraction ($D_{analyze}$) compared with theoretical drug loading (D_{theory}) following Eq. 4.

$$\% \text{ Drug content } = (D_{analyze} / W_{accurate}) \times 100 \quad (3)$$

$$\% \text{ Drug EE } = (D_{analyze} / D_{theory}) \times 100 \quad (4)$$

Moreover, the methanol residues in the LB loaded biomembranes were determined in triplicate by a gas chromatography-flame ionization detector (GC-FID, Agilent HP 6850 GC, USA). The chromatographic column was HP 19091N-113E capillary column (30 m × 320 µm × 0.25 µm). GC oven temperature started at 50 °C and held for 6 min and then was raised to 200 °C. This experiment was done within a few days after the dried film was obtained.

The drug release and permeation of medicated biomembranes were evaluated by modified Franz diffusion cells. The biomembranes were placed on the donor compartment that was separated from the receptor compartment by a dialysis membrane (MW cut-off 12,000) for drug release study and on pig skin for permeation study, respectively. In this skin permeation study, the skin was obtained from the naturally dead newborn pig; therefore, the ethical approval was not needed [34–36]. The skin was thawed and soaked in PBS solution before use. In order to deliver the drug through the skin in transdermal drug delivery, the drug should release and permeate in the blood circulation. In this study, PBS pH 7.4, which is a similar pH to blood, was chosen to be added

in the receptor compartment, with a controlled temperature of 37 °C, and stirred with a magnetic stirrer at 200 rpm. A 1 mL aliquot of the solution was withdrawn from the receptor compartment at predetermined time intervals of 5, 10, 15, 30, 45 min and 1, 2, 3, 4, 6, 8, 12 and 24 h, with an equal volume of PBS added to replace the removed liquid in the receptor. The drug concentration in each sample was determined by HPLC at 254 nm. In vitro drug release and permeation profiles were further analyzed into three types of kinetics including zero order, first order and Higuchi's kinetics. Each sample analysis was performed in triplicate and quintuplicate in kinetics of drug release and drug permeation, respectively. All results were presented as mean ± standard deviation (S.D.). Statistical analysis was calculated using ANOVA with a statistical significance level set at $p < 0.05$, and the post hoc Tukey procedure was done with SPSS.

The stability of medicated biomembranes was studied by keeping them for 3 months at three various temperatures, i.e., 4 ± 1 °C, ambient temperature (≈ 28 ± 4 °C), and 45 ± 1 °C. They were evaluated for changes in appearance, drug content, drug release and permeation profiles by the previously described methods. The replication of each evaluation was the same as above.

Results and Discussion

For film preparations, many parameters were used to decide on a good formula. Physical appearance and film thickness were simple parameters that could be observed immediately. These parameters represented the product's appeal when the user encountered it. Methanol residue was also measured to ensure that the prepared biomembranes were safe to use. Likewise, AFM and SEM also confirmed the microstructures of these films that related to their physical appearances. These also explained the compatibility of the film's ingredients. Moreover, some physical and mechanical properties of films could be important to the use of products. In this study, water uptake, erosion, UTS and elongation at break of developed biomembranes were evaluated to consider the DNRL property for acting as a plasticizer. The water uptake and erosion of films also affected the drug release and stability of biomembranes when used.

Our studies showed that DNRL could be used as a plasticizer. In our preliminary study (data were not shown), the 10, 20, 30, 40, 50, 75 and 100 phg of DNRL were mixed in gelatin/DNRL (GNR) biomembranes. It was found that increasing the amount of DNRL decreased the water uptake and erosion of GNR biomembranes. The 75 and 100 phg of DNRL in GNR provided less water uptake and less-erodable biomembranes. In this study, however, gelatin was chosen as the main polymer, and DNRL was intended for use as a plasticizer only. The developed biomembranes should have the ability to absorb water and release the drugs; therefore, DNRL was further studied in the range of 10–50 phg for preparing gelatin/gelatinized sago starch/DNRL (GSNR) biomembranes. Both the GNR and GSNR biomembranes with or without drug (LB or LH) were prepared by the casting method in the Petri dish. In the drying process at 50 °C, the products in the Petri dish were weighted after 24 h. Then, they were further dried and weighed again every 12 h. The results showed that the weight of GNR biomembranes did not change after 24 h, but those of GSNR biomembranes changed until 48 h and then were constant. These findings indicated that GNR biomembranes could be dried within 24 h, but sago starch blended biomembranes could delay the drying time of films to 48 h. Therefore, these drying conditions were used in further study.

All GNR and GSNR with or without drug biomembranes were homogeneous and opaque in character. The average weight and thickness of all biomembranes were not different as shown in Table 1, because they could be controlled by forecasting the calculated dried weight of components in each Petri dish.

All blank GNR biomembranes showed the highest water uptake at 15–30 min; after that they eroded slowly as shown

Table 1 The weight and thickness of GNR, GSNR and medicated GSNR biomembranes (n = 5)

Sample	Weight ± S.D. (g/4 cm^2)	Thickness ± S.D. (mm)
GNR 10 phg	0.1152 ± 0.0120	0.277 ± 0.027
GNR 20 phg	0.1429 ± 0.0250	0.327 ± 0.037
GNR 30 phg	0.1230 ± 0.0098	0.289 ± 0.038
GNR 40 phg	0.1414 ± 0.0120	0.336 ± 0.017
GNR 50 phg	0.1306 ± 0.0230	0.329 ± 0.038
GS 5 phg NR	0.1296 ± 0.0069	0.368 ± 0.026
GS 10 phg NR	0.1334 ± 0.0093	0.348 ± 0.022
GS 15 phg NR	0.1460 ± 0.0050	0.396 ± 0.028
GS 20 phg NR	0.1444 ± 0.0105	0.374 ± 0.036
GS 25 phg NR	0.1350 ± 0.0085	0.396 ± 0.016
GS 30 phg NR	0.1465 ± 0.0058	0.367 ± 0.027
LB-GS 5 phg NR	0.1257 ± 0.0119	0.320 ± 0.033
LB-GS 10 phg NR	0.1184 ± 0.0163	0.319 ± 0.047
LB-GS 15 phg NR	0.1322 ± 0.0076	0.355 ± 0.023
LB-GS 20 phg NR	0.1447 ± 0.0085	0.358 ± 0.026
LB-GS 25 phg NR	0.1494 ± 0.0043	0.344 ± 0.005
LB-GS 30 phg NR	0.1623 ± 0.0120	0.355 ± 0.023
LH-GS 5 phg NR	0.1487 ± 0.0134	0.350 ± 0.021
LH-GS 10 phg NR	0.1433 ± 0.0112	0.348 ± 0.020
LH-GS 15 phg NR	0.1579 ± 0.0046	0.380 ± 0.007
LH-GS 20 phg NR	0.1397 ± 0.0056	0.349 ± 0.014
LH-GS 25 phg NR	0.1345 ± 0.0042	0.322 ± 0.011
LH-GS 30 phg NR	0.1466 ± 0.0037	0.347 ± 0.007

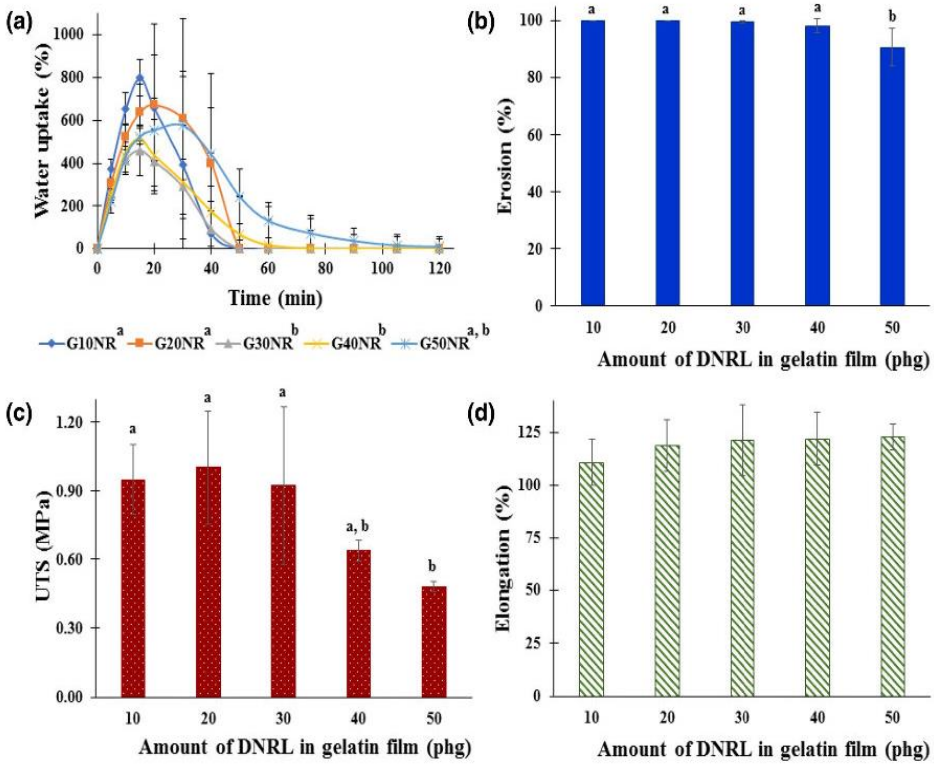


Fig. 1 The **a** water uptake, **b** erosion, **c** UTS and **d** elongation at break of GNR biomembranes (n = 5). (a) and (b) meant the symbol of significant statistics. The different symbols meant the significant difference ($p < 0.05$)

in Fig. 1a. Surprisingly, the GNR biomembranes with 10–20 phg DNRL revealed a significantly higher maximum water uptake than those of 30–40 phg DNRL ($p < 0.05$), while the 50 phg DNRL in GNR biomembrane showed a water uptake with no significant difference between these 2 groups ($p \geq 0.05$). This might be due to the erosion effect that interfered with the water uptake results because both phenomena occurred at the same time. However, the higher DNRL amounts affected the lower erosion. The 40–50 phg of DNRL in GNR biomembranes resulted in retarding the time of erosion and dissolving the blended biomembranes to 2 h as shown in Fig. 1b. The erosion of G50NR was the lowest and was statistically different from the other amounts of DNRL in GNR biomembranes ($p < 0.05$) (Fig. 1b). This indicated that DNRL increased the lipophilicity of gelatin films and might sustain the gelatin film structure in an aqueous medium.

The 50 phg DNRL in GNR biomembranes presented the lowest UTS which was significantly different from those of 10–30 phg DNRL in GNR biomembranes ($p < 0.05$) as shown in Fig. 1c. This indicated that DNRL could improve the elasticity of gelatin films. Increasing the amount of DNRL decreased UTS because of the elastic characteristic of DNRL [37]. However, the values of elongation at break of GNR biomembranes were not different as shown in Fig. 1d. Therefore, the 50 phg DNRL was chosen for use as a plasticizer in gelatin biomembranes. Next, the 5–30 phg gelatinized sago starch was further studied to blend in the GNR biomembranes.

All amounts of gelatinized sago starch in the studied range could be blended in a gelatin film which used 50 phg DNRL as a plasticizer. The characteristics of GSNR biomembranes were also opaque. Increasing the sago starch in biomembranes, the drying time of the biomembrane was longer than that of GNR biomembranes and the appearance was somewhat rough because the gelatinized sago starch could trap the water in its film structure. Similar to GNR, there were no differences in the average weight and thickness of GSNR which had different amounts of sago starch as shown in Table 1. However, GSNR biomembranes showed a slightly higher thickness value than GNR biomembranes. This might be due to the higher water uptake of gelatinized sago starch in the biomembranes, which then provided a lower density of GSNR biomembranes. The increased amounts of gelatinized sago starch could increase the water uptake and decrease the erosion of GSNR biomembranes

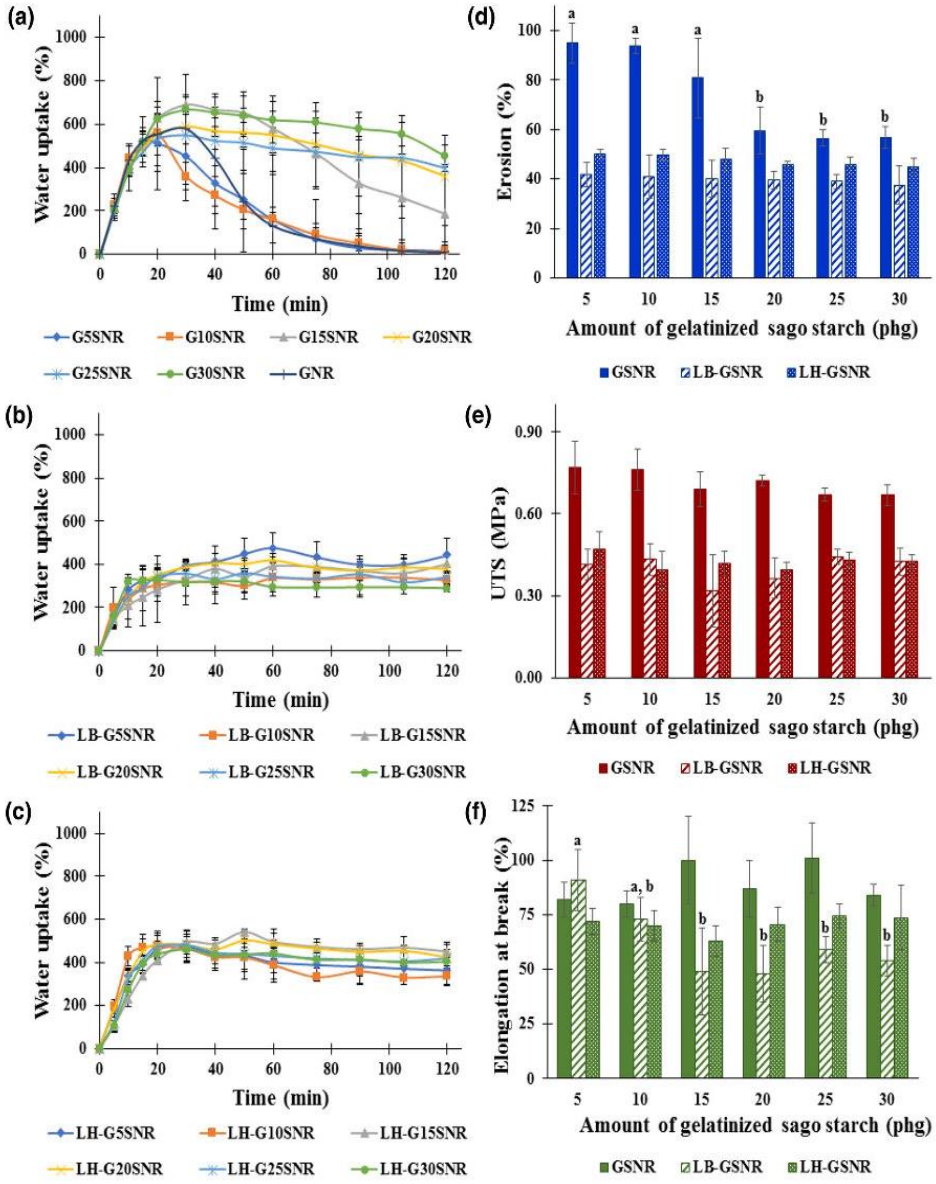


Fig. 2 The water uptake of **a** blank GSNR (n = 5), **b** and **c** LB and LH loaded GSNR biomembranes (n = 3), and **d** erosion, **e** UTS and **f** elongation at break of GSNR, LB-GSNR and LH-GSNR biomembranes (n = 5). (a) and (b) meant the symbol of significant statistics. The different symbols meant the significant difference ($p < 0.05$)

when compared with GNR biomembrane as shown in Fig. 2a, d, respectively. However, GSNR biomembranes showed no significant difference in the maximum water uptake of all amounts of sago starch blends in biomembranes ($p > 0.05$) in both blank GSNR and drug loaded GSNR biomembranes as shown in Fig. 2a–c. The erosion of both medicated LB and LH loaded GSNR biomembranes showed no significant difference ($p > 0.05$) between various amounts of gelatinized sago starch as shown in Fig. 2d. However, the erosion of different amounts of sago starch in blank biomembranes was significantly different in two groups ($p < 0.05$) in which sago starch at 20–30 phg in GSNR biomembranes was significantly lower than sago starch at 5–15 phg in GSNR biomembranes. The gelatinized sago starch might reduce the hygroscopic effect of gelatin in blended biomembranes and then might decrease the erodible rate of film. Normally, the composition of sago starch is reported as 27% amylose and 73% amylopectin [38], and the water uptake property might

be affected by amylose and amylopectin compositions of each starch. Although the amylopectin shows higher hydrophobicity than amylose, it has been reported that amylopectin has better water uptake property of starch because of its network structure [39]. Therefore, in this study, increasing the hydrophobicity by the addition of sago starch could decrease the erosion in biomembranes, but it increased the water uptake because of a higher amylopectin ratio. This result was consistent with the study of Biaopiotrowicz in gel properties of starch [40] which showed that increasing starch concentration decrease the hydrophilicity. This was due to the surface attempt to maintain the hydrophobic character with D-glucose units. In addition, blank GSNR biomembranes had higher water uptake and erosion values than medicated GSNR biomembranes because drug molecules might insert in the polymer networks which could increase the compactness of medicated biomembranes. Therefore, the water molecules were difficult to diffuse into the polymer networks. Moreover, LB-GSNR biomembranes had lower water uptake and erosion properties than LH-GSNR biomembranes. This was due to LB having more hydrophobicity than water soluble LH drugs.

There was no significant difference in elongation at break of blank GSNR biomembranes or medicated LH loaded GSNR biomembranes ($p > 0.05$). However, there was a significant difference between groups in elongation at break of LB-GSNR in which the elongation at break of biomembranes with 15–30 phg sago starch were lower than that of biomembranes with 5 phg sago starch ($p < 0.05$) as shown in Fig. 2f. The UTS of blank GSNR biomembranes was higher than those of medicated LB and LH loaded GSNR biomembranes in all amounts of gelatinized sago starch as shown in Fig. 2e. This might be due to the insertion of drug molecules as the composite biomembranes which decreased the mechanical properties. However, the different amounts of gelatinized sago starch did not significantly affect the physical properties of their biomembranes, but higher amounts of gelatinized sago starch gave a lower erosion rate due to the hydrophobicity of starch. In the next step, therefore, the 5 phg of gelatinized sago starch was further used to prepare the medicated biomembranes and evaluate the other properties. This amount was chosen because of ease of drying, smooth surface, increased water uptake, decreased erosion and good elongation property as described above.

The AFM images of GSNR biomembranes were compared to pure gelatin and DNRL films as shown in Fig. 3a–j. These images indicated the continuous films with a slight roughness on the surface in both upper and lower sides. The roughness results calculated from AFM data are shown in Fig. 3k. Gelatin film and DNRL film had less roughness on both upper and lower side surfaces compared with GSNR biomembranes. This might occur from the smoothness and rigidity of the film of pure material during the drying process. Although the GSNR biomembranes showed slight roughness, blank GSNR and medicated GSNR biomembranes were similar in roughness on the upper surface. The SEM images in Table 2 showed no small particles on the surface of the biomembranes. Nonetheless, the cross section of LH loaded GSNR biomembranes contained small particles. This might be due to the acid properties of LH which could induce the aggregation of DNRL before drying [41]. However, this indicated the non-segregation of ingredients in biomembranes.

Besides SEM as mentioned above, FT-IR, DSC and XRD were also used to confirm the compatibility of the ingredients in the biomembranes. In the FT-IR spectra, the broad band at 3275–3300 cm^{-1} was OH and/or NH stretching. The band at 2930 cm^{-1} was C–H stretching. The bands from 1635 to 1660 and 1525–1550 cm^{-1} were C=O stretching (amide I) and NH bending (amide II), respectively. These spectra could be found in general substances including DNRL, gelatin, starch, and LB and LH drugs. The FT-IR spectra of gelatin showed peaks at 1650 (amide I) and 1541 (amide II) cm^{-1} [8, 42, 43]. The LB and LH showed a similar peak at 1650 cm^{-1}. The LH had two sharp bands at the range 1450–1550 cm^{-1} because of C–N stretching and the higher energy peak was O–C–N bond. On the other hand, LB appeared to only have one peak at the same range due to the two C–N bonds [44]. In the combination biomembranes, all DNRL blended biomembranes showed the peak at 780 and 990 cm^{-1} as shown in Fig. 4. The FT-IR spectra at 1250, 1320 and in the range 450–770 of DNRL and gelatin were also different. The amide I peak of DNRL blended gelatin slightly shifted. Moreover, the intensity of amide I, amide II and OH/NH stretching peaks in DNRL blended biomembranes also changed. These results might indicate that there were interactions between gelatin and DNRL in the biomembrane. DNRL molecules might insert intermolecularly with $–NH_2$ groups, –OH groups or –COOH groups of gelatin [45]. Moreover, the hydroxyl group in gelatin could form hydrogen bonds with water after film preparation as shown in OH stretching bands in FT-IR of blended biomembranes [46]. These were consistent with the mechanical results above that DNRL could improve the elasticity of gelatin films. FT-IR spectra of gelatin and sago starch were quite similar showing a difference in the range 800–1000 cm^{-1}. There was also a slight difference of peak in the range 570–660 cm^{-1} between GNR and GSNR biomembranes. However, sago starch was mixed in a few amounts in biomembrane; therefore, this change was quite small. The results showed that OH stretching of sago starch was also broader than those of gelatin and DNRL alone as shown in Fig. 4. This might indicate that amylopectin in sago starch was disrupted and water molecules could form a hydrogen bond which resulted in higher water uptake of starch blended biomembranes [43, 47].

