Formulation Development of Lidocaine Transdermal Patches from Natural Rubber

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ชื่อวิทยานิพนธ์ การพัฒนาตำรับแผ่นแปะผิวหนังของยาลิโดเคนจากยางธรรมชาติ

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บทคัดย่อ

วัตถุประสงค์หลักของงานวิจัยนี้เพื่อพัฒนาตำรับแผ่นแปะผิวหนังโดยใช้ยางธรรมชาติเป็นส่วนประกอบหลัก เพื่อเป็นการเพิ่มมูลค่าให้กับยางพารา และใช้ยาลิโดเคนเป็นยาต้นแบบ ที่ความเข้มข้นร้อยละ 5 โดยน้ำหนักของเนื้อยาง น้ำยางข้นและยางแห้ง (ยางแท่ง STR-5L) ถูกใช้เป็นตัวแทนของยางธรรมชาติชนิดของเหลวและของแข็งตามลำดับ ศึกษาคุณสมบัติและลักษณะการปลดปล่อยยาของแผ่นแปะที่เตรียมได้ จากผลการทดลองพบว่าแผ่นแปะที่เตรียมจากน้ำยางข้น โดยใช้ปิโตรเลียมเรซินเป็นสารตัวเติม และโดยการใช้น้ำยางธรรมชาตินำหนักโมเลกุลต่ำไม่เสริมการติดผิวหนังของแผ่นแปะ ขณะที่แผ่นแปะจากยางแห้งสามารถให้คุณสมบัติติดผิวหนังได้ จากการศึกษาลักษณะพื้นผิวของแผ่นแปะด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด พบว่าแผ่นแปะจากยางแห้งมีลักษณะพื้นผิวที่เรียบกว่าแผ่นแปะจากน้ำยางข้น แผ่นแปะที่เตรียมจากน้ำยางข้นมีลักษณะพื้นผิวเป็นรอยแตก จากผลการศึกษาด้วยเทคนิคการเลี้ยวเบนของรังสีเอ็กซ์ แผ่นแปะเปล่าจากน้ำยางข้นและยางแห้ง ให้ลักษณะของสเปกตรัมที่กว้างแสดงว่าเป็นอสัณฐาน ขณะที่แผ่นแปะที่มีการผสมยาลิโดเคนเห็นลักษณะของสเปกตรัมที่แสดงความเป็นผลึกของยาที่ต่างกันเมื่อชนิดของยางต่างกัน จากผล การศึกษาด้วยรังสีอินฟาเรดแบบการสะท้อนสะสมทั้งหมดพบว่าไม่เกิดปฏิกิริยาเคมีระหว่างยากับพอลิเมอร์ที่ใช้

ปริมาณยาในแผ่นแปะที่เตรียมน้ำยางข้นและยางแห้ง คือ 1.90±0.09 และ 2.03±0.14 mg/cm^2 ตามลำดับ คิดเป็นประสิทธิภาพของการกักเก็บยาของแผ่นแปะน้ำยางข้นและยางแห้ง คือ 80.56 ±3.80% และ 86.29±5.77% ตามลำดับ ปริมาณยาลดลงเนื่องจากยาอาจแทรกตัวอยู่ในสายโมเลกุลของยางที่พันกัน ทำให้ไม่สามารถถูกสกัดออกมา ซึ่งพบว่าการผสมยาลงในแผ่นแปะมีผลให้การดูดความชื้นของแผ่นแปะลดลง จากการศึกษาการปลดปล่อยนอกกาย พบว่า แผ่นแปะปลดปล่อยยาลิโดเคนเร็วในช่วง 12 ชั่วโมงแรก อุณหภูมิในการเก็บแผ่นแปะมีผลต่อสมบัติของแผ่นแปะซึ่งส่งผลต่อการลดการปลดปล่อยยา การเก็บที่อุณหภูมิ 45 องศาเซลเซียส มีผลต่อการปลดปล่อยยาลดลง เนื่องจากการเรียงแน่นกันมากขึ้นของสายโมเลกุลยางธรรมชาติขณะเก็บ สภาวะการเก็บแผ่นแปะที่เหมาะสม คือที่อุณหภูมิต่ำ (4 องศาเซลเซียส) ปริมาณโปรตีนของแผ่นแปะที่เตรียมจากน้ำยางข้นและยางแห้ง มีค่า 1910.00 ± 441.42 และ 1006.25 ± 365.41μg/dm^2 ตามลำดับ ซึ่งเป็นปริมาณที่ยังไม่สามารถยอมรับได้สำหรับผลิตภัณฑ์ทางการแพทย์ ดังนั้นอาจใช้ยางธรรมชาติโปรตีนต่ำแทนในอนาคต

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ABSTRACT

The main objective of this research was to develop the formulation of transdermal patches using natural rubber as the dominant composition in order to increase the value of natural rubber latex. The lidocaine was used as the model drug in the concentration of 5 %w/w of dry rubber content (DRC). The concentrate natural rubber latex (CNRL) and dry natural rubber (DNR; block rubber STR-5L) were used as polymer bases as the representatives of liquid and solid natural rubber types, respectively. The patches were studied for their properties and drug release characteristics. From the obtained datum, they could be indicated that adding of petroleum resin into CNRL and using low molecular weight of CNRL could not develop adherence property of patches. DNR itself had adherence property. The surface morphology tested by scanning electron microscope (SEM) showed that the DNR and lidocaine-loaded dry natural rubber (L-DNR) patches had smooth surface while CNRL and lidocaine-loaded concentrate natural rubber latex (L-CNRL) patches had crack of surface. In the results of X-ray diffraction (XRD), they were found that blank CNRL and DNR patches presented broad XRD

spectra indicating amorphous property. Drug-loaded CNRL and DNR provided different crystallinity. In attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectra, they had been observed that there was no chemical interaction between the drug and the polymer bases.

The drug content in L-CNRL and L-DNR formulations were 1.90±0.09 and 2.03±0.14 mg/cm^2, respectively. The efficiency of drug entrapment of L-CNRL and L-DNR were about 80.56 ±3.80 and 86.29±5.77 %, respectively. The decrease of drug content might be due to inserting of drug in molecular chain of polymers which could not be extracted. It is also found that the lidocaine-loaded rubber patches provided lower average moisture absorption than their blank counterparts. From *in vitro* release data, the release rates of lidocaine from the patches implied that the patches provided fast drug release during initial 12 h. The storage temperature could age rubber patches resulting in decrease of drug release. When the samples were kept at higher temperatures (45 °C), more decrease in amount of drug release was found due to tighter of molecular chain of natural rubber. The appropriate storage condition for lidocaine-loaded patches was at low temperature (4°C)

Amounts of protein in the CNRL and DNR patches were 1910.00 ± 441.42 and 1006.25 ± 365.41 μg/dm^2, respectively. These amounts were higher than the acceptable protein amount in medical products. In future work, deproteinized rubber should be used in the study.

KEY WORDS: Transdermal patches; Natural rubber; Drug release; Lidocaine

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Hasleena Boontawee

CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF ABBREVIATIONS

ADRs	adverse drug reactions
ANOVA	analysis of variance
ASTM	american Society for Testing and Materials
ATR-FTIR	attenuated total reflection fourier transform infrared
BCA	bicinchoninic acid solution
BSA	bovine serum albumin
cm	centrimeter (s)
cm^2	square centimeter (s)
CNRL	concentrate natural rubber latex
CNS	central nervous system
cont.	continued
CV	constant viscosity
$^{\circ}C$	degree Celsius
dm^2	square decimeter (s)
DNR	dry natural rubber
DRC	dry rubber content
EC	ethyl cellulose
e.g.	exempli gratia, for example

LIST OF ABBREVIATIONS (continued)

Eq.	equation
et al.	et alii, and others
etc.	et cetera, and other things
eV	electron volt
EVA	ethyl vinyl acetate
Fig.	figure
FTIR	fourier transform infrared
g	gram (s)
h	hour (s)
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
i.e.	id est, that is
IPB	isotonic phosphate buffer
keV	kilo electron volt
kg	kilogram (s)
kV	kilovolt
l	liter (s)

LIST OF ABBREVIATIONS (continued)

µg	microgram (s)
µl	microliter (s)
µm	micrometer (s)
M	molar
mA	milliampere
max	maximum
mg	milligram (s)
min	minimum
ml	milliliter (s)
mm	millimeter (s)
mM	millimolar
MW	molecular weight
N	newton
nm	nanometer (s)
No.	number
%	percent
PCP-Cys	polycarbophil-cysteine
pH	the negative logarithm of the hydrogen ion concentration

LIST OF ABBREVIATIONS (continued)

phr	part per hundred of dry rubber
PMMA	polymethyl methacrylate
PSA	pressure sensitive adhesive
PVA	polyvinyl alcohol
PVP	polyvinylpyrrolidone
rpm	revolutions per minute
R^2	coefficient of determination
SDS	sodium dodecyl sulfate solution
S.D.	standard deviation
SEM	scanning electron microscope
STR	standard thai rubber
TDDS	transdermal drug delivery systems
US FDA	united States Food and Drug Administration
UV	ultraviolet
v/v	volume by volumn
w/w	weight by weight
XRD	x-ray diffraction

CHAPTER 1

INTRODUCTION

A transdermal patch or skin patch is a medicated adhesive patch placed on the surface of the skin to deliver a specific dose of medication into lower layers of the skin and the bloodstream. The advantages of transdermal drug delivery systems (TDDS) include avoiding gastro-intestinal drug absorption difficulties, substituting for oral administration of medication, avoiding first-pass effect, avoiding the inconveniences of parenteral therapy and providing capacity to terminate drug effect rapidly (Ansel et al., 1995). Generally, they are prepared from synthetic polymers such as polyvinyl alcohol (PVA) and Eudragit® (Padula et al., 2007; Schoeder et al., 2007) which mostly are not manufactured in Thailand.