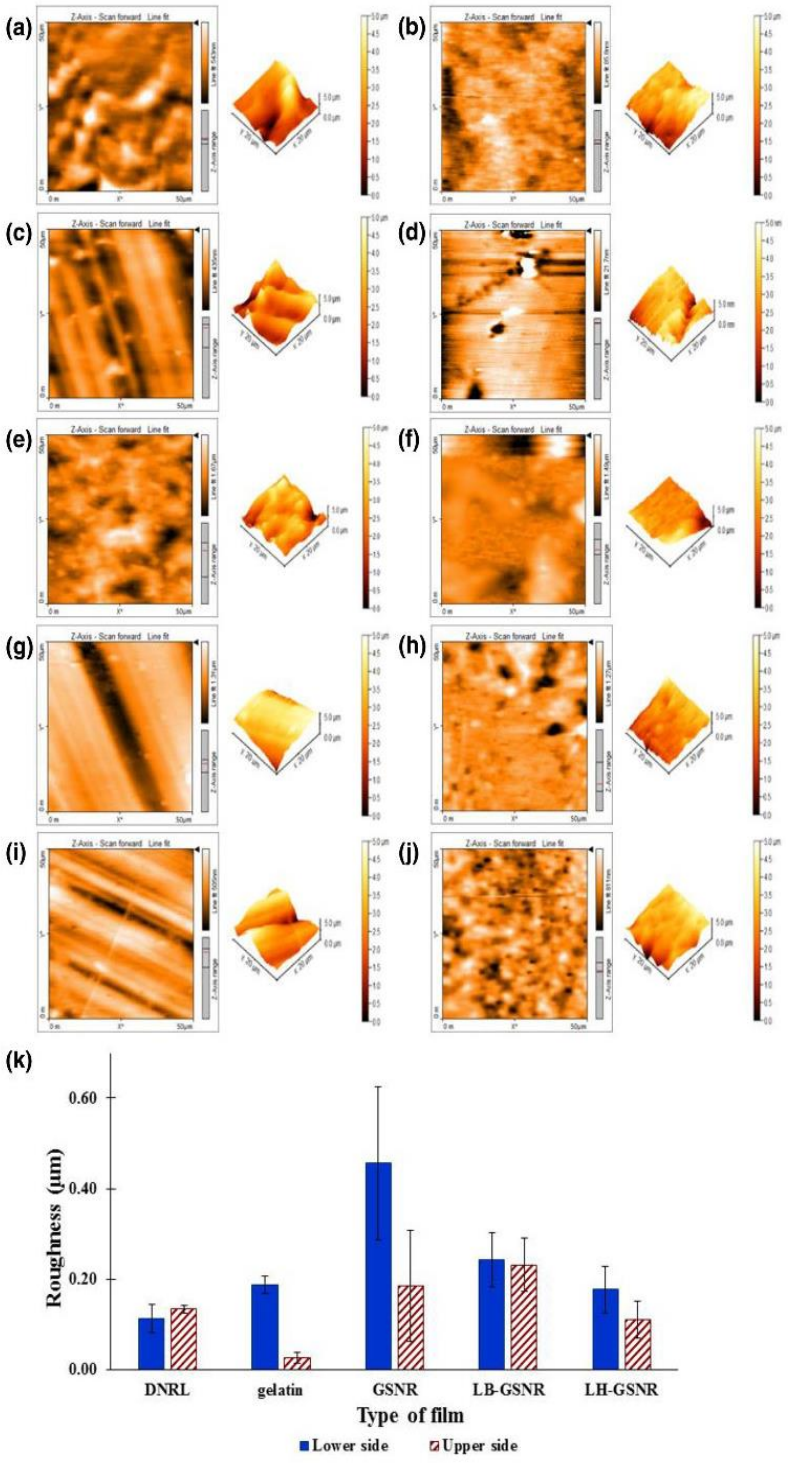
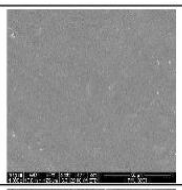
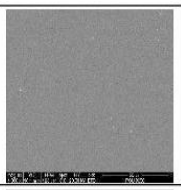
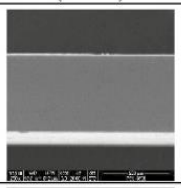
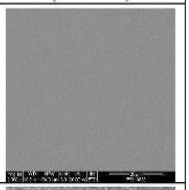
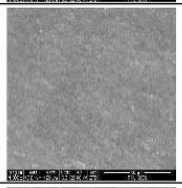
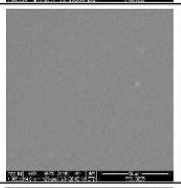
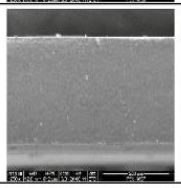
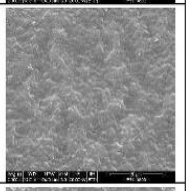
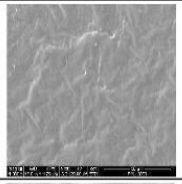
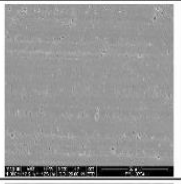
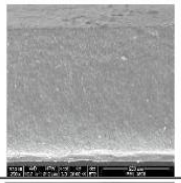
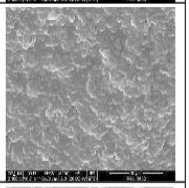
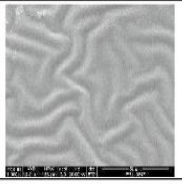
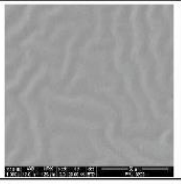
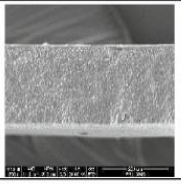
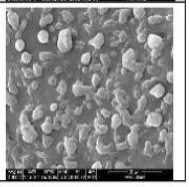


Fig. 3 The lower (**a**, **c**, **e**, **g**, **i**) and upper (**b**, **d**, **f**, **h**, **j**) AFM images of **a**, **b** DNRL films, **c**, **d** gelatin films, **e**, **f** GSNR biomembranes, **g**, **h** LB-GSNR biomembranes, **i**, **j** LH-GSNR biomembranes and **k** the roughness value of the biomembranes calculated from AFM (n = 5)

The DSC thermograms (Fig. 5) showed peaks at 69 and 80 °C which were the intrinsic peaks of LB and LH, respectively. These DSC thermograms showed the results only from room temperature (25 °C) to 250 °C for all samples and 25–95 °C for DNRL sample because there was no significant transition of endothermic peak after these over range

Table 2 SEM images of upper and lower biomembranes at 1000X and cross section at 250X and 2000X

Biomembrane samples	Upper (1000X)	Lower (1000X)	Cross section	
			(250X)	(2000X)
GNR				
GSNR				
LB-GSNR				
LH-GSNR				

temperatures to 450 °C. In this study, the DSC thermogram of DNRL was done only until 95 °C because the previous study concluded that it could not observe the peak over the range 25 to 170 °C [48]. However, peaks of either LB or LH disappeared from the blended GSNR biomembranes. It might be that the amorphous form of drug occurred after being solubilized during film preparation. On the other hand, the amount of drug in the biomembranes was less than 10% which might be difficult to observe as clear results in DSC thermograms. From both FT-IR and DSC results, these might preliminarily confirm the compatibility of gelatin/gelatinized sago starch biomembranes.

The XRD diffractograms are shown in Fig. 6. The XRD patterns of LB and LH revealed many peaks of crystalline characteristic. The highest sharp peak at 16.53° and 24.98° were found for LB, and 16.58° and 25.98° for LH. The raw materials of gelatin and DNRL showed the amorphous patterns of XRD diffractogram, while sago starch presented the crystalline peaks at 15°, 17°, and 23° from both amylose and amylopectin components [49]. The diffractogram of GSNR showed the disappearance of the crystalline peak of sago starch; however, the pattern of the diffractogram was mixed between gelatin, sago starch, and DNRL. This amorphous character was due to the amorphous property of most gelatin and DNRL composition of GSNR biomembrane, and only 5 phg of crystalline sago starch was blended. These indicated the amorphous character of GSNR biomembrane that easily uptakes water and is good for drug release. LH-GSNR exhibited the same broad spectrum as GSNR. No crystallinity of drug was exhibited in LH-GSNR. Even if LB-GSNR appeared as very small crystalline peaks, the intensity of peaks was much lower than the pure LB because of the low amount of LB in biomembranes. The slight crystalline in LB-GSNR biomembranes might occur from the low solubility of LB that could precipitate during the drying process. The crystalline of drug in STR-5L was also found in 10–15% w/w lidocaine-loaded transdermal patch as reported previously [50]. However, this phenomenon did not influence the drug release [51].

From preliminary study, it was found that methanol was a good solvent to extract both LB and LH from biomembranes. For content determination, therefore, methanol was chosen to use as solvent extraction. With the theoretical drug loading in each biomembrane at 5% w/w, the drug extraction results of LB-GSNR and LH-GSNR were 95.38 ± 6.41 and 99.12 ± 10.59%, respectively. These indicated that the biomembranes prepared by casting method could keep all drug content in the biomembrane without any loss. Since

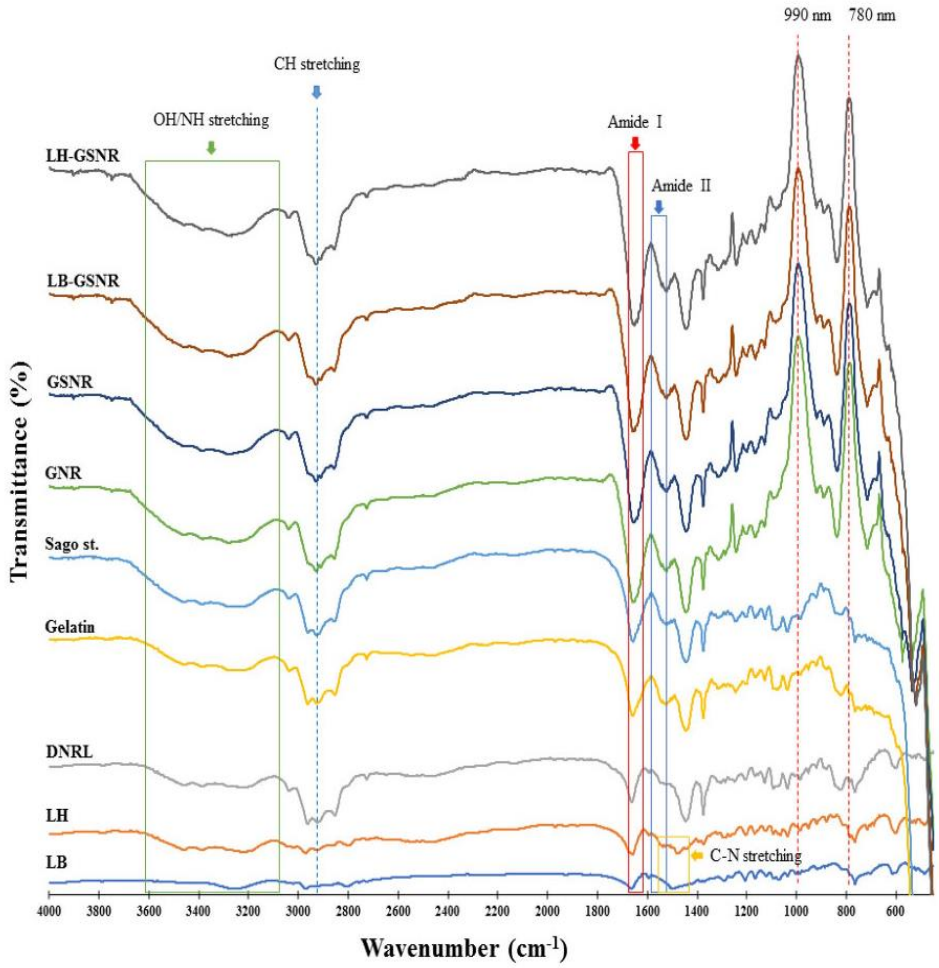


Fig. 4 FT-IR spectra of materials and biomembranes

LB is not soluble in water, in the preparation process, a 1:1 mixture of methanol:water was used in LB-GSNR biomembranes to solubilize the drug before mixing it into the other ingredients and drying. Methanol is a toxic residual solvent which the ICH guideline has limited. The concentration limit of methanol in pharmaceutical products is 3000 ppm [52]. From methanol determination, the result showed that the methanol residue in LB-GSNR biomembrane was less than 125 ppm, and, therefore, the methanol peak could not be detected (LOQ = 125 ppm). This indicated that it was safe to use this biomembrane in pharmaceuticals. During film preparations and other processes of methanol use, our university does not require ethics clearance for methanol pollution in air.

After stability studies at 1 and 3 months, the appearance and color of medicated GSNR biomembranes were quite similar to the initial preparation. The percentage of drug content of both the LB-GSNR and LH-GSNR biomembranes after stability studies in various temperatures including 4 °C, ambient temperature, and 45 °C for 3 months are presented in Fig. 7. It was found that the percentage of drug content of LB in GSNR biomembranes was above 90% until 3 months after storage in 4 °C and room temperature, and 2 months for 45 °C. LB-GSNR biomembranes at 4 °C were the most stable; the drug content had a lower decrease than those of ambient temperature and 45 °C in 1, 2 and 3 months. However, the percentages of drug content of LH in GSNR biomembranes were above 90% only 2 months after storage at 4 °C, but chemical instability of LH in biomembranes was found when stored at room temperature and 45 °C for 1, 2, and 3 months. This might be due to greater hydrophilicity of LH representing higher moisture absorption of LH biomembranes than LB biomembranes and enhanced oxidation of drug. In addition, oxidative reaction could occur at

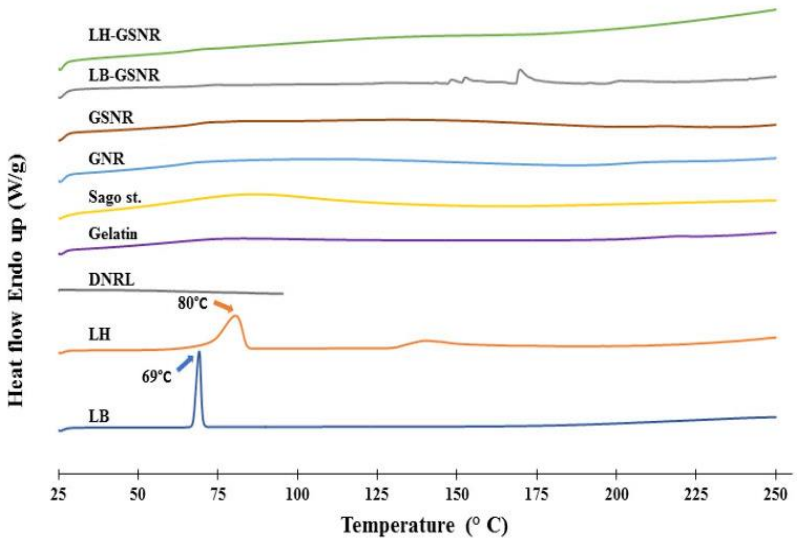


Fig. 5 DSC thermograms of materials and biomembranes

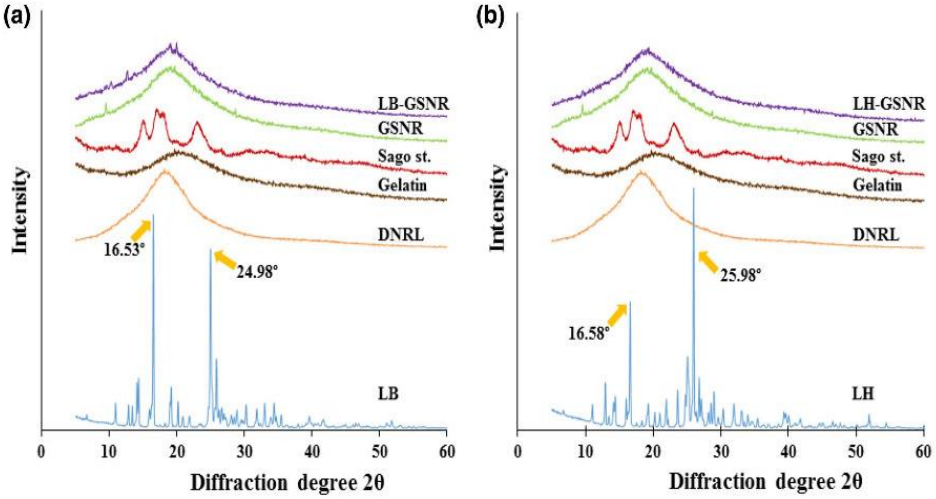


Fig. 6 XRD diffractograms of materials and **a** LB-GSNR and **b** LH-GSNR biomembranes

the nitrogen groups of LH which was suggested in the degradation study by hydrogen peroxide (H_2O_2) for oxidation at room temperature [53]. This indicated that LB was more stable in GSNR biomembranes than LH.

The drug release and permeation studies were important parameters to confirm the utility of this biomembrane as transdermal drug delivery systems. These parameters might depend on many physical, chemical, and mechanical parameters. Some on them are described above. For example, the higher water uptake and erosion films should release the drug from polymer matrix easier than lower water uptake and erosion films. In this study, the drug release and permeation profiles of LB and LH loaded GSNR biomembranes are shown in Fig. 8. For initial preparations, the LH release (Fig. 8c) was higher than LB release (Fig. 8a). This might be due to the aqueous soluble property of LH, which made dissolving and releasing from the membrane easier than LB [54]. However, drug releases in 24 h showed minimal difference. This also confirmed that the slight crystalline form observed in XRD diffractograms did not affect the drug release as described above, and as found in a previous study [51]. On the other hand, the permeation of LB (Fig. 8e) was slightly higher than that of LH (Fig. 8g) because the skin barrier should be more permeable for the lipophilic LB than the hydrophilic LH [55]. The stability of biomembranes after being kept at various temperatures showed that the

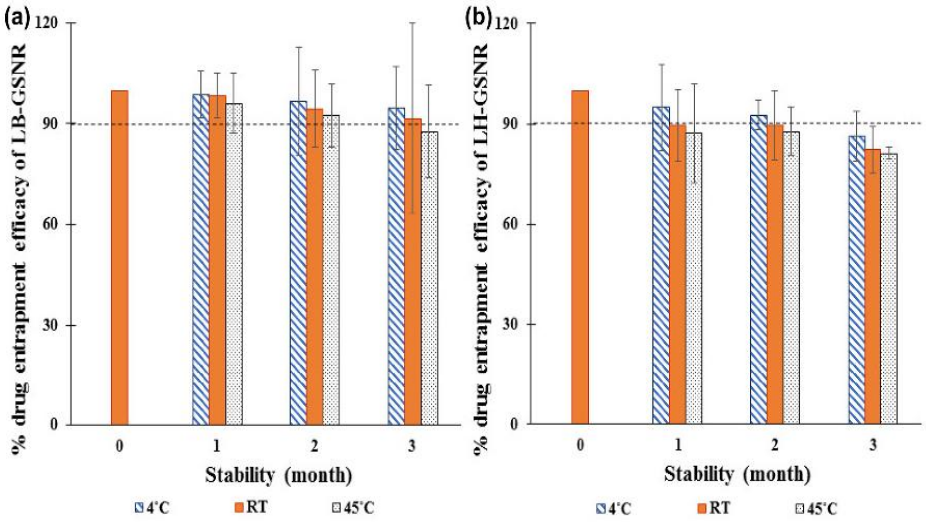


Fig. 7 The percentage of drug EE of **a** LB-GSNR and **b** LH-GSNR biomembranes (n = 3)

release of LB decreased in all GSNR biomembranes especially LB-GSNR biomembranes kept at 45 °C for 3 months (Fig. 8b). However, there was no difference in the release of LH-GSNR biomembranes (Fig. 8d). The permeation of LB and LH loaded GSNR after storage for 1 and 3 months were not significantly different from those of the initial preparations. All permeation results (Fig. 8e–h) showed lower values than the release amounts of drugs from the GSNR biomembranes. This indicated that the skin property was the significant determining step that could limit the permeation amount of the drug through the skin. Nevertheless, drug loaded GSNR biomembranes at 4 °C seemed to be the slowest and least changed drug release and permeation pattern compared to others. Lidocaine is resistant to the temperature in the range of − 20 to 70 °C [56]. In the solution, it is also stable in all regions of pH and resistant to hydrolysis at room temperature. However, lidocaine degrades slowly at higher temperature [57, 58]. Thus, a decrease of drug content might be influenced by high temperatures during the drying and keeping of GSNR biomembranes.

The types of both LB and LH release kinetics from GSNR biomembranes were not different as shown in Table 3. Most of both medicated LB and LH release kinetics from GSNR biomembranes were fitted to first order or Higuchi's kinetics, which they are not different statistically ($p \geq 0.05$); however, these kinetics were significantly different from zero order kinetics ($p \geq 0.05$). The release of first order kinetics depends on drug concentration and can be found in the swellable system with the dissolution of polymer matrix [59]. The Higuchi's model can be exhibited in a transdermal system with hydrophilic drugs that can diffuse through the matrix system [60]. In this study, both gelatin and gelatinized sago starch are hydrophilic polymers that can swell and dissolve in aqueous solution; therefore, both diffusion and dissolution mechanisms of drug release from GSNR could occur. For the kinetics of permeation profiles, LB-GSNR biomembranes kinetics were zero order or first order in different biomembranes which meant the independent or dependent permeations by drug concentration, respectively. These kinetics of LB permeation were significantly different from the Higuchi's model ($p < 0.05$). On the other hand, most LH-GSNR permeation profiles presented no difference in the statistics of 3 types of kinetics ($p \geq 0.05$). The variability of kinetics could have occurred because of either the drug properties or the variability of the skin [61]. This also confirmed that the lidocaine permeation was determined by the skin property as concluded above.