Natural rubber latex is the mucous liquid obtained from *Hevea brasiliensis* or Para rubber tree, the plant which can be plentifully found in the Southeast Asia, especially in the south and east of Thailand. It is a milky colloidal suspension of elastic hydrocarbon polymer. The main composition of rubber latex, cis-1,4-polyisoprene, can form film under suitable formulation (Steward, 1998). Fresh natural rubber latex contains about 35% dry rubber content (DRC) which is not convenient for using due to its bulky. Commercially, concentrate natural rubber latex (CNRL) having 60% DRC is prepared by centrifugation. CNRL can be classified into 2 types according to the type of preservative used, i.e. high ammonia rubber latex (preserved with only

ammonia in the concentration of not lower than 0.60 %w/w) and low ammonia rubber latex (preserved with lower than 0.29 %w/w ammonia, zinc and tetramethylthiuram disulfide). In addition, commercial rubber may be prepared as dry natural rubber (DNR) by coagulation of natural rubber latex with acidic substance and then dry at 100-110°C for 4 h. DNR can be classified into several types depending on manufacturing methods, e.g. crepe rubber, air dried sheet, ribbed smoked sheet, block rubber, and constant viscosity rubber (Robert, 1988).

Most natural rubbers, over one-third of the total in the world, have been produced in Thailand. The 90% of them have been exported as CNRL and DNR. Only 10 percent have been used as a raw material for some kinds of products, such as tyres, gloves and condoms (Natural Rubber Products Technology Transfer Center, 2008). Since finished products can provide higher income than raw material, the research about development of products derived from natural rubber should be done for increasing the value of natural rubber. In present, the natural rubber was less or never studied about applying in pharmaceutics or drug delivery system.

The aims of this study were to formulate transdermal patches using CNRL and DNR as main components, to study physicochemical characteristic and *in vitro* drug release from the obtained patches, and to evaluate the stability of the patches. In this study, CNRL (high ammonia type) and DNR (block rubber STR-5L type) were used as the representatives of liquid and solid natural rubber types, respectively. Additionally, lidocaine, a local anesthetic drug, was used as the model drug.

CHAPTER 2

LITERATURE REVIEWS

2.1 Transdermal drug delivery systems

Transdermal drug delivery systems, also known as "patches", are the dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin, and into the systemic circulation (Ansel et al., 1995). Several TDDS containing drugs such as clonidine, estradiol, fentanyl, nicotine, nitroglycerin, oxybutynin and scopolamine are commercially available (Wokovich et al., 2006). Recently, Lidoderm® (5% lidocaine transdermal patch) has been sold in United states (Endo Pharmaceuticals, 2006)

The development of TDDS is a multidisciplinary activity that encompasses fundamental feasibility studies starting from the selection of a drug molecule to the demonstration of sufficient drug flux in an *ex vivo* and/or *in vivo* model. The fabrication of a drug delivery system that meets all the stringent needs that are specific to the drug molecule (physicochemical and stability factors), the patient (comfort and cosmetic appeal), the manufacturer (scale-up and manufacturability), and most important, the economy (Kandavilli et al., 2002).

2.1.1 Advantages and disadvantages of TDDS

2.1.1.1 Advantages of TDDS (Ansel et al., 1995):

— Avoids gastrointestinal drug absorption difficulties caused by gastrointestinal pH, enzymatic activity, drug interactions with food, drink, or other orally administered drugs.

— Substitutes for oral administration of medication when that route is unsuitable, as in instances of vomiting and/or diarrhea.

— Avoids first-pass effect, that is, the initial pass of a drug substance though the systemic and portal circulation following gastrointestinal absorption (thereby possibly avoiding the drug's deactivation by digestive liver enzymes).

— Avoids the risks and inconveniences of parenteral therapy, the variable absorption and metabolism associated with oral therapy.

— Provides the capacity for multiday therapy with a single application. Thereby improving patient compliance over use of other dosage forms requiring more frequent dose administration.

— Extends the activity of drugs having short half-lives though the reservoir of drug present in the therapeutic delivery system. Its controlled release characteristics.

— Provides capacity to terminate drug effect rapidly (if clinically desired) by re-moval of drug application from the surface of the skin.

— Provides ease of rapid identification of the medication in emergencies (e.g., nonresponsive, unconscious, or comatose patient).

1.1.2 Disadvantages of TDDS (Ansel et al., 1995):

— The transdermal route of administration is unsuitable for drugs that irritate or sensitize the skin.

— Only relatively potent drugs are suitable candidates for transdermal delivery due to the natural limits of drug entry imposed by the skin's impermeability.