Conclusions

DNRL could successfully act as plasticizer for gelatin films. Gelatin or gelatin/gelatinizied sago starch mixed with DNRL produced good films without any problem of brittleness. Increasing the amount of DNRL resulted in decreasing the UTS and erosion of biomembranes. The gelatinized sago starch could increase the water uptake and decrease the erosion of gelatin/gelatinized sago starch biomembranes. LB or LH could be added in the gelatin/gelatinized sago starch biomembranes. However, the water uptake and UTS of medicated LB and LH loaded GSNR biomembranes were lower than those of blank GSNR biomembrane. FT-IR spectra and DSC thermograms showed good compatibility of all ingredients. However, the SEM cross-section image of LH-GSNR

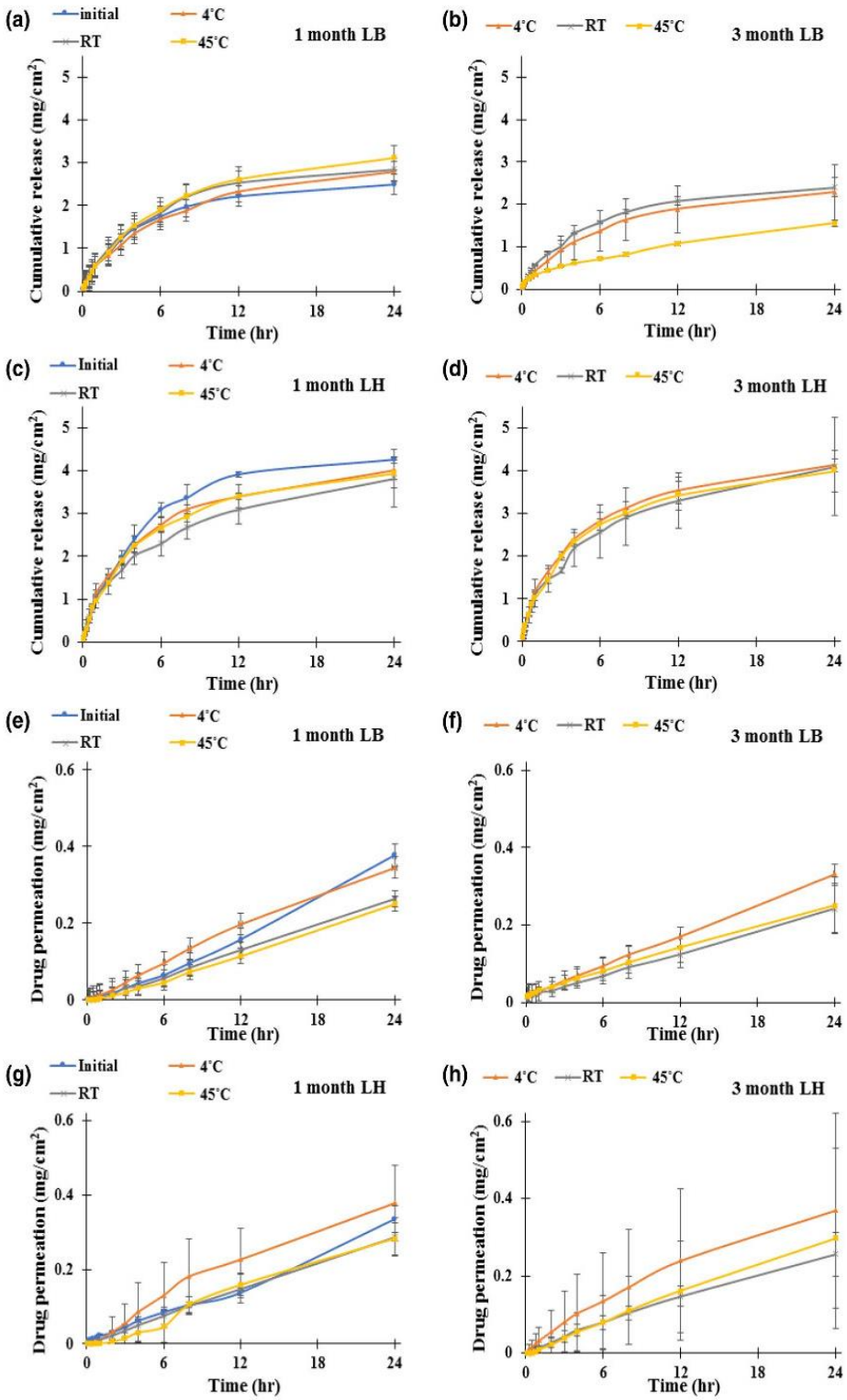


Fig. 8 The drug release profiles (**a**–**d**) (n = 3) and the permeation profiles (**e**–**h**) (n = 5) of (**a**, **e**) LB-GSNR 1 month, (**b**, **f**) LB-GSNR 3 month, (**c**, **g**) LH-GSNR 1 month, (**d**, **h**) LH-GSNR 3 month

Table 3 The kinetics of drug release (n = 3) and permeation (n = 5) in LB-GSNR and LH-GSNR biomembranes

Sample	month	Temp. (°C)	kinetics of drug release (R^2) (mean ± S.D.)			kinetics of drug permeation (R^2) (mean ± S.D.)		
			Zero order	Higuchi's	First order	Zero order	Higuchi's	First order
LB-GSNR	0	RT	0.8810 ± 0.0610^a	0.9789 ± 0.0171^b	0.9939 ± 0.0066^b	0.9763 ± 0.0207^b	0.8859 ± 0.0663^a	0.9572 ± 0.0328^b
	1	4	0.9267 ± 0.0249^a	0.9940 ± 0.0022^b	0.9897 ± 0.0079^b	0.9884 ± 0.0099^b	0.8858 ± 0.0440^a	0.9705 ± 0.0208^b
		RT	0.9072 ± 0.0410^a	0.9870 ± 0.0042^b	0.9892 ± 0.0093^b	0.9686 ± 0.0256^b	0.8771 ± 0.0789^a	0.9632 ± 0.0338^b
		45	0.9259 ± 0.0196^a	0.9904 ± 0.0022^b	0.9962 ± 0.0039^b	0.9860 ± 0.0087^b	0.8612 ± 0.0249^a	0.9679 ± 0.0082^b
	3	4	0.9279 ± 0.0345^a	0.9871 ± 0.0041^b	0.9951 ± 0.0003^b	0.9856 ± 0.0029^b	0.9466 ± 0.0259^a	0.9903 ± 0.0116^b
		RT	0.9023 ± 0.0192^a	0.9898 ± 0.0008^b	0.9947 ± 0.0030^b	0.9546 ± 0.0359	0.9336 ± 0.0202	0.9668 ± 0.0231
		45	0.9100 ± 0.0219^a	0.9915 ± 0.0038^b	0.9696 ± 0.0080^b	0.9701 ± 0.0268	0.9496 ± 0.0270	0.9782 ± 0.0144
LH-GSNR	0	RT	0.9006 ± 0.0190^a	0.9871 ± 0.0064^b	0.9914 ± 0.0081^b	0.9765 ± 0.0115^a	0.9683 ± 0.0055^a	0.9911 ± 0.0071^b
	1	4	0.8645 ± 0.0104^a	0.9822 ± 0.0036^b	0.9777 ± 0.0100^b	0.8888 ± 0.0918	0.7744 ± 0.1684	0.8795 ± 0.0889
		RT	0.8758 ± 0.0135^a	0.9874 ± 0.0043^b	0.9789 ± 0.0130^b	0.9823 ± 0.0192^b	0.8930 ± 0.0716^a	0.9743 ± 0.0313^b
		45	0.8787 ± 0.0297^a	0.9852 ± 0.0072^b	0.9848 ± 0.0137^b	0.9289 ± 0.0952	0.7807 ± 0.1368	0.9164 ± 0.0914
	3	4	0.8607 ± 0.0237^a	0.9826 ± 0.0093^b	0.9502 ± 0.0161^b	0.8999 ± 0.1135	0.8091 ± 0.2262	0.9005 ± 0.1344
		RT	0.8792 ± 0.0072^a	0.9855 ± 0.0068^b	0.9783 ± 0.0164^b	0.8884 ± 0.1945	0.7923 ± 0.2560	0.8917 ± 0.1963
		45	0.8700 ± 0.0346^a	0.9827 ± 0.0090^b	0.9791 ± 0.0202^b	0.9452 ± 0.1011	0.8623 ± 0.1758	0.9436 ± 0.1040

Each datum represents the mean ± S.D.

Supercript letters (a) and (b) in the same row meant the symbol of significant statistics. The different symbols meant the significant difference ($p < 0.05$)

biomembranes had small particles which might occur when LH as an acid induced the aggregation of DNRL. LB and LH could be controlled by the release from the GSNR biomembranes and permeated by a constant rate through pig skin. However, the obtained LB-GSNR biomembranes should be kept at a low temperature for good stability.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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CHAPTER 5

Lidocaine loaded gelatin/gelatinized tapioca starch films for buccal delivery and the irritancy evaluation using chick chorioallantoic membrane[1]

Abstract

The aim of this study was to confirm the feasibility of gelatin/gelatinized tapioca starch (α st) films for buccal delivery and their irritancy. Lidocaine (LB) or lidocaine hydrochloride (LH) was used as drug model and glycerin was used as plasticizer. The scanning electron microscopy, atomic force electron microscopy, X-ray diffraction and thermogravimetric analysis results confirmed the compatibility of gelatin/alpha starch/glycerin (Gαgly) films. Drug releases of LB or LH Gαgly films were evaluated. The drug release profiles of medicated films presented the good patterns in both short time and 8 h drug release. Their permeation study was examined through chick chorioallantoic membrane (CAM) by using modified Franz diffusion cell. Moreover, the irritancy study for buccal films was also examined by hen's egg test on CAM (HET-CAM) model. The results revealed that LB and LH could permeate through CAM, and these Gαgly films were no irritation on HET-CAM. These indicated that the LB and LH Gαgly films are possible to use as buccal films.

Keywords: Gelatin, Gelatinized tapioca starch, Buccal film, Chick chorioallantoic membrane

[1] The content of this chapter has been submitted in Saudi Pharmaceutical Journal.

5.1 Introduction

Buccal drug delivery is one of interesting route to deliver drugs because it has high total blood flow in buccal, can avoid degradation at gastrointestinal and first pass metabolism in the liver and intestine (Aungst, 2000; Harris and Robinson, 1992). In addition, it eases for administration and removal (Senel, *et al.*, 2001). The buccal mucosa structure is similar to the skin and acts as an absorption barrier. The drugs can act either in that local area or absorb to systemic circulation. There are many dosage forms such as tablets, gels, ointments, patches and films which have been developed for buccal drug delivery (Peh and Wong, 1999; Kraisit, *et al.*, 2018).

Hydrophilic polymers are normally chosen to prepare the dissolving buccal films because the films can dissolve and deliver the drug after contact with liquid or saliva (Mahajan, *et al.*, 2011; Irfan, *et al.*, 2016). The polymers can be used alone or combine to gain a good film. There are many types of polymer to make films such as cellulose derivatives, pullulan, sodium alginate, methylmethacrylate copolymer, chitosan and gelatin (Nagar, *et al.*, 2011; Kadajji and Betageri, 2011). In our previous study, biopolymers blended between gelatin and pregelatinized tapioca starch (alpha starch®; α st) was studied, and the effect of three water soluble plasticizers, i.e. polyethylene glycol 400 (PEG400), propylene glycol (PG) or glycerin to improve flexibility of film was observed. Glycerin at 25 part per hundred of gelatin (phg) was chosen to mix with gelatin/α st (Wannaphatchaiyong, *et al.*, 2017). In this study, lidocaine base (LB) or its hydrochloride salts (LH) loaded gelatin/α st/glycerin (Gαgly) was further evaluated to use as anesthetic films. The atomic force microscope (AFM), scanning electron microscope (SEM), thermogravimetric analysis (TGA) and X-ray diffraction (XRD) were further determined for the physical characteristics, thermal stability and decomposition, and compatibility of Gαgly films. For application to buccal mucosa, the anesthetic films should be non-toxic and non-irritant to the buccal membrane (Karki, *et al.*, 2016). Moreover, the films should release and permeate the drug to relief pain. For this reason, the *in vitro* drug release study, *ex vivo* permeation study and irritancy evaluation were studied. Normally, the drug permeability via buccal tissue can be observed in animal buccal tissue such as rabbit (Dowty, *et al.*, 1992), hamster (Tsutsumi, *et al.*, 1999), dog (Galey, *et al.*, 1976), and pig (Artusi, *et al.*, 2003; Marxen, *et al.*, 2018). The porcine buccal mucosa is reported as the nearest to human

tissue, however, it is too small surface in cheek and easy to damage between *ex vivo* membrane preparation. Moreover, it is difficult to supply the fresh pig tissue from the farm at the right time for experiments. Then, chick chorioallantoic membrane (CAM) is alternative membrane of porcine buccal mucosa which is easy to collect and prepare for using (Tay, *et al.*, 2011). In addition, CAM structure is quite same as human buccal membrane but have no mucous layer. CAM can be kept at -20°C up to 14 days for permeation study with no changes in permeation properties (Tay, *et al.*, 2011). Furthermore, hen's egg test-CAM (HET-CAM) can also be provided to evaluate the irritancy of buccal films (Tay, *et al.*, 2012; Kaewbanjong, *et al.*, 2017).

Therefore, the aim of this study was to confirm the physicochemical properties, *in vitro* drug release and *ex vivo* permeation of Gαgly films for buccal delivery of both LB and LH, and the irritancy evaluation using HET-CAM.

5.2 Materials and methods

5.2.1 Materials

Gelatin (160 bloom) was bought from PB Gelatins (Tessenderlo, Belgium). The gelatinized tapioca starch (α st) was kindly gifted from Thaiwah (Bangkok, Thailand). Glycerin was from Sigma-Aldrich (Munich, German). LB and LH were gained from Sigma-Aldrich (Shanghai, China). Methanol, ethanol (RCI Labscan Asia, Bangkok, Thailand), sodium hydroxide (Loba Chemie, Mumbai, India), sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride (Merck, Darmstadt, Germany) were used as supplied. Distilled water was used throughout the experiments. All other solvents and chemicals used were pharmaceutical or analytical grade and used without further modification. The specific pathogen-free (SPF) chicken eggs of White Leghorn were collected from the Animal and Plant Health Center, Agri-Food and Veterinary Authority of Singapore for *ex vivo* permeation and irritancy study.

5.2.2 Preparation of blank and medicated LB or LH Gαgly films

The gelatin solution was prepared by dissolving gelatin powder in distilled water and heating at 45°C until it was homogenous, cooling down at room temperature and adjusted to 15% w/w. For the gelatin/glycerin (Ggly) film, glycerin at 25 phg was mixed in gelatin solution. For Gαgly film, the 5% w/w of α st solution was

produced by dispersing and stirring slowly in distilled water. Then, the starch solution was mixed with gelatin solution at the final concentration of 5 phg, after that 25 phg glycerin was added into gelatin/α st solution. Either the Ggly or Gαgly solution was poured into the petri dish with the aluminum foil supported and dried at 50°C for 24 h. The blank Ggly and Gαgly films were built. Both blank Ggly and Gαgly films were used to evaluate their properties compared with medicated films.

For the medicated films, LB and LH were dissolved in (1:1) methanol:water mixture and distilled water to get 4% w/v of drug solution, respectively. LB or LH solution was slowly added into the Gαgly solution which prepared as previous described. After that, the mixtures were stirred to get the homogenous solution and poured into the petri dish with the aluminum foil supported and dried at 50°C for 24 h. The amount of either LB or LH loading was calculated in advance that there was a final concentration of 5% drug in dry basis.

All dried films were peeled off from the petri dish and stored in desiccators at room temperature before further evaluation.

5.2.3 Characterization of films

In the previous study, the thickness, weight uniformity, swelling and erosion, ultimate tensile strength (UTS), elongation at break, Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) of Gαgly films were reported (Wannaphatchaiyong, *et al.*, 2017). In this study, the medicated Gαgly films were further evaluated for the morphology and thermal stability by using AFM, SEM and TGA. Moreover, the crystallinity of films was determined by XRD. These could confirm the compatibility of drug in Gαgly films.

The morphology of films was observed by using AFM (model nanosurf easyscan2, Switzerland). The AFM cantilever used in this work was silicon probes (ACLA) with the resonance frequency of 160-225 KHz and a force constant of 36-90 N/m (USA.) which the non-contact mode applied to static mode was used. The AFM results were calculated for the roughness of films by using easyscan2 control software and Gwyddion as free program (GNU General Public License). The top surface, the bottom surface and cross section of films were also investigated by using SEM (model FEI: SEM-Quanta 400, USA.).

The thermal stability of films was evaluated by TGA (TGA 7, Perkin Elmer, USA.). The condition of analyzing used nitrogen atmosphere with a flow rate of 20 ml/min. The mass of samples ranged from 8 to 16 mg and the temperature interval of 50-1000 ℃ at a heating rate of 10°C/min was studied. A function of temperature and weight loss was determined.

The X-ray diffactometer (XRD; Empyrean, PANalytical, the Netherlands) was used to study the compounds in materials and films. The parameter of XRD was 40 kV, 35 mA, scan range (2θ) of 5-90º, step size (2θ) of 0.026º and time/step of 70.125 sec.

5.2.4 Extraction of medicated films

In preliminary extraction, LH or LB was extracted from Gαgly films by using different solvents including with methanol, methanol:water (1:1), isotonic phosphate buffer solution (PBS) pH 7.4 and water. The 1 cm × 2 cm medicated film was cut into small pieces and the 10 ml of each solvent was filled. The films were sonicated for 15 min and rested for 24 h. Then, they were sonicated for 1 h before diluted with PBS. The suitable concentrations were analyzed by high performance liquid chromatography (HPLC) with Themo scientific BDS HYPERSIL C18 column. The HPLC conditions were as follows: the mobile phase was 50 mM ammonium acetate with 1% v/v acetic acid and methanol (60:40% v/v) and triethylamine was added as 0.1% v/v of the total volume, the injection volume was 50 µl, the flow rate was 0.8 ml/min, and UV detector wavelength was 254 nm. The drug content (% w/w) was calculated as Eq. 1 by using the ratio between drug extraction ($D_{analyze}$) and accurately weight of film ($W_{accurate}$), and the percentage of drug entrapment efficiency (% Drug EE) was calculated by comparing between the drug extraction ($D_{analyze}$) and the theoretical drug loading (D_{theory}) as the Eq. 2.

$$\% \text{ Drug content} = (D_{analyze} / W_{accurate}) \times 100 \quad (1)$$

$$\% \text{ Drug EE} = (D_{analyze} / D_{theory}) \times 100 \quad (2)$$

5.2.5 Preparation of CAM

All SPF chicken eggs were wiped with povidone iodine and disinfectant (70% v/v ethanol) before placing the blunt end upwards into the egg incubator with an

automatic rotator (Octagon® 20, North Somerset, UK) at 37°C and 60% humidity. After 7 days, the embryo age (EA7) egg was punctured at blunt end. Then, the egg shell and the internal shell membranes were removed in a sterile environment by the cleansphere CA 100 (Safetech Limited, USA.) to reveal the CAM. The egg was covered with parafilm and returned to the incubator without rotation. On days 15, the EA15 CAM was collected by cutting along the length of egg and pouring out of content. The CAM was washed until it was clean with normal saline and stored at -20°C, and used within 14 days.

5.2.6 In vitro drug release and ex vivo permeation studies

In vitro drug release and *ex vivo* permeation studies of medicated Gαgly films were determined by using modified Franz diffusion cell (Hansen Research, Chatsworth, CA, USA.). The receptor compartment was filled with PBS pH 7.4, controlled the temperature at 37±0.5ºC and stirred with a magnetic stirrer of 200 rounds per min (rpm). In drug release, 2 patterns of study were observed, i.e. the short time release without barrier and the 8 h release with barrier. For short time drug release study, the films were place on the receptor compartment directly, and the aliquots of 1 ml sample were kept at 1, 2, 3, 5, 7, 11 and 15 min. For 8 h drug release study, the films were put on the donor compartment which was divided from the receptor compartment by a dialysis membrane (MW cut-off 12000). For permeation study, the dialysis membrane was replaced with CAM, and the filter paper was also used to support the CAM on the hole between donor and receptor compartments. In both 8 h drug release and permeation study, aliquots of 1 ml in receptor fluid were collected at 5, 10, 15, 30, 45 min and 1, 2, 3, 4 ,6 and 8 h. After that, the equivalent aliquoted volumes of PBS were replaced in the receptor fluid. Each sample was evaluated for the drug concentration by HPLC at 254 nm. In vitro short time and 8 h drug release were done in triplicate, and ex vivo permeation was studied in quadruplicate. All of resulted studies were further analyzed into zero order, first order and Higuchi's kinetics (Habib, *et al.*, 2010; Rana and Murthy, 2013)

5.2.7 Stability study of medicated Gαgly films

The medicated Gαgly films were kept for 3 months at 4±1ºC, ambient temperature (≈28±4ºC), and 45±1ºC to determine their stability. They were examined

for changes in appearances, drug contents and drug release. For permeation of stored films, they were studied only after storage at room temperature. All the tests were done as previously described method.

5.2.8 Irritancy evaluation using HET-CAM

The SPF chicken eggs were hatched and opened the shell at EA7 as previously described. HET-CAM was used on EA10 to study the irritation potential of the formulations. The medicated Gαgly film was applied on the CAM surface of opened egg. The positive control and negative control were 0.1 M sodium hydroxide solutions and 0.9% sodium chloride solutions, respectively. The films were cut into 1.5 cm × 1.5 cm which the area of film could cover almost of the CAM surface in case of film test, or 3 ml of each solution was dropped onto the CAM when the liquid sample was tested. The irritation test started after placing the sample and irrigating 20 sec with warm water (37 ºC). After application, the blood vessels were evaluated and scored of irritant effects at 0.5, 2 and 5 min. The pictures of HET-CAM were taken by digital camera microscope (Olympus DP 71, Japan) and zoom stereo microscope (Olympus SZ 61, Japan). Hyperaemia, haemorrhage and clotting of blood vessels were observed and examined by using a score (Luepke, 1985; ICCVAM, 2010; Kaewbanjong, *et al.*, 2017) in Table 1. These scores were evaluated by five referees. The cumulative irritancy score was interpreted in terms of irritation potential as shown in Table 1. Irritancy testing was done in triplicate.

Table 1 Score of irritancy testing and the interpretation as cumulative score for severity of irritation potential (Luepke, 1985; ICCVAM, 2010; Kaewbanjong, *et al.*, 2017)

Irritation effect	Time and score		
	≤ 0.5 min	0.5-2 min	2-5 min
Hyperemia	5	3	1
Hemorrhage	7	5	3
Clotting/coagulation	9	7	5

Interpretation	
Cumulative score	Irritation potential
< 1.0	Negligible
1.0 - 4.9	Slight
5.0 - 8.9	Moderate
9.0 - 21.0	Strong

5.3 Results and discussion

5.3.1 Preparation of films

Several plasticizers have been studied to blend in the films for improving the mechanical properties of glassy films. However, it might have limitation to mix the plasticizer into films because some plasticizers could absorb the humidity then became over-hydrating the film and decreased the adhesive strength of films (Kaur, *et al.*, 2014). Therefore, the appropriated types and amounts of plasticizer should be firstly evaluated. The previous study reported the effect of plasticizers including with PEG400, PG and glycerin in gelatin/α st (Gα) films (Wannaphatchaiyong, *et al.*, 2017). Among of them, the glycerin blended Gα films showed transparent and good properties. The 25 phg of glycerin was suitable to make the Gα films. It showed the lower ultimate tensile strength and higher elongation at break than PG and PEG400 plasticized Gα films. Moreover, the over-hydrated films were not found during storage. Therefore, the Gαgly films were further studied, and then, either LB or LH was interested to study and prepare as the medicated Gαgly buccal films because both drugs have different properties which might present the different properties of Gαgly buccal films.

In the same as previous study, the blank Ggly and Gαgly films, and the medicated LB or LH loaded Gαgly films could be prepared with good visualization and high reproducibility. The transparent thin films were prepared with good physical and mechanical properties as described previously (Wannaphatchaiyong, *et al.*, 2017).

5.3.2 Physiochemical properties of films

The AFM images of blank Ggly and Gαgly films, and medicated Gαgly films are shown in Figure 1. The images revealed that both upper and lower surfaces of films were slightly rough. The AFM data were calculated for their surface roughness as shown in Figure 2. LH-Gαgly presented the lowest roughness both lower and upper sides of film. However, there were not significantly different when compared with Ggly film. The comparison between medicated films showed that the upper and lower surfaces of LH-Gαgly were significantly smoother than LB-Gαgly. This roughness of films might be as a result of the complete dissolving of starch or drug, the drying rate which effect on the upper side of films, and the peeling effect on the lower side of films. However, this roughness of films was too small that could not observe by naked

visualization. The SEM images of medicated Gαgly films are presented in Figure 3. The upper sides of LB and LH loaded Gαgly films were quite smooth. On the other hand, the lower sides of LB and LH loaded Gαgly films were found as some roughness. These might be due to peeling the films from the aluminium foil supporting after drying process. The cross-section images at 250X and 2000X presented that both of LB and LH loaded Gαgly films did not find any particles inside the films. This indicated the good blend of all components in the medicated Gαgly films.

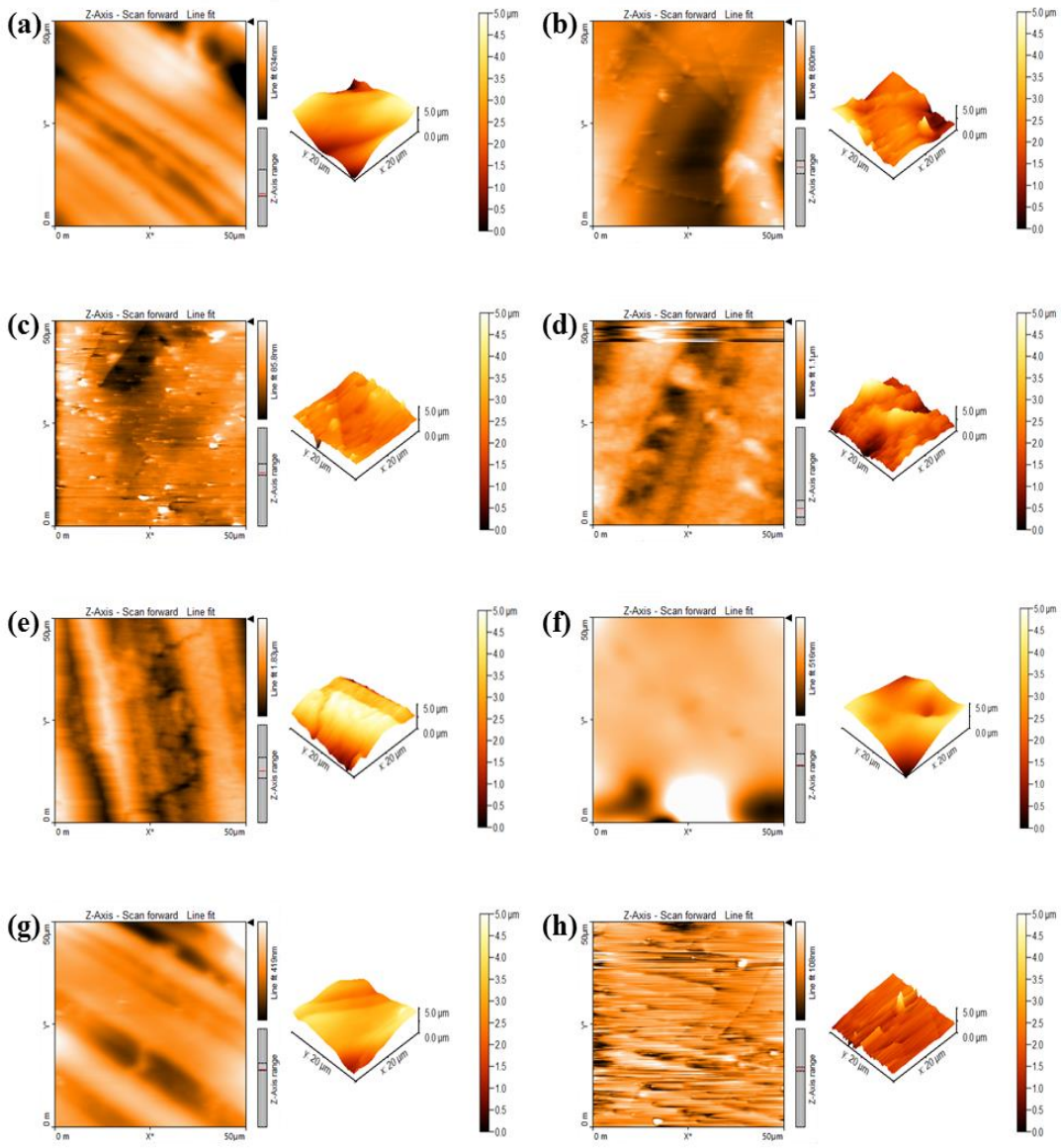

Figure 1 The lower (a, c, e, g) and upper (b, d, f, h) AFM images of (a, b) Ggly films, (c, d) Gαgly films, (e, f) LB-Gαgly films and (g, h) LH-Gαgly films

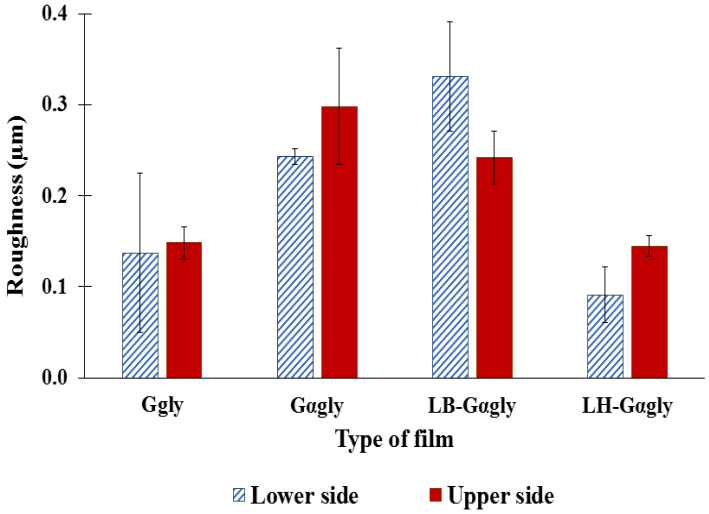

Figure 2 The roughness values of the films calculated from AFM

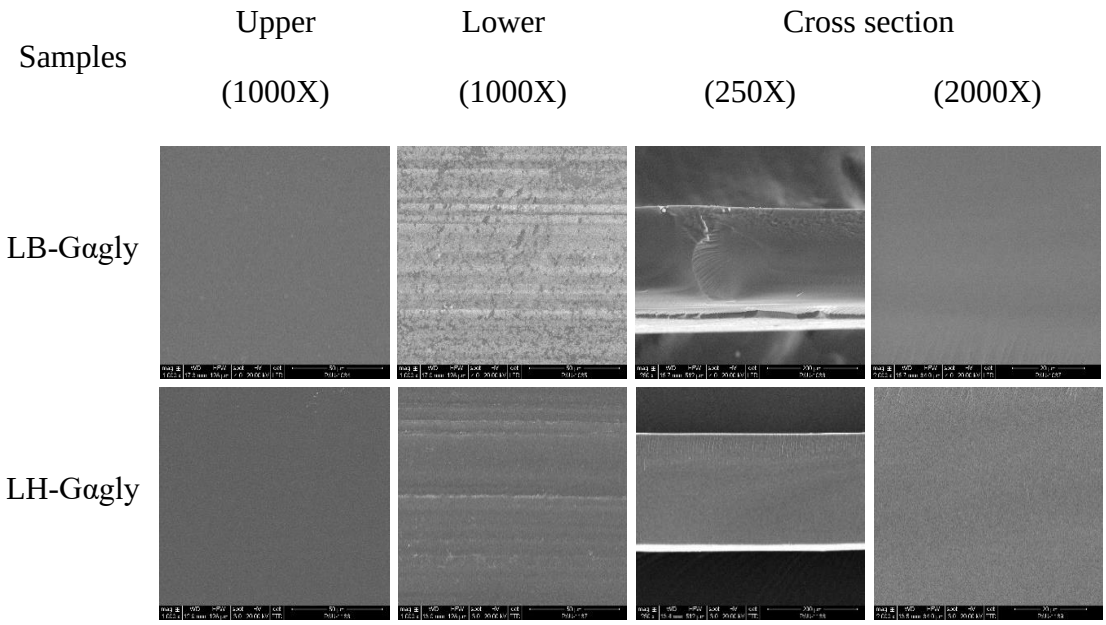

Figure 3 SEM images of upper and lower medicated Gαgly films at 1000X and cross section at 250X and 2000X

The thermal stability of the films observed by TGA is shown in Figure 4. Most of TGA thermograms of materials and medicated Gαgly films revealed two thermal events. The first decreasing of weight occurred immediately after the temperature increase and ends about 300°C. This event occurred from evaporation or dehydration of remained water or other low molecular weight compounds in the sample (Liu, *et al.*, 2009). The initial peak of gelatin also presented around 100-300°C which was the degradation of gelatin chain, and the second peak at 300-600°C referred to the breaking of peptide bonds from amino acids which is more thermally stable structure (Hoque, *et al.*, 2011; Mu, *et al.*, 2012). For the medicated Gαgly films, they exhibited the curve between 50-250°C which might be the degradation of water, glycerin, starch, gelatin or drug. The second curve was 250-500°C which was attributed to the decomposition of polymers of films (Rodríguez-Castellanos, *et al.*, 2015). The LB and LH showed the different decomposed temperatures, but they did not affect the TGA thermograms of their medicated Gαgly films. The TGA thermograms of medicated Gαgly films which were added with glycerin had shifted to lower temperature from the gelatin curve. Gelatin could increase the stability of glycerin. In addition, glycerin might prevent protein-protein interactions which affected the higher heat sensitivity of gelatin films (Hoque, *et al.*, 2011). However, the trend of TGA curves was similar to the original gelatin curve. This could imply that gelatin was the main component in films without any significant change. Therefore, these TGA results could also confirm the compatibility of all components in the medicated Gαgly films.

The XRD patterns of films are presented in Figure 5 which could support the FT-IR and DSC results in the previous study (Wannaphatchaiyong, *et al.*, 2017). As the former study, Gαgly film and medicated Gαgly films showed absent of new peak in FT-IR and the amorphous form of drug in DSC. The XRD diffractograms revealed the crystallographic structure of materials and is used to study the complex of polymers. In the results of this study, the XRD diffractogram of blank Gαgly films exhibited the 2 broad peaks. Normally, the granular structure of starch can appear as crystalline form which the amylose of starch is still in granules and can form the complex in structure (Nakorn, *et al.*, 2009). In this study, however, the pregelatinized starch is a soluble component that was dissolved completely before film was formed. Therefore, the peak of α st in Gαgly film showed as a broad diffractogram indicated the non-crystalline

form of all components after dried film was formed. These 2 broad peaks were also found in the medicated LB-Gαgly and LH-Gαgly films in the same as blank Gαgly films. However, the crystalline patterns in medicated Gαgly films were observed, especially in LB-Gαgly films. LB is the low solubility in water, therefore, it might precipitate in the LB-Gαgly after drying and showed the 2 sharp crystalline characters in XRD diffractogram. While LH is good soluble in water and might completely blend with the other soluble components such as gelatin and α st, therefore, very slight crystalline form was observed in XRD diffractogram. However, these crystalline peaks of both drugs were changeable from the raw drugs. These indicated the different crystalline forms of drug after re-crystallization in the dried films. Moreover, this crystalline character of drug in Gαgly films was not observed by SEM technique as described previously, this indicated that the very small of crystalline drug remained in the medicated films. Although, there were some crystalline peaks in medicated films, they might not affect the drug release behavior which had been already reported (Preis, *et al.*, 2014).

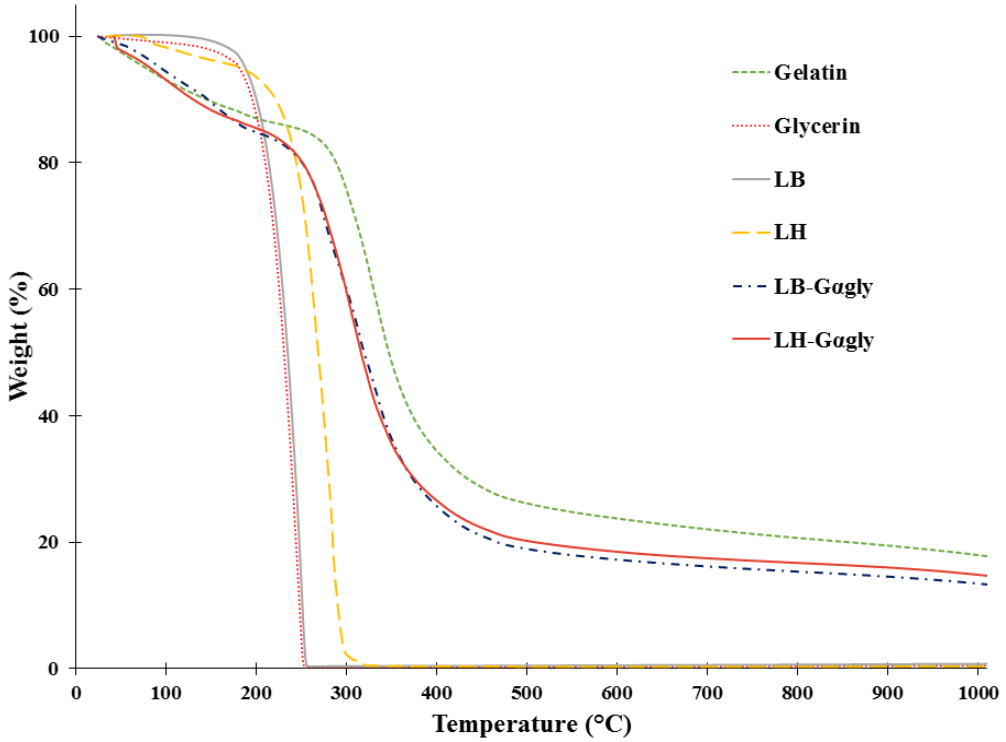

Figure 4 TGA thermograms of materials and films

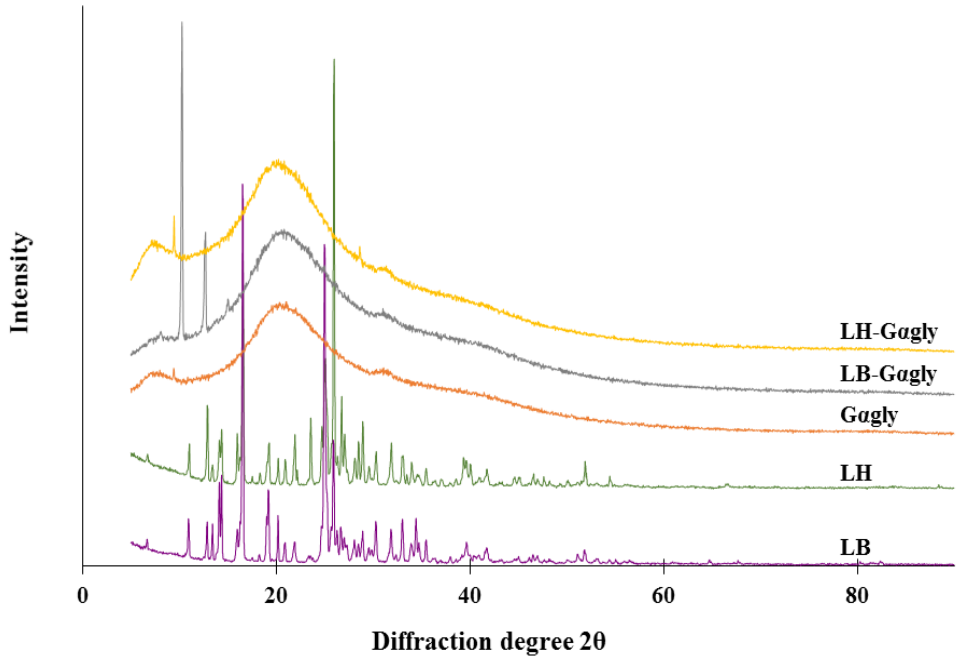

Figure 5 XRD diffractograms of blank film and medicated films

5.3.3 Drug extraction

The 5% w/w of the theoretical drug was loaded in each film during preparation process. The results of drug extraction are presented in Table 2. The best solvent for LB and LH extractions from medicated Gαgly films was PBS pH 7.4 and water, respectively. For LB, PBS might be mixed with water and salts which the gelatin in film was swelled and dissolved, after that LB could be dissolved (Østergaard, *et al.*, 2011) and extracted from the film higher than methanol:water, methanol and water, respectively. For LH, water could extract the drug greater than PBS pH 7.4, methanol and methanol:water, respectively. The water could be absorbed into the film, and it also could dissolve LH from the film. The percentages of drug EE in different solvents of LB-Gαgly and LH-Gαgly were 80-95% and 89-98%, respectively. However, the extraction values lower than 100% might be due to the entrapment of partial drug molecules in the structure of either gelatin or starch that could not be completely extracted by the solvents. These implied that the medicated Gαgly films prepared by casting method could preserve drug in the film without any loss. After that, PBS pH 7.4 and water were used as solvent for determination of the drug EE in LB and LH loaded Gαgly films in the stability test, respectively.

Table 2 The percentages of drug content and drug EE of medicated films extracted with different solvents (n=5)

Solvent	LB-Gαgly		LH-Gαgly	
	% Drug content (mean±S.D.)	% Drug EE (mean±S.D.)	% Drug content (mean±S.D.)	% Drug EE (mean±S.D.)
Methanol	4.039±0.390	80.789±7.795	4.606±0.541	92.125±10.824
Methanol:Water (1:1)	4.209±0.175	84.183±3.504	4.455±0.730	89.109±14.594
PBS pH 7.4	4.768±0.643	95.370±12.854	4.662±0.535	93.238±10.708
Water	3.984±0.347	79.676±6.948	4.904±0.301	98.098±6.207

5.3.4 Stability study of medicated LB or LH Gαgly films

The characteristic and color of medicated Gαgly films after stability study at 1 and 3 months were quite similar to the initial preparations. The percentages of drug content were calculated after stability study at different temperatures which were 4ºC, ambient temperature and 45ºC for 3 months (Figure 6). The percentages of drug content of LB in Gαgly films were above 90% when stored at 4ºC for 3 months, and at room temperature and 45ºC for 2 months. In LH-Gαgly films, the percentages LH content retained above 90% when stored at 4ºC and room temperature for 3 months, and at 45ºC for 2 months. The medicated Gαgly films were suitable to store in 4ºC because the decreasing of drug was the lowest which was the most stable of drug content in the films. The chemical instability of the drug was found after kept in room temperature and 45ºC. The degradation of LB and LH would be explained with drug release and permeation study.

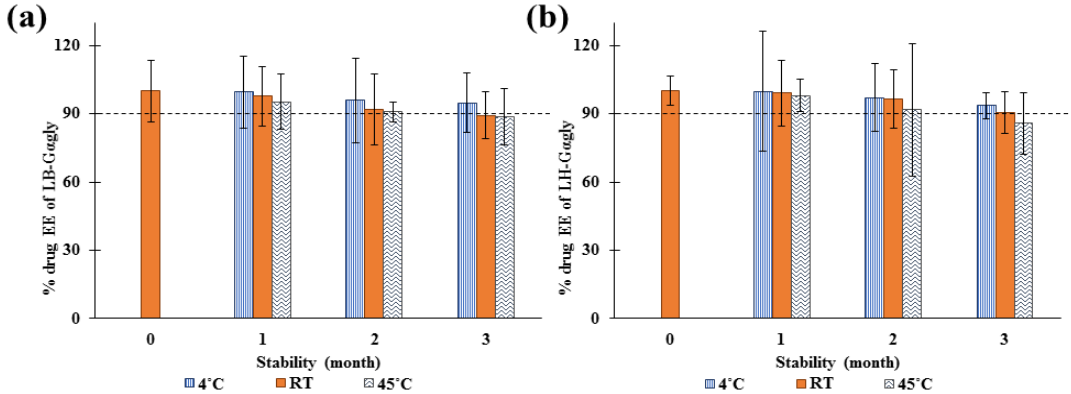

Figure 6 The percentages of drug EE of (a) LB-Gαgly and (b) LH-Gαgly films (n=5)

5.3.5 Drug release and permeation study

The drug release and permeation profiles of LB and LH loaded Gαgly films are presented in Figure 7. In the freshly prepared films, both short time and 8 h drug release profiles of LH film were slightly higher than those of LB film. This might be due to the better solubility of LH in aqueous medium than in LB (Gröningsson, *et al.*, 1985); thus, LH could dissolve and release from the films easier than LB. LH blended in Gαgly films also increased the hydrophilicity of the film. Moreover, the drug permeation from LH-Gαgly film was also slightly higher than from LB-Gαgly film owing to the higher drug content and release from the Gαgly films, and the higher concentration gradient resulting in the higher drug permeation. In fact, both LH and LB drugs could change their forms to the same lidocaine form when being in PBS pH 7.4. Therefore, the permeability of both LB and LH in the same medium should be the same. The slight differences of permeation results should display from the different concentrations of drug release. After the films were stored at various temperatures, the drug release from films exposure to 45ºC decreased higher than that exposure to 4ºC and room temperature for both LB and LH Gαgly films. The release profiles of LB and LH Gαgly films, which were kept at 45ºC for 3 months, were lowest in each film. Moreover, drug loaded Gαgly films at 4ºC presented the least changing of drug release when compared to others. For drug permeation profiles of films storage at room temperature, the longer period of storage, the lower amount the drug was permeated. However, it was not significantly different permeation in LB-Gαgly film. Even LB and LH is resistant to temperature, acid or base in aqueous solutions, however, the films were dried in an oven at 50ºC for 24 h to reduce the moisture content which the hydrolysis could be occurred for LB (Repka, *et al.*, 2005) and the slightly change of ^{1}H-NMR spectrum in LH was also observed in oxidation reaction study at room temperature (Kadioglu, *et al.*, 2013). Thus, these reasons might affect the stability, drug release and permeation study.