— Technical difficulties are associated with the adhesion of the systems to different skin types and under various environmental conditions as well as the development of rate-controlling drug delivery features.

2.1.2 Types of TDDS

Commercially available TDDS can be categorized as (i) reservoir systems, (ii) matrix systems without a rate-controlling membrane and (iii) matrix systems with a rate-controlling membrane as illustrated in Fig. 1. Reservoir systems consist of three major components: the drug reservoir, the rate-controlling membrane and the adhesive. Typically, the drug reservoir contains the drug and excipients. The drug permeates through the membrane and the adhesive to reach the skin. The adhesive component of the TDDS responsible for skin adhesion may either completely cover the drug release area (continuous adhesive) or may form a

perimeter around a non-adhering drug release surface (peripheral adhesive). In a matrix systems, the drug is in the adhesive. The adhesive performs the roles of formulation foundation and skin adhesions. In matrix systems with a rate-controlling membrane, the systems contain a membrane between the drug-in-adhesive layers (Wokovich et al., 2006).

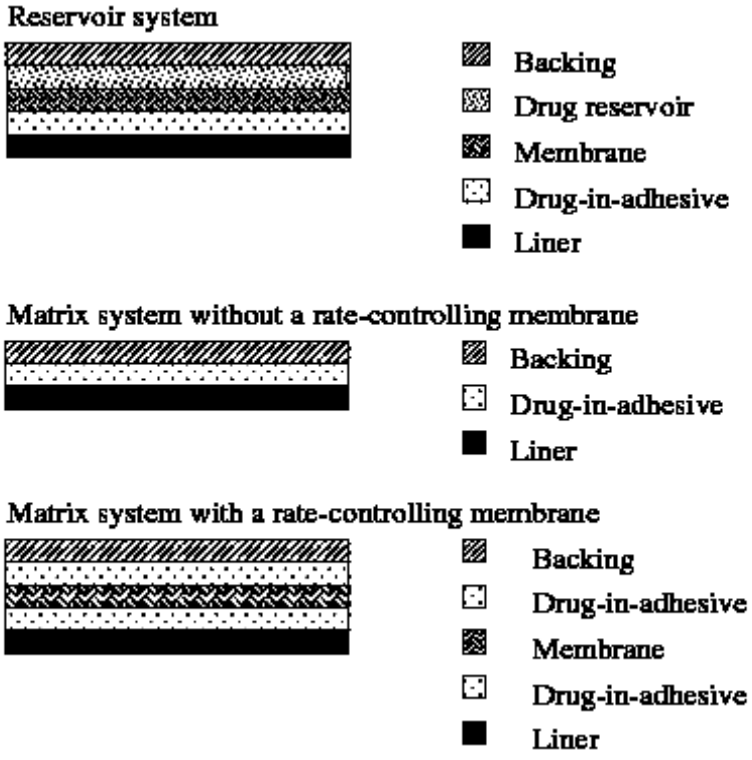

Fig. 1. Types of TDDS (Wokovich et al., 2006).

2.2 Polymers used in transdermal patches

Polymers are the backbone of TDDS. Advances in the field of polymer science have paved the way for TDDS designs that have considerable flexibility. An impressive amount of technical know-how has been gained in this area of research.

TDDS technology represents one of the most rapidly advancing areas of novel drug delivery. This growth is catalyzed by developments in the field of polymer science. Polymers are used in TDDS in various ways, including as :

- matrix formers
- rate-controlling membranes
- pressure sensitive adhesives (PSA)
- backing layers
- release liners.

2.2.1 Matrix formers

Polymer selection and design must be considered when striving to meet the diverse criteria for the fabrication of effective TDDS. The main challenge is in the design of a polymer matrix and the optimization of the drug loaded matrix not only in terms of release

properties, but also with respect to its adhesion–cohesion balance, physicochemical properties, compatibility and stability with other components of the system as well as with skin (Wolff, 2000). A monolithic solid-state design often is preferred for passive TDDS because of manufacturing considerations and cosmetic appeal.

2.2.1.1 Cross-linked poly(ethylene glycol) (PEG) networks

Biocompatibility of PEGs makes them as the polymers of choice for numerous biomedical applications. Proteins can be delivered by PEGs cross-linked with tris(6-isocyanatohexyl) isocyanurate by means of a urethane–allophanate bond to obtain polymer networks capable of swelling in phosphate-buffered saline or ethanol and forming gels. These systems have been shown to release the solutes in a biphasic manner (Bromberg, 1996).

2.2.1.2 Acrylic-acid matrices

Acrylic-acid matrices with plasticizers have been used to make drug–polymer matrix films for TDDS. Some of the polymers that have been reported are Eudragit® RL PM, Eudragit® S-100, Eudragit® RS PM, and Eudragit® E-100 (Röhm America, Piscataway, NJ). Eudragit® NE-40D (a copolymer of ethyl acrylate and methyl methacrylate), a non adhesive hydrophobic polymer, also has been used as a matrix former. The release rates of drugs from

these matrix systems are more closely described by the square-root-of-time model (Costa et al., 1997).