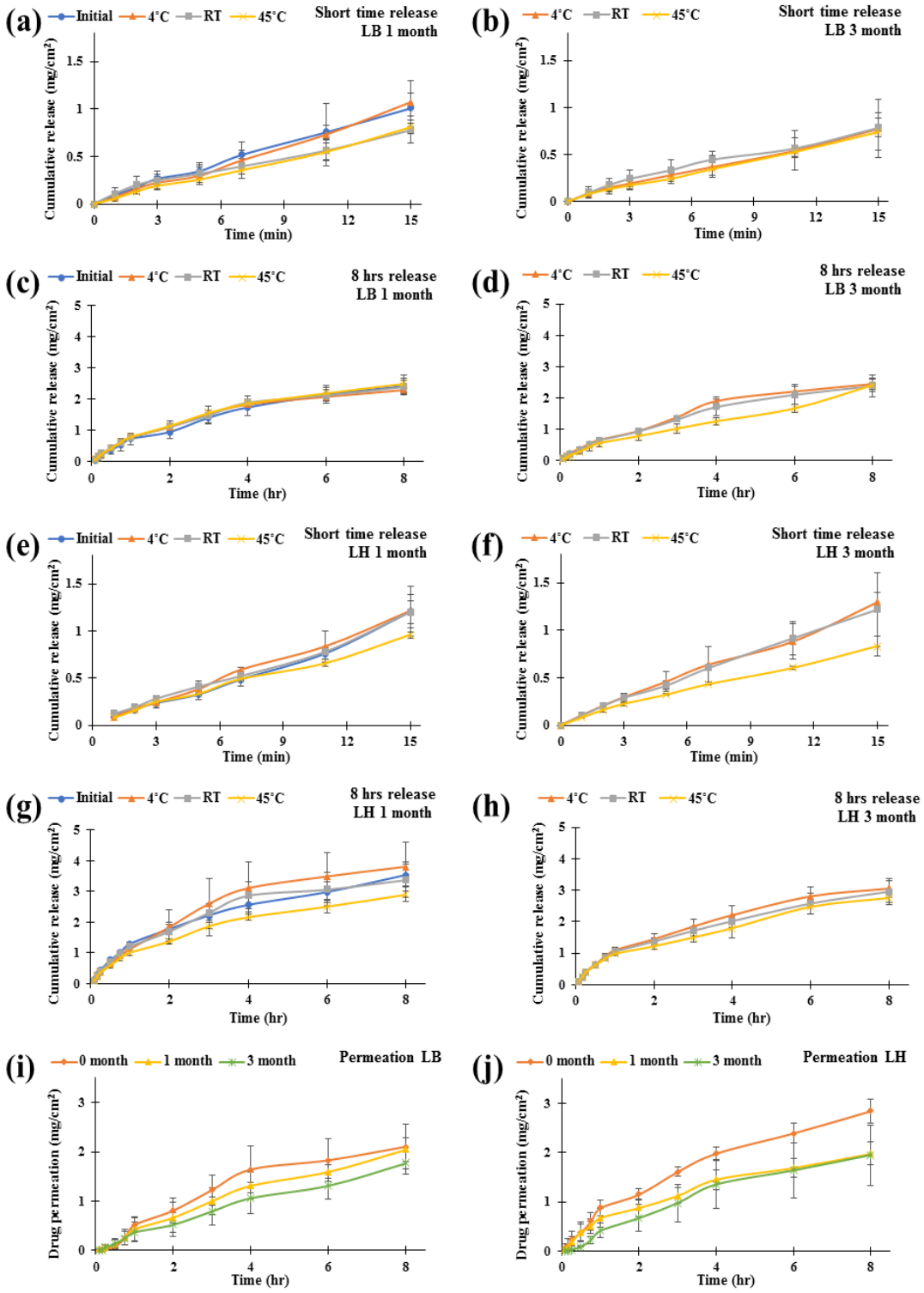

Figure 7 The short time (n=3) (a, b, e, f) and 8 hrs drug release profiles (n=3) (c, d, g, h) of (a, c) LB-Gαgly 1 month, (b, d) LB-Gαgly 3 month, (e, g) LH-Gαgly 1 month, (f, h) LH-Gαgly 3 month and the permeation profiles (n=4) (i-j) of (i) LB-Gαgly and (j) LH-Gαgly

5.3.6 Kinetics

The kinetics of drug release profiles (Table 3) in short time and 8 h drug release were different. For short time drug release, the drug release from most of films were fitted well to zero order kinetics. This could be demonstrated that drug release was independent with concentration in the first 15 min, or the drug dissolved, partitioned and diffused from film (Bruschi, 2015). In this short time drug release study, the medicated Gαgly film directly contacted to the receptor medium, and then, fast dissolving of whole films occurred. However, the drug release from LH-Gαgly films after 3 months storage was fitted with first order equation that because the condensed films might occur after storage and thereafter retarded the film dissolving. However, most of short time drug release kinetics in medicated Gαgly film was not statistically different ($p \geq 0.05$). For 8 h drug release, most of LB and LH Gαgly films were fitted to first order kinetics; however, LH-Gαgly after 3 months storage was fitted to Higuchi's for all storage conditions. Most of 8 h drug release kinetics was not statistically different in Higuchi's and first order kinetics ($p \geq 0.05$). Fitting well with the first order or Higuchi's kinetics indicated that the drug release depended on concentration or diffusion taken place in matrix (Bansal, *et al.*, 2013; Ramteke, *et al.*, 2014), respectively. For 8 h drug release study, the medicated Gαgly film and the receptor medium was separated by a dialysis membrane (MW cut-off 12000), and then, the whole films could not dissolve into the lower compartment. The drug release should occur by diffusion and some dissolution of matrix films. Moreover, the condensed films occurred after storage could also retard the film dissolving, and the diffusion kinetics was dominant. For the permeation profiles (Table 4), most of drug permeation kinetics was not statistically different in three types of kinetics. Some films was appropriated with first order or Higuchi's kinetic indicating that the drug permeation depended on concentration of drug or diffusion from matrix that referred to the drug release from the films.

Table 3 The kinetics of drug release (n=3) in LB-Gαgly and LH-Gαgly films

Sample	Month	Temperature (°C)	Kinetics of short time drug release; R^2 (mean±S.D.)			Kinetics of 8 hrs drug release; R^2 (mean±S.D.)		
			Zero order	Higuchi's	First order	Zero order	Higuchi's	First order
LB-Gαgly	0	RT	0.9902±0.0010[a]	0.9262±0.0201[b]	0.9675±0.0146[a]	0.9620±0.0135	0.9782±0.0128	0.9822±0.0052
	1	4	0.9783±0.0098	0.8979±0.0669	0.9515±0.0533	0.9263±0.0258[a]	0.9848±0.0077[b]	0.9929±0.0062[b]
		RT	0.9500±0.0339	0.9592±0.0570	0.9705±0.0260	0.9341±0.0189[a]	0.9818±0.0061[b]	0.9866±0.0104[b]
		45	0.9879±0.0100[a]	0.9245±0.0204[b]	0.9737±0.0223[a]	0.9692±0.0092	0.9805±0.0085	0.9682±0.0202
	3	4	0.9609±0.0183	0.9413±0.0428	0.9698±0.0271	0.9678±0.0066	0.9710±0.0053	0.9720±0.0052
		RT	0.9301±0.0596	0.9447±0.0479	0.9626±0.0303	0.9676±0.0189	0.9775±0.0112	0.9753±0.0190
		45	0.9614±0.0285	0.9070±0.0889	0.9554±0.04821	0.9554±0.0333	0.9722±0.0330	0.9790±0.0172
LH-Gαgly	0	RT	0.9576±0.0241	0.8995±0.1120	0.9588±0.0630	0.9097±0.0191[a]	0.9907±0.0031[b]	0.9914±0.0070[b]
	1	4	0.9721±0.0283	0.9105±0.0588	0.9735±0.0118	0.9382±0.0126[a]	0.9784±0.0134[b]	0.9880±0.0003[b]
		RT	0.9705±0.0288	0.9400±0.0572	0.9774±0.0166	0.9133±0.0299[a]	0.9803±0.0063[b]	0.9718±0.0111[b]
		45	0.9788±0.0171[a]	0.9396±0.0191[a, b]	0.9852±0.0121[b]	0.9173±0.0090[a]	0.9866±0.0092[b]	0.9882±0.0048[b]
	3	4	0.9869±0.0057[a]	0.9392±0.0169[b]	0.9900±0.0057[a]	0.9358±0.0339	0.9870±0.0068	0.9597±0.0410
		RT	0.9788±0.0242	0.9143±0.0655	0.9605±0.0314	0.9325±0.0141[a]	0.9926±0.0004[b]	0.9801±0.0103[b]
		45	0.9841±0.0110[a]	0.9537±0.0168[b]	0.9894±0.0060[a]	0.9356±0.0182[a]	0.9831±0.0074[b]	0.9413±0.0208[a]

Each datum represents the mean±S.D.

a and **b** in the same row meant the symbol of significant statistics. The different symbols meant the significant difference ($p<0.05$).

Table 4 The kinetics of drug permeation (n=4) in LB-Gαgly and LH-Gαgly films

Sample	Month	Temperature (°C)	Kinetics of short time drug release; R^2 (mean±S.D.)		
			Zero order	Higuchi's	First order
LB-Gαgly	0	RT	0.9262±0.0332	0.9528±0.0217	0.9763±0.0245
	1	4	N.D.	N.D.	N.D.
		RT	0.9683±0.0234	0.9549±0.0322	0.9697±0.0290
		45	N.D.	N.D.	N.D.
	3	4	N.D.	N.D.	N.D.
		RT	0.9700±0.0272	0.9500±0.0382	0.9828±0.0172
		45	N.D.	N.D.	N.D.
LH-Gαgly	0	RT	0.9517±0.0134[a]	0.9830±0.0110[b]	0.9926±0.0039[b]
	1	4	N.D.	N.D.	N.D.
		RT	0.9233±0.0402[a]	0.9834±0.0043[b]	0.9825±0.0081[a, b]
		45	N.D.	N.D.	N.D.
	3	4	N.D.	N.D.	N.D.
		RT	0.9582±0.0180	0.9702±0.0130	0.9861±0.0079
		45	N.D.	N.D.	N.D.

Abbreviation: N.D. = not determined. Each datum represents the mean±S.D.

a and **b** in the same row meant the symbol of significant statistics. The different symbols meant the significant difference ($p<0.05$).

5.3.7 Irritancy evaluation using HET-CAM

The irritancy test is shown in Figure 8. The irritation potential of medicated Gαgly films was negligible. However, LB-Gαgly film found hyperemia in one sample. This might be due to the formulation was mixed with glycerin which it was slightly irritant on HET-CAM and there was reported as moderate irritant chemical (Sindhu, *et al.*, 2014). Moreover, the CAM is very sensitive, and glycerin is also hygroscopic property. The glycerin blended film was tightly attached with CAM in some experiments, and the peeling off the film from CAM might affect or damage CAM. However, no observation of hemorrhage and clotting was found in LB and LH loaded Gαgly films. This demonstrated LB and LH loaded Gαgly films are safe to use. This could be used as buccal delivery systems.

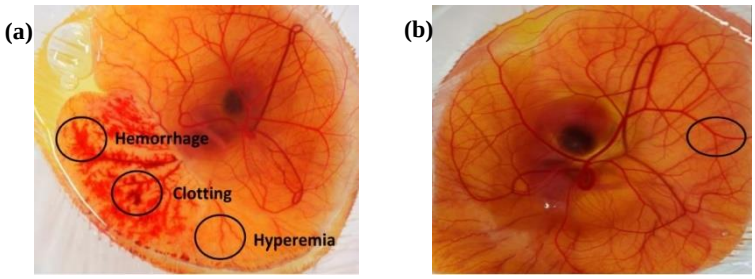

Figure 8 HET-CAM model (a) positive control with hyperemia, hemorrhage and clotting and (b) negative control (Al-Kinani, et al., 2018)

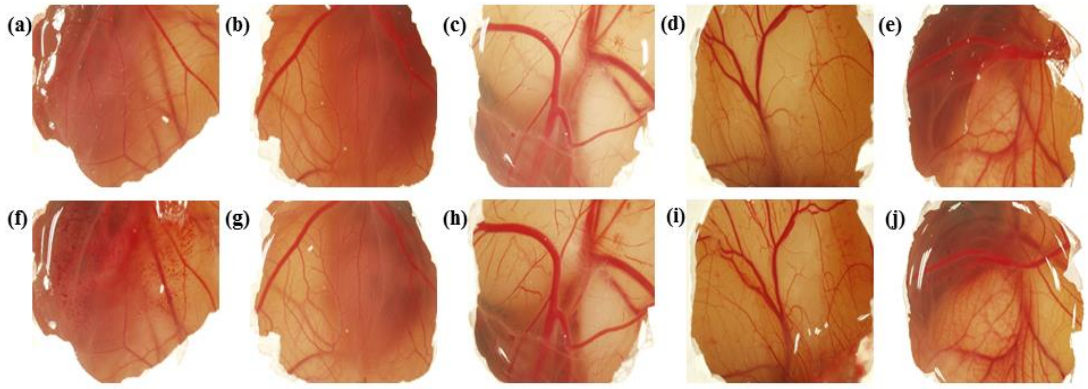

Figure 9 The blood vessels of HET-CAM at EA10 (a-e) before applying the formulation or chemical and after applying (f) 0.1 M sodium hydroxide solution at 0.5 min as positive control, (g) 0.9% sodium chloride solution at 5 min as negative control, (h) LB-Gαgly at 5 min, (i) LH-Gαgly at 5 min, (j) glycerin at 2 min

5.4 Conclusion

LB and LH loaded Gαgly films gave the good properties for buccal drug delivery. The AFM results showed that the surface of medicated Gαgly films was slightly rough on upper and lower side. However, the SEM images in upper side and cross section of both LB and LH Gαgly films presented smooth surface. The TGA thermograms of medicated Gαgly films revealed the same trend as the original gelatin curve. This confirmed the compatibility of all components in the medicated Gαgly films, same as the FT-IR patterns and DSC thermograms as reported previously. However, very slight crystalline form of drugs was observed in XRD diffractograms, especially in LB loaded Gαgly films. LB and LH could release from Gαgly films and permeate through CAM which can be the buccal model. The stability test implied that medicated Gαgly films recommended to store at low temperature. Moreover, the irritation test in HET-CAM indicated that the medicated Gαgly films were safe and could use as buccal delivery. In conclusion, gelatin and pregelatinized tapioca starch could be prepared as the transparent thin film using glycerin as plasticizer, and LB and LH could be loaded into Gαgly with good properties to use as buccal films.

5.5 Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

5.6 Acknowledgements

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CHAPTER 6

CONCLUSIONS

Gelatin has been used as a material in food and pharmaceutical industries. It presents many good properties such as biocompatibility, biodegradability, and film forming property. Moreover, it is safe and edible. In this study, then, gelatin was selected as the main polymer. Starch is widely used in daily life which is a food ingredient. It exhibits good film forming behavior. Then, the film formulation was prepared by blending gelatin with several starches to develop as films or patches. In this study, gelatin and starch was not only used in TDDS, but it was also applied in OTD. However, gelatin/starch films were very brittle. Therefore, plasticizer was necessary to improve the mechanical properties of gelatin/starch films.

Glycerin (GLY), propylene glycol (PG), or polyethylene glycol 400 (PEG400) was used as a plasticizer in gelatin films. The different amounts (5-30 phg) of plasticizer were evaluated. The 30 phg GLY in gelatin gave too softy film and could not be peeled off from Petri dish. Then, the 25 phg GLY was the maximum amount of plasticizer to add into gelatin film. Increasing the amount of plasticizer resulted in increasing water uptake and elongation at break of gelatin films, while their UTS values decreased. To improve the brittleness of gelatin films, therefore, 25 phg of all types of plasticizer was better than the other amounts of plasticizer.

The native starches (rice starch, glutinous starch, and sago starch) and modified starches (pregelatinized maize starch (starch 1500®) and pregelatinized tapioca starch (alpha starch®, α st)) were selected to blend in gelatin films. The native starches were prepared by simple mixing and gelatinized mixing method. While the modified starches were prepared only by simple mixing method. The gelatinized native starches and modified starches blends gave good appearance films, but some of them showed the precipitation of starch granules in the bottom side of films. From these starch blended films, various amounts of α st in gelatin blended films presented the homogenous films when using GLY, PG, PEG400 as a plasticizer. Moreover, gelatinized sago starch could also blend with gelatin/DNRL (GNR) in various amounts

of starch and gave homogenous film. Then, α st and gelatinized sago starch were chosen for further study.

The α st was selected to mix with gelatin. It could disperse and form gel in cold water. The amounts of α st at 5-30 phg could be mixed into gelatin solution. GLY, PG, or PEG400 at 25 phg was added into gelatin/α st solution as plasticizer. The types of plasticizer related to appearance of films. Which GLY and PG blends gave the transparent films and PEG400 blends presented opaque films. Among these plasticizers, 25 phg of GLY was the best plasticizer to improve the elasticity of gelatin/α st films. Moreover, the addition of lidocaine base (LB) or lidocaine hydrochloride (LH) in gelatin/α st/GLY (Gαgly) films could be mixed in the films and gave the good properties in flexibility and erosion of films. The amounts of α st related to the mechanical properties of medicated and non-medicated films. Increasing the amount of α st could increase water uptake and UTS, but it decreased erosion and elongation at break in both medicated and non-medicated films. The 5 phg α st in medicated Gαgly films provided better properties in UTS and elongation at break than non-medicated films and the 10-30 phg of α st in medicated films. Thus, 5 phg α st was used to prepare the medicated films for evaluation of the physiochemical properties. The compatibility of each ingredient in the films was confirmed by physical appearances, FT-IR spectra, DSC thermograms, AFM images, SEM images, TGA thermograms, and XRD diffractograms. Even LB-Gαgly exhibited slightly crystalline pattern in XRD diffractograms, however, LB could release from Gαgly films and permeate through chick chorioallantoic membrane (CAM) which could be the buccal model as same as those of LH products. The drug release both in short time (15 min) and 8 h and permeation profiles of LH-Gαgly films were slightly higher than those of LB-Gαgly films. In short time and 8 h, the drug release profiles presented the different kinetics of drug release. Most of films fitted to zero order kinetics at short time drug release and first order kinetics at 8 h drug release. After stability study, only the kinetics at 3 months of LH-Gαgly drug release changed to first order kinetics and Higuchi's kinetics in short time and 8 h drug release, respectively. This might be due to the condensed films after storage. The kinetics of both LB-Gαgly and LH-Gαgly permeations fitted to first order equatuion. After the stability study, the medicated Gαgly films were suitable to keep at low temperature (4°C). When medicated film was stored at higher temperature, the

amount of drug loss was higher. Both LB-Gαgly and LH-Gαgly presented the good stability with drug amount greater than 90% for 3 months at 4°C and room temperature. Moreover, the irritation of medicated Gαgly films using hen's egg-chorioallantoic membrane test (HET-CAM) indicated the safety to apply on buccal membrane. These could be summarized that gelatin comprising 5 phg α st plasticized with glycerin could be used as ingredients to prepare the transparent films. In addition, LB and LH could be loaded into Gαgly films with the good properties to be used as buccal film for OTD.

DNRL has many good properties such as a high tensile strength and elasticity, biocompatibility, and easy to prepare films. Then, DNRL was chosen to improve the flexibility in gelatin and gelatin/pregelatinized sago starch films. The various amounts of DNRL at 10-50 phg were used in GNR films. All GNR films were opaque. Increasing the amount of DNRL resulted in the decrease of erosion and UTS. The erosion of gelatin/50 phg DNRL (G50NR) was lowest among the others amount DNRL in GNR films. Moreover, G50NR showed the lowest UTS. This indicated that DNRL could improve the flexibility of gelatin films. The different amounts of gelatinized sago starch at 5-30 phg could be blended in gelatin film and 50 phg DNRL was added as plasticizer. All gelatin/pregelatinized sago starch/DNRL (GSNR) patches were opaque. Increasing the amount of sago starch resulted in increasing the drying time of patches and appeared a slight roughness. In addition, it could increase the water uptake and decrease the erosion of patches. Therefore, drug-loaded 5 phg gelatinized sago starch/gelatin film was evaluated for the physicochemical properties because it could be prepared with short drying time and it showed the smoother surface of patch. The AFM, SEM, FT-IR, DSC, and XRD were examined to confirm the compatibility of the ingredients in the films. FT-IR spectra and DSC thermograms indicated the compatibility of the ingredients in patches. However, the SEM cross-section image of LH-GSNR appeared the small particles. Moreover, LB-GSNR showed the small peak indicating crystalline pattern which was similar to LB-Gαgly. The drug release from LH-GSNR was higher than that from LB-GSNR because of the higher water solubility of LH. On the other hand, LB could permeate through newborn pig skin higher than LH. Both LB and LH release were well fitted to first order or Higuchi’s kinetics. The kinetics of permeation profiles of LB-GSNR could be fitted in both zero order and first order kinetics. However, LH-GSNR presented no different of kinetics permeation. For

the stability study, medicated GSNR patches were recommended to store at low temperature. The drug content of only LB-GSNR at 4°C and room temperature was above 90% for 3 months. These could be concluded that gelatin and gelatinized sago starch could be prepared into the films by using DNRL as plasticizer. LB and LH could release and permeate through pig skin. Thus, LB-GSNR posed as a potential transdermal patch in TDDS because it provided the higher drug permeation through the skin.

From this research, either GLY or DNRL could be used as plasticizer in gelatin film. Starch could be blended with gelatin and plasticizer to provide the pleasant films. LB and LH could be incorporated into Gαgly films and GSNR films. Moreover, the drugs could release and permeate through the buccal or skin model. The medicated Gαgly films and LB-GSNR patches were stable after storage at low temperature and suitable for OTD and TDDS, respectively.

Suggestion for further study:

1. These developed biomembranes could not adhere on the skin by themselves. Then, the adhesive liner should be included for improving the adhesive property.
2. The anesthetic property of lidocaine patches should be further evaluated in *in vivo* study, ex. by Tail-flick test or hot plate test in rat model (Langerman, *et al*., 1995).