2.2.1.3 Ethyl cellulose (EC) and polyvinylpyrrolidone (PVP)

EC and PVP matrix films with 30% dibutyl phthalate as a plasticizer have been fabricated to deliver diltiazem hydrochloride and indomethacin. The addition of hydrophilic components such as PVP to an insoluble film former such as EC tends to enhance its release-rate constants. This outcome can be attributed to the leaching of the soluble component, which leads to the formation of pores and thus a decrease in the mean diffusion path length of drug molecules to release into the dissolution medium. Substances such as PVP act as antinucleating agents that retard the crystallization of a drug. Thus they play a significant role in improving the solubility of a drug in the matrix by sustaining the drug in an amorphous form so that it undergoes rapid solubilization by penetration of the dissolution medium (Ramarao and Diwan, 1998).

2.2.1.4 Hydroxypropyl methylcellulose (HPMC)

HPMC, a hydrophilic swellable polymer widely used in oral controlled drug delivery, also has been explored as a matrix former in the design of patches of propranolol hydrochloride. HPMC has been shown to yield clear films because of the adequate solubility of

the drug in the polymer. Matrices of HPMC without rate-controlling membranes exhibited a burst effect during dissolution testing because the polymer was hydrated easily and swelled, leading to the fast release of the drug (Guyot and Fawaz, 2000).

2.2.1.5 Organogels

Some nonionic surfactants such as sorbitan monostearate, lecithin, and Tween® tend to associate into reverse micelles (Florence and Attwood, 1982). These surfactants in an organic solvent, upon the addition of water, undergo association reorientation to form a gel. These organogels can be used as a matrix for the TDDS with greater influx. Walde proposed a reverse micelle-based microemulsion of soy lecithin in isooctane gelled with water as a vehicle for propranolol TDDS. The transdermal flux of propranolol from this organogel increased 10-fold over a vehicle composed of petrolatum (Walde, 1990). Willimann et al. also described that organogels obtained when small amounts of water were added to a solution of lecithin in organic solvents, used as matrices for the transdermal transport of drugs. The gels obtained in this manner are isotropic and thermoreversible (liquefy at >40 °C) and can solubilize lipophilic, hydrophilic, and amphoteric substances, including enzymes. They are biocompatible and are stable for a long time. Organogels can cause slight disorganization of the skin, an outcome that is attributable to the organic solvent that is used to make the gel. Thus, organogels can enhance the permeation of various substances (Willimann et al., 1992). Pluronic lecithin organogels also have been used as

TDDS because both hydrophobic and hydrophilic drugs can be incorporated into them. Oil-soluble drugs are miscible with the lecithin phase, and water-soluble drugs are miscible with the aqueous phase (Kandavilli et al., 2002).

The effect of a combination of the adhesive polymethyl methacrylate (PMMA) with cellulose ethers or PVP was evaluated by a peel adhesion test. The addition of PVP resulted in a 40-fold improved creep compliance. The significant increase of the matrix cohesion was due to interactions between the amide groups of PVP and the carboxylic acid groups of PMMA. The adhesive properties of the matrix cohesion could be modified by changing the component ratios of PVP and PMMA (Minghetti et al., 2003).

Transdermal patches are also promising candidates for veterinary use. A formulation with controlled release based on silicone as carrier for ivermectin, a veterinary drug, was designed. With this formulation, a linear release of ivermectin was obtained. Additional PEG 4000 accelerated the ivermectin release (Kandavilli et al., 2002).

Percutaneous absorption studies with of patches containing verapamil hydrochloride with Eudragit® RL, Eudragit® RS100, hydroxypropyl methylcellulose (HPMC) and ethyl cellulose (EC) of varying degrees of hydrophilicity and hydrophobicity were carried out. The pharmacokinetic parameters calculated from blood levels of the drug revealed a profile typical of a sustained release formulation with the ability to maintain adequate plasma levels for the patch containing Eudragit® RL and HPMC in the ratio of 8:2 (Kusum et al., 2003).

In an *in vitro* study, polyurethane matrix containing terpenes was tested. Release of the terpenes directly to the receptor fluid as well as through isolated human epidermis and dermis was studied. The permeation enhancement of terpenes was only limited by the stratum corneum (Cal et al., 2001). Polycarbophil-cysteine (PCP-Cys) conjugate, a partly thiolated polymer, was tested as a matrix for TDDS with the advantage of good cohesiveness within the polymer film due to its the excellent adhesiveness on skin, no additional adhesive was required. In studies with porcine skin, higher progesterone permeation could be demonstrated from PCP-Cys compared to PVP/HPMC and PVP/PVA formulations. The hydrophilic thiolated polymers might be promising candidates as carriers for TDDS (Valenta et al., 2001). In addition to hydrophilic-based, lipophilic-based polymers e.g. silicones can also be used in transdermal patches. One interesting study employed such a silicone polymer incorporated with coumarin, different amounts of excipients were added and evaluated *in vivo*. From all tested formulations, the area under the blood level-time curve of the propylene glycol containing system was twice of that from the device without propylene glycol (Ritschel and Nayak, 1987). Glycerol, ethylene glycol or PEG 400 were also able to influence the properties of silicone matrix significantly (Wagner, 1998).