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APPENDICES

PROCEEDING 1

Rubber latex as plasticizer for gelatin/gelatinized sago starch biomembranes

The 3rd International Congress on Advance Materials (AM2016)

November 27th-30th, 2016

Centara Grand at Central Plaza Ladprao, Bangkok, Thailand

POL-P-23

Rubber latex as plasticizer for gelatin/gelatinized sago starch biomembranes

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Abstract:

This study aimed to develop the biodegradable gelatin/gelatinized sago starch films as drug delivery biomembranes by casting method. However, gelatin film was brittle and lack of elasticity. Therefore, deproteinized natural rubber latex (DNRL) was chosen to blend as plasticizer. Effect of DNRL amounts (10-50 part per hundred of gelatin; phg) on mechanical properties of gelatin films was investigated. The opaque films were formed. Increase the amounts of DNRL resulted in decreased tensile strength and increased elongation at break that could improve the brittle problem of films. However, these DNRL blended films still swelled and dissolved in water, and eroded quickly in 2 hours. The 50 phg DNRL was further chosen for biomembrane preparations. Then, the 5-30 phg in-house preparing gelatinized sago starch was added. These gelatin/gelatinized sago starch biomembranes were homogenous and opaque character. They showed good swelling property that the highest swelling time occurred in 20-30 minutes and then films eroded slowly. Increase the amounts of sago starch in biomembranes gave the higher swelling rate but showed the lower erosion properties. Their tensile strengths were not different between films with and without starch blends. Their elongations at break increased when increasing the amounts of starch but still were lower than that of gelatin/DNRL film. The morphology, Fourier transform infrared spectroscopy and differential scanning calorimeter confirmed the compatibility of ingredients in biomembranes. Lidocaine and its hydrochloride salt were successfully incorporated in these biomembranes with good properties.

Keywords: Gelatin, Sago starch, Natural rubber latex, Biomembranes

PROCEEDING 2

Gelatin films and its pregelatinized starch blends: Effect of plasticizers

The 9th International Conference on Materials Science and Technology (MSAT9)

December 14th-15th, 2016

Swissôtel Le Concorde, Bangkok, Thailand

POL-P-11

Gelatin Films and Its Pregelatinized Starch Blends: Effect of Plasticizers

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Keywords: Gelatin, Pregelatinized starch, Plasticizer, Film

Green biofilms are attractive materials to use in many applications including packaging, agriculture, food, pharmaceuticals and medicals. In this study, the erodible gelatin biofilms and gelatin/commercially pregelatinized tapioca starch blended biofilms were developed which pregelatinized starch might reduce the hygroscopic property of gelatin and then could decrease the degradation rate of films. However, these films were brittle and lack of elasticity. Therefore, several plasticizer blends were investigated to prove this problem. The aim of this study was to investigate the effect of types and amounts of well-known safety plasticizers on the properties of both gelatin and pregelatinized starch blended biofilms. Films were prepared by casting method. Each of glycerol, propylene glycol (PG), or polyethylene glycol 400 (PEG400) was added into gelatin solution in different amounts (5-30 part per hundred of gelatin; phg) and the mixtures were then dried in 50˚C. It was found that all types and amounts of plasticizer could be blended into gelatin solution and the transparent films were formed, except the PEG400 blended film gave opaque character. However, 30 phg glycerol blended film could not be peeled off because it was too softy. When the amount of plasticizer blends increased, the tensile strength decreased and the elongation at break increased. These gelatin films dissolved and eroded very quickly. Pregelatinized starch was also blended, and the amounts of starch (5-30 phg) and types of plasticizer (25 phg) were studied. Pregelatinized starch dispersions mixed well in the gelatin solution and gave the homogenous films. These blended films swelled and eroded in water completely in 2 hours. Increase the amount of starch gave longer swelling time and decreased the degradation rate of blended films. The tensile strength of glycerol blended films slightly increased when increasing the amount of starch but those of PEG400 and PG blended films were not different. The elongation at break of all plasticizers blended films decreased when the amount of starch increased. The morphology, Fourier transform infrared spectroscopy and differential scanning calorimeter confirmed their compatibilities in these films. In application, either lidocaine or its hydrochloride salt was mixed in these gelatin films to use as local anesthetic on the pain skin, and their physicochemical properties were evaluated.

PETTY PATENT 1

This petty patent presents gelatin blended with natural rubber as transdermal patch. This invention relates to preparation gelatin blended with deproteinized natural rubber latex and development of these patches for transdermal drug delivery. This petty patent No. 13750 was filed on November 26, 2016.

เลขที่อนุสิทธิบัตร 13750

อสป/200 - ข

อนุสิทธิบัตร

อาศัยอำนาจตามความในพระราชบัญญัติสิทธิบัตร พ.ศ. 2522
แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ. 2542
ธิบดีกรมทรัพย์สินทางปัญญาออกอนุสิทธิบัตรฉบับนี้ให้แก่

มหาวิทยาลัยสงขลานครินทร์

สำหรับการประดิษฐ์ตามรายละเอียดการประดิษฐ์ ข้อถือสิทธิ และรูปเขียน (ถ้ามี)
ปรากฏในอนุสิทธิบัตรนี้

เลขที่คำขอ	1703000995
วันรับอนุสิทธิบัตร	26 พฤศจิกายน 2559
ผู้ประดิษฐ์	นายวิวัฒน์ พิชญากร และคณะ
การประดิษฐ์ที่แสดงถึงการประดิษฐ์	แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา

ให้ผู้ทรงอนุสิทธิบัตรมีสิทธิและหน้าที่ตามกฎหมายว่าด้วยสิทธิบัตรทุกประการ

ออกให้ ณ วันที่ 4 เดือน เมษายน พ.ศ. 2561

หมดอายุ ณ วันที่ 25 เดือน พฤศจิกายน พ.ศ. 2565

(ลงชื่อ).....
(นายดิเรก ปุญแท้)
รองอธิบดีกรมทรัพย์สินทางปัญญา ปฏิบัติราชการแทน
อธิบดีกรมทรัพย์สินทางปัญญา
ผู้ออกอนุสิทธิบัตร

พนักงานเจ้าหน้าที่

หมายเหตุ
1. ผู้ทรงอนุสิทธิบัตรต้องชำระค่าธรรมเนียมรายปีเริ่มแต่ปีที่ 5 ของอายุสิทธิบัตร มิฉะนั้น อนุสิทธิบัตรจะสิ้นอายุ
2. ผู้ทรงอนุสิทธิบัตรจะขอชำระค่าธรรมเนียมรายปีล่วงหน้าโดยชำระทั้งหมดในคราวเดียวก็ได้
3. ภายใน 90 วันก่อนวันสิ้นอายุอนุสิทธิบัตร ผู้ทรงอนุสิทธิบัตรมีสิทธิขอต่ออายุอนุสิทธิบัตรได้ 2 คราว มีกำหนดคราวละ 2 ปี โดยยื่นคำขอต่ออายุ ต่อพนักงานเจ้าหน้าที่
4. การอนุญาตให้ใช้สิทธิตามอนุสิทธิบัตรและการโอนอนุสิทธิบัตรต้องทำเป็นหนังสือและจดทะเบียนต่อพนักงานเจ้าหน้าที่

034229

ข้อมูลส่วนที่ 1		
เลขที่คำขอ : 1703000995	วันที่ขอ : 26 Nov 2559	วันที่รับคำขอ : 07 Jun 2560
เลขที่ประกาศ :	วันที่ประกาศ :	เล่มที่ประกาศ :
เลขที่สิทธิบัตร : 13750	วันที่จดทะเบียน : 04 Apr 2561	เอกสารประกาศโฆษณา :

ข้อมูลส่วนที่ 2

ผู้ขอจดทะเบียนสิทธิบัตร : มหาวิทยาลัยสงขลานครินทร์		IPC/ID A61K 9/70 A61K 36/46
ตัวแทน : นายจิตดิยุทธ เยี่ยมยกกุล		
ผู้ประดิษฐ์/ออกแบบ : นายวิวัฒน์ พิชญากร, นางสาวประภาพร บุญมี, นางสาวศุจิภา วรรณภาสชัยยง, นายวิรัช ทวีปรีดา		
ชื่อผลิตภัณฑ์/สิ่งประดิษฐ์ : แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา		
สถานะสุดท้าย : ชำระค่ารับจดทะเบียนและประกาศโฆษณาคำขอรับอนุสิทธิบัตร	วันที่ตามสถานะ : 04 Apr 2561	

บทสรุปการประดิษฐ์ซึ่งจะปรากฏบนหน้าประกาศโฆษณา **Read File** : หน้า 1 ของจำนวน 1 หน้า บทสรุปการประดิษฐ์ แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามการประดิษฐ์นี้ สามารถเตรียมได้จากสารละลาย เจลาดิน น้ำยางพาราโปรตีนต่ำ และด้วยาสำคัญ ซึ่งอาจผสมเพิ่มเติมได้ด้วยแป้งกระจายตัวในน้ำชนิดต่างๆ ผสม เข้ากันด้วยกระบวนการคนผสมแบบง่ายจากส่วนประกอบที่เป็นของเหลว ทำให้เกิดฟิล์มโดยการเทส่วนผสมใน ภาชนะพื้นผิวเรียบและอบให้แห้ง สามารถควบคุมความหนาด้วยการกำหนดปริมาณน้ำหนักแห้งของฟิล์มในแต่ละ ขนาดพื้นที่ แผ่นฟิล์มที่ได้มีความยืดหยุ่น อีกทั้งด้วยาสามารถปลดปล่อยจากแผ่นฟิล์มได้

ข้อถือสิทธิ์ (ข้อที่หนึ่ง) ซึ่งจะปรากฏบนหน้าประกาศโฆษณา : ------15/09/2560------(OCR) หน้า 1 ของจำนวน 1 หน้า ข้อถือสิทธิ 1. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ที่ซึ่งประกอบด้วย - สารละลายเจลาดิน ร้อยละ 65-85 โดยน้ำหนักของผลิตภัณฑ์ - น้ำยางพาราโปรตีนต่ำ ร้อยละ 5-30 โดยน้ำหนักของผลิตภัณฑ์ - ด้วยาสำคัญ ร้อยละ 0.01-5 โดยน้ำหนักของผลิตภัณฑ์ 2. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งอาจประกอบเพิ่มเติมด้วย แป้ง กระจายตัวในน้ำ ร้อยละ 3-20 โดยน้ำหนักของผลิตภัณฑ์ 3. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที

แท็ก :

ข้อมูลส่วนที่ 3

เอกสารข้อถือสิทธิ์ Read File	เอกสารแบบพิมพ์คำขอ Read File	เอกสารรายละเอียดการประดิษฐ์ Read File
ภาพเขียนRead File	แบบพิมพ์คำขอ Read File	

ข้อถือสิทธิ์ (ทั้งหมด) ซึ่งจะไม่ปรากฏบนหน้าประกาศโฆษณา :

-----15/09/2560-----(OCR) หน้า 1 ของจำนวน 1 หน้า ข้อถือสิทธิ 1. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ที่ซึ่งประกอบด้วย - สารละลายเจลาดิน ร้อยละ 65-85 โดยน้ำหนักของผลิตภัณฑ์ - น้ำยางพาราโปรตีนต่ำ ร้อยละ 5-30 โดยน้ำหนักของผลิตภัณฑ์ - ตัวยาสำคัญ ร้อยละ 0.01-5 โดยน้ำหนักของผลิตภัณฑ์ 2. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งอาจประกอบเพิ่มเติมด้วย แป้ง กระจายตัวในน้ำ ร้อยละ 3-20 โดยน้ำหนักของผลิตภัณฑ์ 3. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งสารละลายเจลาดิน เลือกได้จาก เจลาดินที่ได้จาก โค กระบือ สุกร หรือ ปลา ที่ละลายในน้ำให้มีความเข้มข้นร้อยละ 5-20 โดยน้ำหนักและที่ดี ที่สุดเข้มข้นร้อยละ 10-15 โดยน้ำหนัก 4. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งน้ำยางพาราโปรตีนต่ำ ได้แก่ น้ำยาง ธรรมชาติที่ผ่านกระบวนลดปริมาณโปรตีนด้วยกระบวนการต่างๆ เพื่อให้มีปริมาณโปรตีนเหลือน้อยที่สุดและ ปลอดภัยต่อการนำมาใช้ทางยา เครื่องสำอาง รวมถึงวัสดุทางการแพทย์ โดยมีปริมาณเนื้อยางร้อยละ 30-60 โดยน้ำหนักและที่ดีที่สุดในช่วงร้อยละ 35-40 โดยน้ำหนัก 5. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 2 ที่ซึ่งแป้งกระจายตัวในน้ำ ได้แก่ (1) แป้ง ดิบ ซึ่งหมายถึง แป้งข้าวเจ้า, แป้งข้าวเหนียว, แป้งข้าวโพด, แป้งถั่วเขียว, แป้งมันสำปะหลัง, แป้งมันฝรั่ง และ แป้งสาคู (2) แป้งดัดแปร ซึ่งเตรียมจากการดัดแปรทางเคมี, ความร้อน หรือทางชีวภาพ ซึ่งรวมถึง สตาร์ช 1500, อัลฟาสตาร์ช และรวมถึงแป้งดัดแปรที่เตรียมขึ้นเองด้วยวิธีการดังกล่าว ซึ่งการใช้แป้งดังกล่าวสามารถ ใช้ได้ทั้งในรูปแป้งชนิดเดียว และการใช้แป้งเหล่านี้ผสมรวมกันมากกว่า 1 ชนิดก็ได้โดยนำแป้งมากระจายตัวใน น้ำ ให้มีลักษณะเป็นน้ำแป้ง หรือแป้งเปียก ที่มีปริมาณเนื้อแป้งร้อยละ 2.5-10 โดยน้ำหนักก่อนการผสมกับ ส่วนประกอบอื่นๆ 6. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งตัวยาสำคัญ ได้แก่ ตัวยาที่สามารถ ซึมผ่านชั้นผิวหนังและแพร่เข้าสู่กระแสเลือดได้ เลือกได้จาก ลิโดเคน, เบนโซเคน, มีลอกซิแคม, ไดโคลฟีแนค, เมโทรนิดาโซล, ซิโปรฟลอกซาซิน, นิโคดิน, สโคโปลามีน, เฟนทานิล, โคลนิดีน, ไนโตรกลีเซอรีน, เอสทราไดออล, เทสโทสเตอโรน, ดิโตโรแลค, บิวพรีนอร์ฟีน, ซิลิจิลีน, ไรวาสติกมีน รวมทั้งสารสกัดและวิตามิน ต่างๆ และหมายรวมถึงการใช้ยาเดี่ยวและยาผสมร่วมกัน โดยมีปริมาณเข้มกับปริมาณที่ต้องการใช้ในการรักษา ในแต่ละครั้งของการบริหารยาชนิดนั้นๆ ---------- 1. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ที่ซึ่งประกอบด้วย - สารละลายเจลาดิน ร้อยละ 65-85 โดยน้ำหนัก - น้ำยางพาราโปรตีนต่ำ ร้อยละ 5-30 โดยน้ำหนัก - ตัวยาสำคัญ ร้อยละ 0.01-5 โดยน้ำหนัก 2. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งอาจประกอบเพิ่มด้วย แป้ง กระจายตัวในน้ำ ร้อยละ 3-20 โดยน้ำหนัก 3. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งสารละลายเจลาดิน เลือกได้จาก เจลาดินที่ได้จาก โค กระบือ สุกร หรือ ปลา ที่ละลายในน้ำให้มีความเข้มข้นร้อยละ 5-20 โดยน้ำหนัก และที่ดี ที่สุดเข้มข้นร้อยละ 10-15 โดยน้ำหนัก 4. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งน้ำยางพาราโปรตีนต่ำ ได้แก่ น้ำยาง ธรรมชาติที่ผ่านกระบวนการลดปริมาณโปรตีนด้วยกระบวนการต่างๆ เพื่อให้มีปริมาณโปรตีนเหลือน้อยที่สุดและ ปลอดภัยต่อการนำมาใช้ทางยา เครื่องสำอาง รวมถึงวัสดุทางแพทย์ โดยมีปริมาณเนื้อยางร้อยละ 30-60 โดยน้ำหนัก และที่ดีที่สุดในช่วงร้อยละ 35-40 โดยน้ำหนัก 5. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 2 ที่ซึ่งแป้งกระจายตัวในน้ำ ได้แก่ (1) แป้ง ดิบ ซึ่งหมายถึง แป้งข้าวเจ้า,แป้งข้าวเหนียว,แป้งข้าวโพด,แป้งถั่วเขียว,แป้งมันสำปะหลัง,แป้งมันฝรั่ง และ แป้งสาคู (2) แป้งดัดแปร ซึ่งเตรียมจากการดัดแปรทางเคมี,ความร้อน หรือทางชีวภาพ ซึ่งหมายถึง สตาร์ช 1,500 อัลฟาสตาร์ช ที่มีจำหน่ายเชิงการค้า และรวมถึงแป้งดัดแปรที่เตรียมขึ้นเองด้วยวิธีการดังกล่าว ซึ่งการใช้ แป้งดังกล่าวสามารถใช้ได้ทั้งในรูปแป้งชนิดเดียว และการใช้แป้งเหล่านี้ผสมรวมกันมากกว่า 1 ชนิดก็ได้ โดยนำ แป้งมากระจายตัวในน้ำ ให้มีลักษณะเป็นน้ำแป้ง หรือแป้งเปียก ที่มีปริมาณเนื้อแป้งร้อยละ 2.5-10 โดยน้ำหนัก ก่อนการผสมกับส่วนประกอบอื่นๆ 6. แผ่นยาแปะผิวหนังสูตรผสมเจลาดินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งตัวยาสำคัญ ได้แก่ ตัวยาที่สามารถ ซึมผ่านชั้นผิวหนังและแพร่เข้าสู่กระแสเลือดได้ เลือกได้จาก ลิโดเคน,เบนโซเคน,มีลอกซิแคม,ไดโคลฟีแนค,เมโทรนิดาโซล,ซิโปรฟลอกซาซิน,นิโคดิน,สโคโปลามีน,เฟนทานิล,โคลนิดีน,ไนโตรกลีเซอรีน, เอสทราไดออล,เทสโทสเตอโรน,ดิโตโรแลค,บิวพรีนอร์ฟีน,ซิลิจิลีน,ไรวาสติกมีน รวมทั้งสารสกัดและวิตามิน ต่างๆ และหมายรวมถึงการใช้ยาเดี่ยวและยาผสมร่วกัน โดยมีปริมาณเข้มกับปริมาณที่ต้องการใช้ในการรักษา ในแต่ละครั้งของการบริหารยาชนิดนั้นๆ

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หน้า 1 ของจำนวน 2 หน้า

คำขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ การประดิษฐ์ ยื่นทางไปรษณีย์

☐ การออกแบบผลิตภัณฑ์

☑ อนุสิทธิบัตร ออกงานแสดง

ข้าพเจ้าผู้ลงลายมือชื่อในคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ขอรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ.2522 แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 2) พ.ศ.2535 และพระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ.2542

สำหรับเจ้าหน้าที่	
วันรับคำขอ – 7 มิ.ย. 2560	เลขที่คำขอ 1703000995
วันยื่นคำขอ 26 พ.ย. 2559	
สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	
ใช้กับแบบผลิตภัณฑ์ ประเภทผลิตภัณฑ์	
วันประกาศโฆษณา	เลขที่ประกาศโฆษณา
วันออกสิทธิบัตร/อนุสิทธิบัตร	เลขที่สิทธิบัตร/อนุสิทธิบัตร
ลายมือชื่อเจ้าหน้าที่	

1. ชื่อที่แสดงถึงการประดิษฐ์/การออกแบบผลิตภัณฑ์

แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา

2. คำขอรับสิทธิบัตรการออกแบบผลิตภัณฑ์นี้เป็นคำขอสำหรับแบบผลิตภัณฑ์อย่างเดียวกันและเป็นคำขอลำดับที่ ในจำนวน คำขอ ที่ยื่นในคราวเดียวกัน

3. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร และที่อยู่ (เลขที่ ถนน ประเทศ)

มหาวิทยาลัยสงขลานครินทร์ 15 ถนนกาญจนวณิชย์ อำเภอหาดใหญ่ จังหวัดสงขลา 90110

3.1 สัญชาติ	ไทย
3.2 โทรศัพท์	0-7428-9322
3.3 โทรสาร	0-7428-9339
3.4 อีเมล์	jittiyut.y@psu.ac.th

4. สิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ ผู้ประดิษฐ์/ผู้ออกแบบ ☑ ผู้รับโอน ☐ ผู้ขอรับสิทธิโดยเหตุอื่น

5. ตัวแทน (ถ้ามี) ที่อยู่ (เลขที่ ถนน จังหวัด ประเทศ รหัสไปรษณีย์)

นายจิตติยุทธ เยี่ยมยกกุล ศูนย์ทรัพย์สินทางปัญญา อุทยานวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ 15 ถนนกาญจนวณิชย์ อำเภอหาดใหญ่ จังหวัดสงขลา 90110

5.1 ตัวแทนเลขที่	2266
5.2 โทรศัพท์	074-289322
5.3 โทรสาร	074-289339
5.4 อีเมล์	jittiyut.y@psu.ac.th

6. ผู้ประดิษฐ์/ผู้ออกแบบผลิตภัณฑ์ และที่อยู่ (เลขที่ ถนน ประเทศ)

1.นายวิวัฒน์ พิชญากร 2.นางสาวประภาพร บุญมี 3.นางสาวสุจิภา วรรณภาสชัยยง คณะเภสัชศาสตร์ และ 4.นายวิรัช ทวีปรีดา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ 15 ถนนกาญจนวณิชย์ อำเภอหาดใหญ่ จังหวัดสงขลา 90110

7. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม

ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ในวันเดียวกับคำขอรับสิทธิบัตรเลขที่ วันยื่น เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ

☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

หมายเหตุ ในกรณีที่ไม่อาจระบุรายละเอียดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้ โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย

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หน้า 2 ของจำนวน 2 หน้า

8. การยื่นคำขอนอกราชอาณาจักร

วันยื่นคำขอ	เลขที่คำขอ	ประเทศ	สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	สถานะคำขอ
8.1				
8.2				
8.3				

8.4 ☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอสิทธิให้ถือว่าได้ยื่นคำขอนี้ในวันที่ได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรในต่างประเทศเป็นครั้งแรกโดย

☐ ได้ยื่นเอกสารหลักฐานพร้อมคำขอนี้ ☐ ขอยื่นเอกสารหลักฐานหลังจากวันยื่นคำขอนี้

9. การแสดงการประดิษฐ์ หรือการออกแบบผลิตภัณฑ์ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรได้แสดงการประดิษฐ์ที่หน่วยงานของรัฐเป็นผู้จัด

วันแสดง **26-29 พฤศจิกายน 2559** วันเปิดงานแสดง **26 พฤศจิกายน 2559** ผู้จัด **คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ร่วมกับ สมาคมวิทยาศาสตร์แห่งประเทศไทยในพระบรมราชูปถัมภ์ และ** The international Union of Advanced Materials and the Chinese Advanced Materials Society

10. การประดิษฐ์เกี่ยวกับจุลชีพ

10.1 เลขทะเบียนฝากเก็บ	10.2 วันที่ฝากเก็บ	10.3 สถาบันฝากเก็บ/ประเทศ

11. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอยื่นเอกสารภาษาต่างประเทศก่อนในวันยื่นคำขอนี้ และจะจัดยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ที่จัดทำเป็นภาษาไทยภายใน 90 วัน นับจากวันยื่นคำขอนี้ โดยขอยื่นเป็นภาษา

☐ อังกฤษ ☐ ฝรั่งเศส ☐ เยอรมัน ☐ ญี่ปุ่น ☐ อื่น ๆ

12. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้อธิบดีประกาศโฆษณาคำขอรับสิทธิบัตร หรือรับจดทะเบียน และประกาศโฆษณาอนุสิทธิบัตรนี้ หลังจากวันที่ เดือน พ.ศ.

☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอให้ใช้รูปเขียนหมายเลข ในการประกาศโฆษณา

13. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ประกอบด้วย

ก. แบบพิมพ์คำขอ 2 หน้า

ข. รายละเอียดการประดิษฐ์ หรือคำพรรณนาแบบผลิตภัณฑ์ 6 หน้า

ค. ข้อถือสิทธิ 1 หน้า

ง. รูปเขียน 2 รูป 2 หน้า

จ. ภาพแสดงแบบผลิตภัณฑ์

☐ รูปเขียน รูป หน้า

☐ ภาพถ่าย รูป หน้า

ฉ. บทสรุปการประดิษฐ์ 1 หน้า

14. เอกสารประกอบคำขอ

☑ เอกสารแสดงสิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ หนังสือรับรองการแสดงการประดิษฐ์/การออกแบบผลิตภัณฑ์

☐ หนังสือมอบอำนาจ

☐ เอกสารรายละเอียดเกี่ยวกับจุลชีพ

☐ เอกสารการขอนับวันยื่นคำขอในต่างประเทศเป็นวันยื่นคำขอในประเทศไทย

☐ เอกสารขอเปลี่ยนแปลงประเภทของสิทธิ

☐ เอกสารอื่น ๆ

15. ข้าพเจ้าขอรับรองว่า

☑ การประดิษฐ์นี้ไม่เคยยื่นขอรับสิทธิบัตร/อนุสิทธิบัตรมาก่อน

☐ การประดิษฐ์นี้ได้พัฒนาปรับปรุงมาจาก.....

16. ลายมือชื่อ (☐ ผู้ขอรับสิทธิบัตร / อนุสิทธิบัตร; ☑ ตัวแทน)

จิตติยุทธ เยี่ยมยกกุล

(นายจิตติยุทธ เยี่ยมยกกุล)

ตัวแทนผู้รับมอบอำนาจ

หมายเหตุ บุคคลใดยื่นคำขอรับสิทธิบัตรการประดิษฐ์หรือการออกแบบผลิตภัณฑ์ หรืออนุสิทธิบัตร โดยการแสดงข้อความอันเป็นเท็จแก่พนักงานเจ้าหน้าที่ เพื่อให้ได้ไปซึ่งสิทธิบัตรหรืออนุสิทธิบัตร ต้องระวางโทษจำคุกไม่เกินหกเดือน หรือปรับไม่เกินห้าพันบาท หรือทั้งจำทั้งปรับ

รายละเอียดการประดิษฐ์

ชื่อที่แสดงถึงการประดิษฐ์

แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา

สาขาวิทยาการที่เกี่ยวข้องกับการประดิษฐ์

เคมีและเภสัชศาสตร์ ในส่วนที่เกี่ยวข้องกับแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา

ภูมิหลังของศิลปะหรือวิทยาการที่เกี่ยวข้อง

การนำส่งยาทางผิวหนัง สามารถนำส่งได้หลายรูปแบบ เช่น เจล (gel), ครีม (cream), อิมัลชัน (emulsion) หรือแผ่นแปะ (patch) เป็นต้น สำหรับแผ่นแปะผิวหนังที่ใช้ทางยา คือ แผ่นแปะที่มีตัวยาสำคัญซึ่งสามารถวางบนผิวหนังในบริเวณที่ต้องการและนำส่งตัวยาสำคัญที่มีขนาดยาที่จำเพาะ ผ่านผิวหนังเข้าสู่ระบบไหลเวียนโลหิต ทำให้สามารถออกฤทธิ์ในการรักษาโรคต่างๆ ได้ตามคุณสมบัติของตัวยานั้นๆ ซึ่งการนำส่งยาทางผิวหนังมีข้อดีหลายประการ คือ หลีกเลี่ยงการถูกทำลายของยาที่ทางเดินอาหารและที่ตับได้ สามารถควบคุมการปลดปล่อยของยา ผู้ป่วยสามารถบริหารยาได้ด้วยตัวเอง ลดผลข้างเคียงจากการใช้ยาได้ เป็นต้น

พอลิเมอร์ซึ่งเป็นส่วนประกอบของแผ่นแปะผิวหนัง เป็นส่วนที่มีความสำคัญมากที่สุดสำหรับการเตรียมแผ่นแปะผิวหนังที่ใช้ทางยา เนื่องจากเป็นส่วนที่ทำหน้าที่ควบคุมการปลดปล่อยตัวยา และส่งผลต่อลักษณะและความน่าใช้ของผลิตภัณฑ์โดยตรง นอกจากนี้พอลิเมอร์ควรมีความปลอดภัย ไม่ก่อให้เกิดการแพ้หรือการระคายเคืองต่อผิวหนัง

เจลาติน (gelatin) เป็นโปรตีนที่ได้จากการแปรรูปคอลลาเจน (collagen) ที่มีอยู่ในผิวหนัง กระดูก และเนื้อเยื่อเกี่ยวพันของสัตว์ เช่น โค กระบือ สุกร และ ปลา เป็นต้น โดยการไฮโดรไลซ์ด้วยความร้อนหรือใช้สารอื่น เช่น กรดหรือเบส ทำให้โครงสร้างของคอลลาเจนถูกทำลายและเปลี่ยนแปลงเป็นเจลาติน เจลาตินจะหลอมเหลวเมื่อได้รับความร้อนและแข็งตัวกลับเมื่อได้รับความเย็น นอกจากนี้ยังสามารถก่อเป็นเจลแบบกึ่งแข็งเมื่อละลายเจลาตินในน้ำ เจลาตินถูกนำมาใช้เป็นส่วนประกอบของผลิตภัณฑ์หลายชนิด เช่น เครื่องสำอาง อาหาร ยา เป็นต้น โดยทางเภสัชกรรมจะใช้เจลาตินในการเคลือบเม็ดยา ผลิตแคปซูล เป็นสารเพิ่มความหนืดในตำรับยา เป็นต้น ซึ่งเจลาติน มีความปลอดภัยต่อร่างกาย เข้ากับเนื้อเยื่อในร่างกายได้ และไม่ก่อให้เกิดการระคายเคืองต่อผิวหนัง อย่างไรก็ตาม แผ่นฟิล์มเจลาตินจะมีลักษณะเปราะ แตกหักได้ง่าย จึงจำเป็นต้องมีการผสมสารชนิดอื่นๆ เพื่อปรับปรุงคุณสมบัติดังกล่าว

แป้ง (starch) เป็นพอลิแซคคาไรด์ ที่ประกอบด้วยส่วนประกอบหลัก คือ อะไมโลส (amylose) และอะไมโลเพคติน (amylopectin) แป้งโดยทั่วไปประกอบด้วยอะไมโลส ร้อยละ 20-25 ซึ่งเกี่ยวข้องกับคุณสมบัติการเกิดฟิล์ม และอะไมโลเพคติน ร้อยละ 75-80 อัตราส่วนระหว่างอะไมโลสและอะไมโลเพคตินที่ต่างกันในแป้งแต่ละชนิดส่งผลต่อคุณสมบัติของแป้ง นอกจากยังมีแป้งดัดแปร (modified starch) ซึ่งเตรียมได้จากหลายวิธี เช่น การโฮโดรไลซิสบางส่วน (partial hydrolysis) การเชื่อมขวาง (cross-linking) การแทนที่ (substitution) เป็นต้น เพื่อปรับปรุงคุณสมบัติของแป้ง เช่น การละลายได้ในน้ำเย็น การเกิดเป็นเจล เป็นต้น แป้งเป็นสารอีกชนิดที่สามารถผสมเข้ากันได้กับเจลาตินเพื่อทำให้เกิดเป็นแผ่นฟิล์มสำหรับใช้เตรียมเป็นแผ่นแปะผิวหนังได้ เพื่อปรับปรุง

คุณสมบัติของเจลาตินให้แตกต่างไปจากแผ่นฟิล์มเจลาตินเดี่ยวๆ แต่เจลาตินผสมแป้งอาจยังคงให้แผ่นฟิล์มที่มีลักษณะเปราะ แตกหักได้ง่าย

พลาสติไซเซอร์ (plasticizer) เป็นสารเพิ่มความยืดหยุ่นที่สามารถใช้ปรับปรุงความแข็งและเปราะของแผ่นฟิล์มได้ โดยสามารถเติมผสมลงในพอลิเมอร์ก่อนการขึ้นรูปเป็นแผ่นฟิล์ม สารพลาสติไซเซอร์ที่นิยมใช้ในแผ่นฟิล์มเจลาติน ได้แก่ กลีเซอรีน (glycerin), โพรพิลีนไกลคอล (propylene glycol), ไดบิวทิลพทาเลต (dibutyl phthalate), ไดบิวทิลซีบาเคต (dibutyl sebacate), โอลิอิลโอลีเอต (oleil oleate), ซอร์บิทอล (sorbitol) เป็นต้น

มีการเปิดเผยการประดิษฐ์หรืองานวิจัยเกี่ยวกับแผ่นแปะผิวหนังเจลาตินที่ใช้ทางยาในหลายรูปแบบ เช่น

สิทธิบัตรอเมริกา เลขที่ 8920831 B2 (2014) เรื่อง Lidocaine-containing hydrogel patch โดย Kubo และคณะ ได้เปิดเผยสิ่งประดิษฐ์แผ่นแปะไฮโดรเจลที่มีลิโดเคน (lidocaine) ซึ่งประกอบด้วยชั้นที่อยู่นอกสุดของแผ่นแปะ (support layer) และชั้นที่ทำให้ติดบนผิวหนัง (adhesive layer) ซึ่งชั้นนี้ประกอบด้วยพอลิเมอร์ที่ละลายน้ำได้ ได้แก่ เจลาติน และพอลิเมอร์อื่นอีกหลายชนิด ยาลิโดเคนหรือเกลือของยา แอลกอฮอล์ชนิดหลายประจุ ได้แก่ กลีเซอรีน โพรพิลีนไกลคอล พอลิเอทิลีนไกลคอน บิวทิลีนไกลคอล ซอร์บิทอล นอกจากนี้ยังมีกรดโอลีอิค (oleic acid) เพื่อลดการเป็นผลึกของตัวยาลิโดเคน และกำหนดความเป็นกรดด่างให้อยู่ในช่วง 6.8-7.4 ซึ่งเป็นช่วงที่เหมาะสมสำหรับการซึมผ่านของยาสู่ผิวหนัง ซึ่งต่างจากการประดิษฐ์ที่มีการใช้สารเพิ่มความยืดหยุ่นชนิดอื่นที่ไม่ใช่แอลกอฮอล์ชนิดหลายประจุ ซึ่งทำให้แผ่นแปะที่ละลายน้ำช้าลง และคงสภาพเป็นแผ่นฟิล์มได้นานขึ้น

Jadhav และคณะ (Int. J. Chem. Tech. Res.: 2 (2010)) ทำการวิจัยแผ่นแปะผิวหนังไดโคลฟีแนคโซเดียม (diclofenac sodium) โดยใช้เจลาตินเป็นพอลิเมอร์หลัก และใช้กลีเซอรีนเป็นพลาสติไซเซอร์ ซึ่งเตรียมโดยการให้ความร้อนแก่เจลาตินจนละลายเป็นของเหลว เติมสารอื่นๆ และตัวยาสำคัญ แล้วขึ้นรูปในภาชนะ พบว่าได้แผ่นฟิล์มที่มีความยืดหยุ่นดี มีบางตำรับสามารถควบคุมการปลดปล่อยยาได้ และแผ่นแปะผิวหนังเจลาตินไม่ก่อให้เกิดการแพ้ในอาสาสมัครที่เป็นมนุษย์ด้วย แต่แผ่นฟิล์มจะละลายในน้ำได้อย่างรวดเร็ว

นอกจากนี้ยังมีงานวิจัยเกี่ยวกับการใช้เจลาตินผสมกับพอลิเมอร์ชนิดอื่นๆ ในการเตรียมแผ่นแปะผิวหนังเพื่อนำส่งยา โดย Subramanian และคณะ (IJPSR: 5 (2014)) ได้เตรียมแผ่นฟิล์มไคโตซาน (chitosan) ผสมเจลาติน โดยมียาทีโอฟีลีน (theophylline) เป็นตัวยาสำคัญ และ Hima และคณะ (Int. J. Drug. Deliv.: 2 (2010)) เตรียมแผ่นแปะผิวหนังไคโตซานผสมเจลาติน และใช้เอทิลีนไกคอล (ethylene glycol) เป็นสารให้ความยืดหยุ่น โดยมียาซิโปรฟลอกซาซิน (ciprofloxacin) เป็นตัวยาสำคัญเพื่อใช้ในการรักษาแผลได้ ซึ่งแผ่นฟิล์มของพอลิเมอร์ผสมจะละลายน้ำได้ช้าลง สามารถควบคุมการปลดปล่อยยาได้นานขึ้น แต่ยังจำเป็นต้องมีพลาสติไซเซอร์อื่นเพื่อเพิ่มความยืดหยุ่นของแผ่นฟิล์ม

น้ำยางธรรมชาติ เป็นพอลิเมอร์ที่มีส่วนประกอบหลัก คือ ซิส-1,4-พอลิไอโซพรีน (cis-1,4 polyisoprene) ซึ่งมีคุณสมบัติที่ดีคือ เป็นสารก่อฟิล์มที่ดีและมีความยืดหยุ่นสูง แต่ในน้ำยางธรรมชาติ มีโปรตีนหลายชนิดปนอยู่ ซึ่งอาจก่อให้เกิดการแพ้ได้ จึงจำเป็นต้องมีการลดปริมาณโปรตีนในน้ำยาง เพื่อทำให้ได้น้ำยางโปรตีนต่ำ สำหรับใช้เป็นวัตถุดิบในทางยาและผลิตภัณฑ์เพื่อสุขภาพอื่นๆ ซึ่งน้ำยางโปรตีนต่ำสามารถนำมาใช้เป็นส่วนประกอบเพื่อลดความเปราะและเพิ่มความยืดหยุ่นให้กับแผ่นแปะผิวหนังของเจลาตินได้

ตัวอย่างสิ่งประดิษฐ์ที่มีการจดสิทธิบัตรเกี่ยวกับการใช้น้ำยางเพื่อทำเป็นแผ่นฟิล์ม ได้แก่ สิทธิบัตรอเมริกา เลขที่ 20080221246 A1 (2008) เรื่อง Water soluble films from latex โดย Imam และคณะ ได้เปิดเผยสิ่งประดิษฐ์แผ่นฟิล์มละลายน้ำจากยาง ประกอบด้วย ยางและพอลิเมอร์ และฟิล์มยางผสมพอลิเมอร์เพิ่มความยืดหยุ่นด้วยสารเพิ่มความยืดหยุ่น สามารถเตรียมฟิล์มโดยการผสมน้ำยางและพอลิเมอร์ที่อุณหภูมิห้อง หลังจากนั้นขึ้นรูปด้วยวิธีต่างๆ ที่เหมาะสม ซึ่งมีการผสมสารสี กลิ่น สารทำความสะอาด สารเคมีอื่นๆ เพื่อใช้เป็นพวกแผ่นฟิล์มสำหรับทำความสะอาด พวกบรรจุภัณฑ์ หรือการนำส่งยา แต่แผ่นฟิล์มชนิดนี้เมื่อสัมผัสกับน้ำโดยตรงจะเกิดการแตกตัวและละลายหมดภายใน 2-3 นาที ซึ่งหากใช้ในการนำส่งยาในรูปแบบแผ่นแปะผิวหนัง อาจไม่สามารถปลดปล่อยยาเป็นเวลานานได้

ลักษณะและความมุ่งหมายของการประดิษฐ์

การประดิษฐ์นี้เกี่ยวข้องกับแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ที่ประกอบด้วย เจลาติน น้ำยางพาราโปรตีนต่ำ และตัวยาสำคัญ ซึ่งอาจผสมเพิ่มเติมได้ด้วยแป้งกระจายตัวในน้ำชนิดต่างๆ ส่วนประกอบเหล่านี้สามารถเตรียมเป็นผลิตภัณฑ์ได้โดยการผสมส่วนประกอบทั้งหมดเข้าด้วยกัน แล้วทำให้เกิดเป็นฟิล์มโดยการเทลงในภาชนะที่มีพื้นผิวเรียบ จากนั้นอบให้แห้ง จะได้ผลิตภัณฑ์แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา

ความมุ่งหมายของการประดิษฐ์นี้ คือ พัฒนาแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพาราเพื่อให้มีลักษณะที่ดี สามารถนำส่งยาผ่านทางผิวหนังเพื่อให้ตัวยาสามารถออกฤทธิ์ได้ โดยการประดิษฐ์นี้มีข้อเด่นในการใช้วัสดุจากธรรมชาติเป็นส่วนประกอบของสิ่งประดิษฐ์ซึ่งมีความเป็นมิตรต่อสิ่งแวดล้อม มีการใช้น้ำยางพาราโปรตีนต่ำเป็นสารลดความเปราะและเพิ่มความยืดหยุ่นให้กับแผ่นแปะผิวหนัง นอกจากนี้การลดปริมาณโปรตีนในน้ำยางส่งผลให้ลดการแพ้หรือการระคายเคืองกับผิวหนังด้วย นอกจากนี้ยังเป็นการส่งเสริมการใช้เจลาตินและยางพารา รวมถึงแป้งจากธรรมชาติชนิดต่างๆ เพื่อช่วยในการสนับสนุนการใช้วัตถุดิบที่มีภายในประเทศอีกด้วย

คำอธิบายรูปเขียนโดยย่อ

รูปที่ 1 ร้อยละของการพองตัวของตัวอย่างที่ 1 และตัวอย่างที่ 2 เทียบกับฟิล์มเจลาติน (กลุ่มควบคุม)

รูปที่ 2 การปลดปล่อยยาลิโดเคนไฮโดรคลอไรด์จากตัวอย่างที่ 1 และตัวอย่างที่ 2 เทียบกับฟิล์มเจลาติน (กลุ่มควบคุม)

การเปิดเผยการประดิษฐ์โดยสมบูรณ์

ตามการประดิษฐ์นี้ได้มีการเสนอแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ประกอบด้วย

- สารละลายเจลาติน	ร้อยละ	65-85	โดยน้ำหนักของผลิตภัณฑ์
- น้ำยางพาราโปรตีนต่ำ	ร้อยละ	5-30	โดยน้ำหนักของผลิตภัณฑ์
- ตัวยาสำคัญ	ร้อยละ	0.01-5	โดยน้ำหนักของผลิตภัณฑ์

และมีส่วนประกอบเพิ่มเติมได้ คือ

- แป้งกระจายตัวในน้ำ	ร้อยละ	3-20	โดยน้ำหนักของผลิตภัณฑ์

แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารานี้ มีส่วนประกอบหลักจากสารละลายเจลาติน ซึ่งมีพอลิเมอร์เจลาตินเป็นโครงสร้างหลักในแผ่นแปะ และน้ำยางพาราโปรตีนต่ำใช้เป็นสารเพิ่มความยืดหยุ่นของแผ่นแปะผิวหนัง โดยอาจมีแป้งกระจายตัวในน้ำเป็นเป็นพอลิเมอร์ร่วมผสมเข้ากับสารละลายเจลาตินและเป็นสารก่อ

ฟิล์มด้วยก็ได้ และตัวยาสำคัญซึ่งเป็นสารออกฤทธิ์ในการบรรเทาหรือรักษาอาการต่างๆ โดยมีวัตถุประสงค์ดังกล่าวและวัตถุประสงค์อื่นๆ ของการประดิษฐ์นี้จะเป็นที่ชัดเจนจากคำอธิบายในการเปิดเผยการประดิษฐ์โดยสมบูรณ์ดังต่อไปนี้

สารละลายเจลาติน เลือกได้จาก เจลาตินที่แปรรูปจากคอลลาเจนของ โค กระบือ สุกร และ ปลา โดยทำการละลายในน้ำ อาจใช้ความร้อนในการช่วยละลายด้วยก็ได้ ให้มีความเข้มข้นของสารละลายเจลาตินร้อยละ 5-20 โดยน้ำหนัก และที่ดีที่สุดเข้มข้นร้อยละ 10-15 โดยน้ำหนัก และสามารถนำสารละลายเจลาตินดังกล่าวมาผสมเพื่อเตรียมแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ในสัดส่วนร้อยละ 65-85 โดยน้ำหนักของผลิตภัณฑ์