2.2.2 Rate-controlling membranes

Reservoir-type TDDS contain an inert membrane enclosing an active agent that diffuses through the membrane at a finite, controllable rate. The release rate–controlling membrane can be nonporous so that the drug is released by diffusion directly through the material, or the material may contain fluid-filled micropores — in which case the drug may additionally diffuse through the fluid, thus filling the pores. In the case of nonporous membranes, the rate of passage of drug molecules depends on the solubility of the drug in the membrane and the membrane thickness. Hence, the choice of membrane material must conform to the type of drug being used. By varying the composition and thickness of the membrane, the dosage rate per area of the device can be controlled (Kandavilli et al., 2002).

2.2.2.1 Ethyl vinyl acetate (EVA)

EVA frequently is used to prepare rate-controlling membranes in transdermal delivery systems because it allows the membrane permeability to be altered by adjusting the vinyl acetate content of the polymer. For example, when ethylene is copolymerized with vinyl acetate, which is not isomorphous with ethylene, the degree of crystallinity and the crystalline melting point decreases and amorphousness increases. As the solutes permeate easily through the amorphous regions, the permeability increases. The copolymerization also results in an increase

in polarity. Hence, an increase in the vinyl acetate content of a copolymer leads to an increase in solubility and thus an increase in the diffusivity of polar compounds in the polymers. However, at vinyl acetate levels >60% by weight, the glass-transition temperature, Tg, of polymer increases from ~ -25 °C to ~ -35 °C. An increase in Tg reflects a decrease in the polymer-chain mobility and hence the solute diffusivity. The effect of these structural changes on membrane permeability is shown in the permeation of camphor through a series of poly(ethylene vinyl acetate) copolymers, which has exhibited a maximum of limiting flux at ~60% vinyl acetate content (Gale and Spitze, 1981).

2.2.2.2 Silicone rubber

The silicone rubber has been used in many controlled-release devices. These polymers have an outstanding combination of biocompatibility, ease of fabrication, and high permeability to many important classes of drugs, particularly steroids. The high permeability of this material is attributed to the free rotation around the silicone rubber backbone, which leads to very low microscopic viscosities within the polymer (Kandavilli et al., 2002).

2.2.2.3 Polyurethane

Polyurethane is the general term used for a polymer derived from condensation of polyisocyanates and polyols having an intramolecular urethane bond or carbamate ester bonds (—NHCOO—). The polyurethanes synthesized from polyether polyol are termed polyether urethanes, and those synthesized from polyester polyol are termed polyester urethanes. Although most polyurethanes presently used are of the polyether type because of their high resistance to hydrolysis, polyester polyurethanes recently have become the focus of attention because of their biodegradability. These polyester or polyether urethanes are rubbery and relatively permeable. The hydrophilic–hydrophobic ratio in these polymers can be balanced to get the optimum permeability properties. Polyurethane membranes are suitable especially for hydrophilic polar compounds having low permeability through hydrophobic polymers such as silicone rubber or EVA membranes (Kandavilli et al., 2002).

2.2.3 Pressure sensitive adhesive (PSA)

An approach to explain the adhesive properties of PSA is based on the belief that the PSA will adhere to a substrate, because of interatomic and intermolecular attractive forces (Minghetti et al., 1999). To obtain this degree of contact, the material must be able to deform under slight pressure, giving rise to the term “pressure sensitive”. Adhesion involves a liquid-like flow resulting in wetting of the skin surface upon the application of pressure, and when pressure

is removed, the adhesive sets in that state. PSA wets and spreads onto skin when it surface energy is less than that of skin (Repka and McGinity, 2001). After the initial adhesion, the PSA/skin bond can be built by stronger interactions (e.g. hydrogen bonding), which depends on skin characteristics and other parameters. Widely used PSA polymers in TDDS are polyisobutylene-based adhesives, acrylics and silicone-based PSA. The PSA can be used to affix the TDDS to the skin and/or as a carrier for the drug. The PSA can be located around the edge of the TDDS or be laminated as a continuous adhesive layer on the TDDS surface (Spencer et al., 1990). The PSA should be compatible with the drug and excipients since their presence can modify the mechanical characteristics of the PSA and the drug delivery rate. When selecting PSA for TDDS, several properties need to be evaluated that may influence adhesive performance i.e. solubility of drug and excipients, effect of dissolved/ dispersed additives on adhesion, long-term stability of dissolved/dispersed components, compatibility of the backing layer and release liner with these components, application period and effect of moisture (Muny, 1999). An ideal requirement of TDDS adhesive is that it should be nonirritating and nonsensitizing to the skin, adhere to varying skin types, adhere strongly to the skin for the prescribed application period, be easily removable without trauma, leave no residue on the skin upon removal and be comfortable to wear (Minghetti et al., 2003).