น้ำยางพาราโปรตีนต่ำ เลือกได้จาก น้ำยางธรรมชาติที่ได้จากการกรีดเปลือกต้นยางพารา และผ่านการเตรียมเพื่อกำจัดโปรตีนออกไปด้วยกระบวนการต่างๆ เพื่อให้มีปริมาณโปรตีนเหลือน้อยที่สุดและปลอดภัยต่อการนำมาใช้ทางยา เครื่องสำอาง รวมถึงวัสดุทางการแพทย์ ซึ่งกระบวนการในการลดโปรตีนมีหลายวิธี ได้แก่ การปั่นด้วยเครื่องหมุนเหวี่ยงความเร็วสูงเพื่อกำจัดโปรตีนที่ละลายน้ำออก การเติมสารให้เกิดครีมของเนื้อยางเพื่อแยกโปรตีนออกจากเนื้อยาง การย่อยสายโปรตีนด้วยเอนไซม์เพื่อให้สายโปรตีนสั้นลงและละลายในตัวกลางที่เป็นน้ำ จากนั้นทำการปั่นเหวี่ยงเพื่อแยกออกหรืออาจใช้หลายวิธีร่วมกันเพื่อลดปริมาณโปรตีนในน้ำยางลง โดยมีปริมาณเนื้อยางในน้ำยางพาราโปรตีนต่ำในช่วงร้อยละ 30-60 โดยน้ำหนัก และที่ดีที่สุดในช่วงร้อยละ 35-40 โดยน้ำหนัก และสามารถนำน้ำยางพาราโปรตีนต่ำดังกล่าวมาผสมเพื่อเตรียมแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพาราในสัดส่วนร้อยละ 5-30 โดยน้ำหนักของผลิตภัณฑ์

ตัวยาสำคัญ เลือกได้จาก สารออกฤทธิ์ ที่สามารถบรรเทาอาการหรือรักษาโรค ที่ออกฤทธิ์เฉพาะที่หรือทั่วร่างกาย ซึ่งสามารถใช้นำส่งทางผิวหนังได้ ได้แก่ ลิโดเคน (lidocaine) เบนโซเคน (benzocaine) มีลอกซิแคม (meloxicam) ไดโคลฟีแนค (diclofenac) เมโทรนิดาโซล (metronidazole) ซิโปรฟลอกซาซิน (ciprofloxacin) นิโคติน (nicotine) สโคโปลามีน (scopolamine) เฟนทานีล (fentanyl) โคลนิดีน (clonidine) ไนโตรกลีเซอรีน (nitroglycerin) เอสทราไดออล (estradiol) เทสโทสเตอโรน (testosterone), คีโตโรแลค (ketorolac) บิวพรีนอร์ฟีน (buprenorphine) ซิลิจิลีน (selegiline) ไรวาสติกมีน (rivastigmine) และ/หรือยาอื่นๆ ที่สามารถใช้นำส่งทางผิวหนังได้ รวมทั้งสารสกัดและวิตามินต่างๆ และหมายรวมถึงการใช้ยาเดี่ยวและยาผสมร่วมกัน โดยปริมาณตัวยาสำคัญแต่ละชนิดในตำรับจะมีปริมาณที่แตกต่างกันในช่วงร้อยละ 0.01-5 โดยน้ำหนักของผลิตภัณฑ์ขึ้นกับปริมาณที่ต้องการใช้ในการรักษาในแต่ละครั้ง (dose) ของการบริหารยาชนิดนั้นๆ

แป้งกระจายตัวในน้ำ เลือกได้จาก พอลิแซคคาไรด์ที่ประกอบด้วย อะไมโลส และ อะไมโลแพคติน เป็นองค์ประกอบหลัก ได้จากส่วนต่างๆ ของพืช ได้แก่ (1) แป้งดิบ (native starch) ได้แก่ แป้งข้าวเจ้า แป้งข้าวเหนียว แป้งข้าวโพด แป้งถั่วเขียว แป้งมันสำปะหลัง แป้งมันฝรั่ง และแป้งสาคู (2) แป้งดัดแปร (modified starch) ซึ่งทำได้หลายวิธี เช่น การไฮโดรไลซิสบางส่วน (partial hydrolysis) การเชื่อมขวาง (cross-linking) การแทนที่ (substitution) เป็นต้น เพื่อปรับปรุงคุณสมบัติของแป้ง เช่น อัลฟ่าสตาร์ช (alpha starch) สตาร์ช 1500 (starch 1500) ที่มีจำหน่ายเชิงการค้า และรวมถึงแป้งดัดแปรที่เตรียมขึ้นเองด้วยวิธีการดังกล่าว เป็นต้น ซึ่งการใช้แป้งดังกล่าวสามารถใช้ได้ทั้งในรูปแป้งชนิดเดียว และการใช้แป้งเหล่านี้ผสมรวมกันมากกว่า 1 ชนิดก็ได้ โดยนำแป้งมากระจายตัวในน้ำ ให้มีลักษณะเป็นน้ำแป้ง หรือแป้งเปียก ที่มีปริมาณเนื้อแป้งร้อยละ 2.5-10 โดยน้ำหนักก่อนการผสมกับส่วนประกอบอื่นๆ และสามารถผสมแป้งกระจายตัวในน้ำดังกล่าวเพื่อเตรียมแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ในสัดส่วนร้อยละ 3-20 โดยน้ำหนักของผลิตภัณฑ์

ตัวอย่างที่ 1 แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ซึ่งมีลิโดเคนเป็นสารสำคัญ

- สารละลายเจลาติน เข้มข้นร้อยละ 15	ปริมาณร้อยละ	82.7	โดยน้ำหนักของผลิตภัณฑ์
- น้ำยางพาราโปรตีนต่ำ ที่มีเนื้อยางร้อยละ 37	ปริมาณร้อยละ	16.3	โดยน้ำหนักของผลิตภัณฑ์
- ลิโดเคน (รูปเกลือไฮโดรคลอไรด์)	ปริมาณร้อยละ	1.0	โดยน้ำหนักของผลิตภัณฑ์

ตัวอย่างที่ 2 แผ่นยาแปะผิวหนังสูตรผสมเจลาติน แป้ง และยางพารา ซึ่งมีลิโดเคนเป็นสารสำคัญ

- สารละลายเจลาติน เข้มข้นร้อยละ 15	ปริมาณร้อยละ	73.6	โดยน้ำหนักของผลิตภัณฑ์
- น้ำยางพาราโปรตีนต่ำ ที่มีเนื้อยางร้อยละ 37	ปริมาณร้อยละ	14.5	โดยน้ำหนักของผลิตภัณฑ์
- แป้งสาคูกระจายตัวในน้ำ เข้มข้นร้อยละ 5	ปริมาณร้อยละ	11.0	โดยน้ำหนักของผลิตภัณฑ์
- ลิโดเคน (รูปเกลือไฮโดรคลอไรด์)	ปริมาณร้อยละ	0.9	โดยน้ำหนักของผลิตภัณฑ์

กรรมวิธีการเตรียมแผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามการประดิษฐ์นี้ สามารถเตรียมโดยกระบวนการคนผสมแบบง่าย แล้วขึ้นรูปแผ่นฟิล์มด้วยการเทลงในภาชนะที่มีผิวเรียบ จากนั้นนำไปอบให้แห้ง ดังมีรายละเอียดดังนี้

1. การเตรียมน้ำยางพาราโปรตีนต่ำ จากน้ำยางสด ที่ได้จากการกรีดต้นยางพาราและปราศจากการเติมแต่งสารใดๆ นำน้ำยางกรองผ่านตะแกรงเพื่อกำจัดสิ่งสกปรกออกก่อน จากนั้นเติมสารละลายโซเดียมโดดีซิลซัลเฟส (sodium dodecylsulfate) เพื่อลดการจับตัวกันเป็นก้อนของน้ำยาง เติมยูนิเฟนพี 23 (uniphen P-23) เพื่อเป็นสารกันเสียในตำรับ และเติมอัลคาเลสเอนไซม์ (alcalase enzyme) เพื่อย่อยสายโปรตีนให้สั้นลง โดยสารเติมเหล่านี้จะละลายอยู่ในตัวกลางที่เป็นน้ำ ปรับพีเอชทั้งหมดให้ได้ 7-8 โดยใช้สารละลายโซเดียมไฮดรอกไซด์ (Sodium hydroxide) ความเข้มข้น 5 โมลาร์ จากนั้นบ่มที่ 37 องศาเซลเซียส เป็นเวลา 48 ชั่วโมง เพื่อให้เอนไซม์ทำงานได้อย่างเต็มประสิทธิภาพ ก่อนทำการปั่นเหวี่ยงด้วยความเร็วสูงเพื่อล้างโปรตีนออก และเก็บส่วนของเนื้อยางไว้ เจือจางด้วยน้ำให้มีปริมาณเนื้อยางในช่วงร้อยละ 30-60 โดยน้ำหนัก เพื่อใช้ในการเตรียมสิ่งประดิษฐ์ต่อไป

2. การละลายเจลาตินในน้ำ โดยให้ความร้อนที่อุณหภูมิ 40 องศาเซลเซียส ค่อยๆ โปรยผงเจลาตินลงในน้ำ และคนจนกระทั่งเจลาตินละลายจนหมด ตั้งทิ้งไว้ให้เย็นเพื่อให้สารละลายเจลาตินมีอุณหภูมิเท่ากับอุณหภูมิห้อง โดยให้ความเข้มข้นของเจลาตินเป็นร้อยละ 5-20 โดยน้ำหนัก

3. การเติมแป้งกระจายตัวในน้ำ โดยอาจเตรียมในรูปของผงแป้งที่กระจายตัวในน้ำ หรือแป้งเปียกที่เตรียมโดยการกระจายแป้งในน้ำแล้วให้ความร้อนจนแป้งเกิดเป็นเจลอย่างสมบูรณ์และรอให้เย็นที่อุณหภูมิห้องก่อนนำมาใช้ โดยมีปริมาณเนื้อแป้งร้อยละ 2.5-10 โดยน้ำหนัก จากนั้นจึงค่อยๆ เติมแป้งกระจายตัวในน้ำดังกล่าวลงในสารละลายเจลาติน คนผสมให้เข้ากันตลอดเวลาเพื่อให้ส่วนประกอบเข้ากัน

4. จากนั้นจึงเติมน้ำยางพาราโปรตีนต่ำที่ได้จากขั้นตอนที่ 1 ลงในของผสมอย่างช้าๆ คนผสมให้เข้ากันตลอดเวลาเพื่อให้ส่วนประกอบเข้ากันและลดการจับตัวเป็นก้อนของยาง จะได้เป็นของผสมพอลิเมอร์ในรูปของเหลวข้นหนืด

5. การเติมตัวยาสำคัญ ยาที่มีลักษณะเป็นของแข็งจะถูกละลายในตัวทำละลายปริมาณเล็กน้อยที่สามารถเข้ากันได้กับส่วนประกอบอื่นๆ ในตำรับ ก่อนที่จะผสมลงในตำรับต่อไป ส่วนยาที่มีลักษณะเป็นของเหลวสามารถเติมลงในของผสมในขั้นตอนที่ 4 ได้โดยตรง

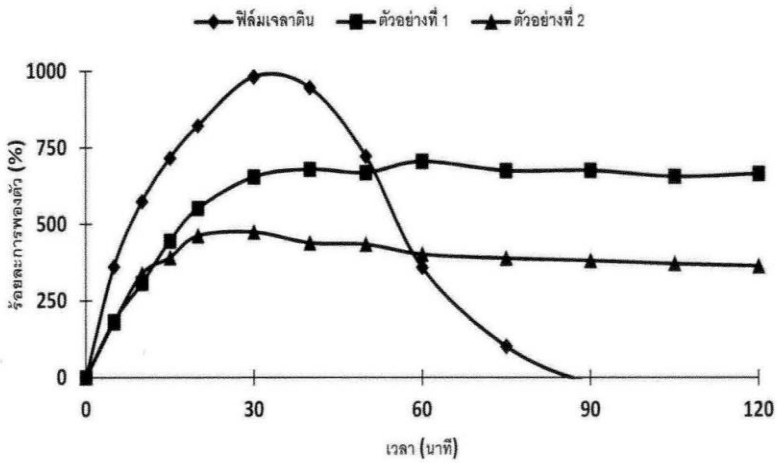
6. นำของผสมที่ได้จากขั้นตอนที่ 5 มาขึ้นรูปแผ่นฟิล์ม ทำโดยการเทส่วนผสมทั้งหมดลงในภาชนะผิวเรียบ ให้มีปริมาณน้ำหนักแห้งของฟิล์มเท่ากันทุกแผ่น เพื่อควบคุมให้ได้ความหนาที่สม่ำเสมอกัน โดยสามารถควบคุมความหนาด้วยการกำหนดปริมาณน้ำหนักแห้งของฟิล์มในแต่ละขนาดพื้นที่ จากนั้นอบให้แห้งในตู้อบ ฟิล์มที่แห้งจะถูกเก็บในภาชนะบรรจุที่ปิดมิดชิดเพื่อป้องกันแผ่นฟิล์มจากสิ่งแวดล้อม

สิ่งประดิษฐ์ที่ได้ จะมีลักษณะเป็นแผ่นฟิล์มบางที่มีความหนาประมาณ 200-500 ไมโครเมตร มีสีเหลือง มีลักษณะยืดหยุ่นได้ ไม่เปราะ มีความสามารถในการดูดความชื้นได้ดี แต่ละลายน้ำได้ช้าลงกว่าฟิล์มเจลาติน (ดังแสดงในรูปที่ 1) นอกจากนี้เมื่อทดสอบการปลดปล่อยของตัวยาจากสิ่งประดิษฐ์ พบว่ายาสามารถปลดปล่อยจากสิ่งประดิษฐ์ได้ช้าลงกว่าฟิล์มเจลาติน และเมื่อมีการผสมแป้งจะทำให้การปลดปล่อยยาเร็วขึ้น (ดังแสดงในรูปที่ 2) ซึ่งแสดงให้เห็นว่าสิ่งประดิษฐ์นี้ สามารถใช้ในการบรรเทาหรือรักษาตามการออกฤทธิ์ของตัวยานั้นๆ ได้

<u>วิธีการในการประดิษฐ์ที่ดีที่สุด</u>

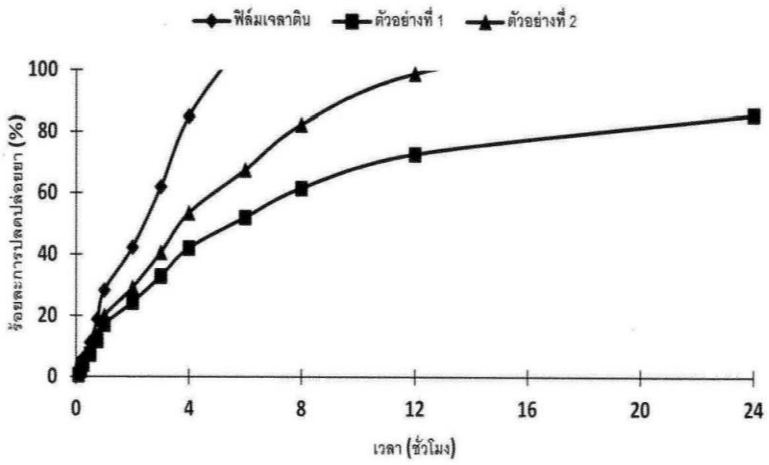
ดังที่ได้กล่าวไว้แล้วในหัวข้อการเปิดเผยการประดิษฐ์โดยสมบูรณ์

หน้า 1 ของจำนวน 2 หน้า



รูปที่ 1

หน้า 2 ของจำนวน 2 หน้า



รูปที่ 2

ข้อถือสิทธิ

1. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ที่ซึ่งประกอบด้วย

- สารละลายเจลาติน	ร้อยละ	65-85	โดยน้ำหนักของผลิตภัณฑ์
- น้ำยางพาราโปรตีนต่ำ	ร้อยละ	5-30	โดยน้ำหนักของผลิตภัณฑ์
- ตัวยาสำคัญ	ร้อยละ	0.01-5	โดยน้ำหนักของผลิตภัณฑ์

2. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งอาจประกอบเพิ่มเติมด้วย แป้งกระจายตัวในน้ำ ร้อยละ 3-20 โดยน้ำหนักของผลิตภัณฑ์

3. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งสารละลายเจลาติน เลือกได้จากเจลาตินที่ได้จาก โค กระบือ สุกร หรือ ปลา ที่ละลายในน้ำให้มีความเข้มข้นร้อยละ 5-20 โดยน้ำหนักและที่ดีที่สุดเข้มข้นร้อยละ 10-15 โดยน้ำหนัก

4. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งน้ำยางพาราโปรตีนต่ำ ได้แก่ น้ำยางธรรมชาติที่ผ่านกระบวนลดปริมาณโปรตีนด้วยกระบวนการต่างๆ เพื่อให้มีปริมาณโปรตีนเหลือน้อยที่สุดและปลอดภัยต่อการนำมาใช้ทางยา เครื่องสำอาง รวมถึงวัสดุทางการแพทย์ โดยมีปริมาณเนื้อยางร้อยละ 30-60 โดยน้ำหนักและที่ดีที่สุดในช่วงร้อยละ 35-40 โดยน้ำหนัก

5. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามข้อถือสิทธิที่ 2 ที่ซึ่งแป้งกระจายตัวในน้ำ ได้แก่ (1) แป้งดิบ ซึ่งหมายถึง แป้งข้าวเจ้า, แป้งข้าวเหนียว, แป้งข้าวโพด, แป้งถั่วเขียว, แป้งมันสำปะหลัง, แป้งมันฝรั่ง และแป้งสาคู (2) แป้งดัดแปร ซึ่งเตรียมจากการดัดแปรทางเคมี, ความร้อน หรือทางชีวภาพ ซึ่งรวมถึง สตาร์ช 1500, อัลฟาสตาร์ช และรวมถึงแป้งดัดแปรที่เตรียมขึ้นเองด้วยวิธีการดังกล่าว ซึ่งการใช้แป้งดังกล่าวสามารถใช้ได้ทั้งในรูปแป้งชนิดเดียว และการใช้แป้งเหล่านี้ผสมรวมกันมากกว่า 1 ชนิดก็ได้ โดยนำแป้งมากระจายตัวในน้ำ ให้มีลักษณะเป็นน้ำแป้ง หรือแป้งเปียก ที่มีปริมาณเนื้อแป้งร้อยละ 2.5-10 โดยน้ำหนักก่อนการผสมกับส่วนประกอบอื่นๆ

6. แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามข้อถือสิทธิที่ 1 ที่ซึ่งตัวยาสำคัญ ได้แก่ ตัวยาที่สามารถซึมผ่านชั้นผิวหนังและแพร่เข้าสู่กระแสเลือดได้ เลือกได้จาก ลิโดเคน, เบนโซเคน, มีลอกซิแคม, ไดโคลฟีแนค, เมโทรนิดาโซล, ซิโปรฟลอกซาซิน, นิโคติน, สโคโปลามีน, เฟนทานีล, โคลนิดีน, ไนโตรกลีเซอรีน, เอสทราไดออล, เทสโทสเตอโรน, คีโตโรแลค, บิวพรีนอร์ฟีน, ซิลิจิลีน, ไรวาสติกมีน รวมทั้งสารสกัดและวิตามินต่างๆ และหมายรวมถึงการใช้ยาเดี่ยวและยาผสมร่วมกัน โดยมีปริมาณขึ้นกับปริมาณที่ต้องการใช้ในการรักษาในแต่ละครั้งของการบริหารยาชนิดนั้นๆ

บทสรุปการประดิษฐ์

แผ่นยาแปะผิวหนังสูตรผสมเจลาตินและยางพารา ตามการประดิษฐ์นี้ สามารถเตรียมได้จากสารละลายเจลาติน น้ำยางพาราโปรตีนต่ำ และตัวยาสำคัญ ซึ่งอาจผสมเพิ่มเติมได้ด้วยแป้งกระจายตัวในน้ำชนิดต่างๆ ผสมเข้ากันด้วยกระบวนการคนผสมแบบง่ายจากส่วนประกอบที่เป็นของเหลว ทำให้เกิดฟิล์มโดยการเทส่วนผสมในภาชนะพื้นผิวเรียบและอบให้แห้ง สามารถควบคุมความหนาด้วยการกำหนดปริมาณน้ำหนักแห้งของฟิล์มในแต่ละขนาดพื้นที่ แผ่นฟิล์มที่ได้มีความยืดหยุ่น อีกทั้งตัวยาสามารถปลดปล่อยจากแผ่นฟิล์มได้

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Bachelor of Pharmacy	Prince of Songkla University	2013

Scholarship Awards during Enrolment

1. Scholarship Awards for Thai Ph.D. Students under Thailand's Education Hub for Southern Region of ASEAN Countries, Graduate school, Prince of Songkla University.
2. Research Grant for Thesis, Graduate school, Prince of Songkla University.
3. Scholarship for supporting exchange students and international credit transfer within ASEAN community, Graduate school, Prince of Songkla University.
4. Conference scholarship Faculty of Pharmaceutical Sciences, Prince of Songkla University.

List of Publication and Proceeding

Publications

Wannaphatchaiyong, S., Boonme, P., Pichayakorn, W. 2017. Gelatin films and its pregelatinized starch blends: Effect of plasticizers. *Key Engineering Materials*. 751: 230-235.

Wannaphatchaiyong, S., Suksaeree, J., Waiprib, R., Kaewpuang, A., Saelee, W., Pichayakorn, W. 2019. Gelatin/gelatinized sago starch biomembranes as a drug delivery system using rubber latex as plasticizer. *Journal of Polymers and the Environment*. https://doi.org/10.1007/s10924-019-01510-2.

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Wannaphatchaiyong, S., Boonme, P., Taweepreda, W., Suksaeree, J., Pichayakorn, W. 2016. Rubber latex as plasticizer for gelatin/gelatinized sago starch biomembranes, The 3rd International Congress on Advanced Materials (AM2016) November 27-30, 2016, Centara Grand at Central Plaza Ladprao,

Bangkok, Thailand, The 3rd International Congress on Advanced Materials (AM2016), Programs and abstract book, p. 230.

Wannaphatchaiyong, S., Boonme, P., Pichayakorn, W. 2016. Gelatin films and its pregelatinized starch blends: Effect of plasticizers. The 9th International Conference on Materials Science and Technology (MSAT9) December 14-15, 2016, Swissôtel Le Concorde, Bangkok, Thailand, The 9th International Conference on Materials Science and Technology (MSAT9), Abstract book, p. 285.

Petty patent

วิวัฒน์ พิชญากร, ประภาพร บุญมี, **ศุจิภา วรรณภาสชัยยง**, วิรัช ทวีปรีดา. 2561. อนุสิทธิบัตรไทย *เลขที่ 13750*. แผ่นแปะผิวหนังสูตรผสมเจลาตินและยางพารา. กรุงเทพฯ: กรมทรัพย์สินทางปัญญา กระทรวงพาณิชย์.