2.2.4 Backing layer

When designing a backing layer, the developer must give chemical resistance of the material foremost importance. Excipient compatibility also must be seriously considered because the prolonged contact between the backing layer and the excipients may cause the additives to leach out of the backing layer or may lead to diffusion of excipients, drug, or penetration enhancer through the layer. However, an over emphasis on the chemical resistance often may lead to stiffness and high occlusivity to moisture vapor and air, causing patches to lift and possibly irritate the skin during long-term wear. The most comfortable backing may be the one that exhibits the lowest modulus or high flexibility, good oxygen transmission, and a high moisture-vapor transmission rate (Godbey, 1996). In a novel modification to the conventional design, a patch was fabricated in which the backing itself acted as a reservoir for the drug. The upper internal portion of the drug reservoir infiltrated the porous backing and became solidified therein after being applied so that the reservoir and the backing were unified. This modification enabled the backing itself to act as a storage location for the medication-containing reservoir (Rolf et al., 2000).

2.2.5 Release liner

During storage, the patch is covered by a protective liner that is removed and discharged immediately before the application of the patch to the skin. It is therefore regarded as a part of the primary packaging material rather than a part of the dosage form delivering the active principle (Santoro et al., 2000). However, because the liner is in intimate contact with the delivery system, it should comply with specific requirements regarding the chemical inertness and permeation to the drug, penetration enhancer, and water. In case cross-linking is induced between the adhesive and the release liner, the force required to remove the liner will be unacceptably high (Pfister and Hsieh, 1990). 3M, for example, manufactures release liners made of fluoro polymers (Scotchpak® 1022 and Scotchpak® 9742, 3M Drug Delivery Systems, St. Paul, MN).

2.3 Characterization of patches

2.3.1 Scanning electron microscope (SEM)

SEM is a type of electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electrons with the sample's surface. The type of signals gathered in a SEM varies and can include secondary electrons, characteristic x-rays, and back scattered

electrons. These signals come not only from the primary beam impinging upon the sample, but from other interactions within the sample near the surface. The SEM is capable of producing high-resolution images of a sample surface in its primary use mode, secondary electron imaging. Due to the manner in which this image is created, SEM images have great depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. This great depth of field and the wide range of magnifications are the most familiar imaging mode for specimens in the SEM. Characteristic x-rays are emitted when the primary beam causes the ejection of inner shell electrons from the sample and are used to tell the elemental composition of the sample. The back-scattered electrons emitted from the sample may be used alone to form an image or in conjunction with the characteristic x-rays as atomic number contrast clues to the elemental composition of the sample (Danilatos, 1988).

In a typical SEM, electrons are emitted from a tungsten or lanthanum hexaboride (LaB_6) cathode and are accelerated towards an anode, alternatively, electrons can be emitted via field emission . Tungsten is used because it has the highest melting point and lowest vapour pressure of all metals, thereby allowing it to be heated for electron emission. The electron beam, which typically has an energy ranging from a few hundred eV to 100 keV, is focused by one or two condenser lenses into a beam with a very fine focal spot sized 0.4 nm to 5 nm. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron optical column, typically in the objective lens, which deflect the beam horizontally and vertically so that it scans in a raster fashion over a rectangular area of the sample surface. When the primary electron beam

interacts with the sample, the electrons lose energy by repeated scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 µm into the surface. The size of the interaction volume depends on the electrons' landing energy, the atomic number of the specimen and the specimen's density. The energy exchange between the electron beam and the sample results in the emission of electrons and electromagnetic radiation, which can be detected to produce an image as shown in Fig. 2. (Danilatos, 1988).

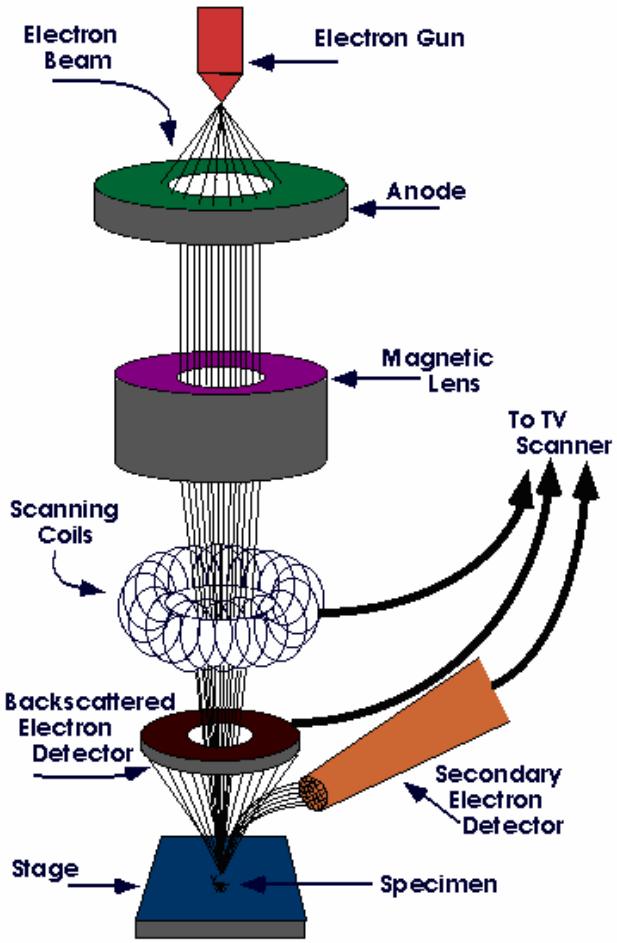

Fig. 2. Schematic diagram of SEM (Radiological and Environmental Management, 2008)

2.3.2 X-ray diffraction (XRD)

XRD is a tool for the investigation of the structure of matter. X -rays are scattered by interaction with the electrons of the atoms in the material being investigated. The technique began when Von Laue discovered that crystals diffracted x-rays in 1912. Since then it has been applied to chemical analysis, stress and strain measurement, the study of phase equilibria, measurement of particle size, as well as crystal structure (Bowen and Tanner, 1998).

The investigation of thin film diffraction refers not to a specific technique but rather a collection of XRD techniques used to characterize thin film samples grown on substrates. XRD have important technological applications in microelectronic and optoelectronic devices, where high quality epitaxial films are critical for device performance. Thin film diffraction methods are used as important process development and control tools, as hard x-rays can penetrate through the epitaxial layers and measure the properties of both the film and the substrate. There are several special considerations for using XRD to characterize thin film samples. First, reflection geometry is used for these measurements as the substrates are generally too thick for transmission. Second, high angular resolution is required because the peaks from semiconductor materials are sharp due to very low defect densities in the material. Consequently, multiple bounce crystal monochomators are used to provide a highly collimated x-ray beam for these measurements as shown in Fig. 3. (Bowen et al., 1998).

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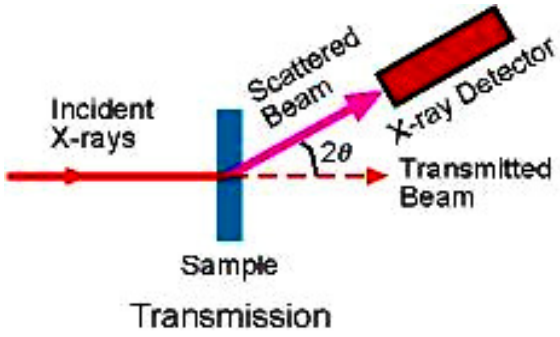


Fig. 3. Schematic diagram of XRD (Materials Research Laboratory, 2001).

2.3.3 Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy

Transmission infrared spectroscopy, either dispersive or FTIR, has been widely used in characterizing substance. Most studies have been done using the KBr pressed-pellet method for solid substance. If substances cannot be crushed with KBr, the technique of ATR can be used instead. ATR technique involves the collection of radiation reflected from the interface between sample and a prism, in which the evanescent wave penetrated from the prism into the sample is absorbed by substances in the sample as shown in Fig. 4 (Tanaka et al., 2001). ATR-FTIR was principally applied to characterize of interaction between additive substance with natural rubber (Sanguansap et al., 2001). This technique is also known as a surface analysis technique. Pliable solids such as rubber and plastics are easily studied via internal reflection spectroscopy. This method works well with samples that are too opaque or too thick for standard

transmission methods. In this experiment, the ATR-FTIR spectra of several polymer samples were obtained to demonstrate the technique (Phan, 2007).

FTIR is the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample as shown in Fig. 5. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. An infrared spectrum represents a fingerprint, of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present (Ellis and Goodacre, 2006).

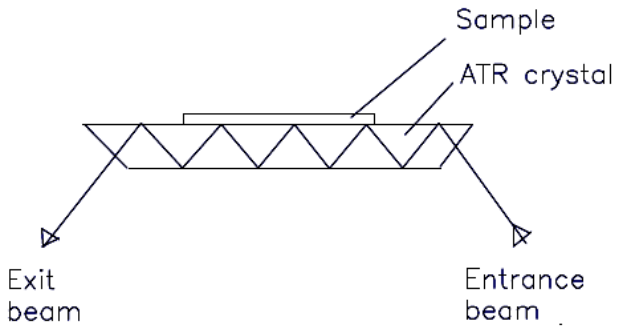


Fig. 4. Schematic representation of the method of ATR-FTIR (Phan, 2007).

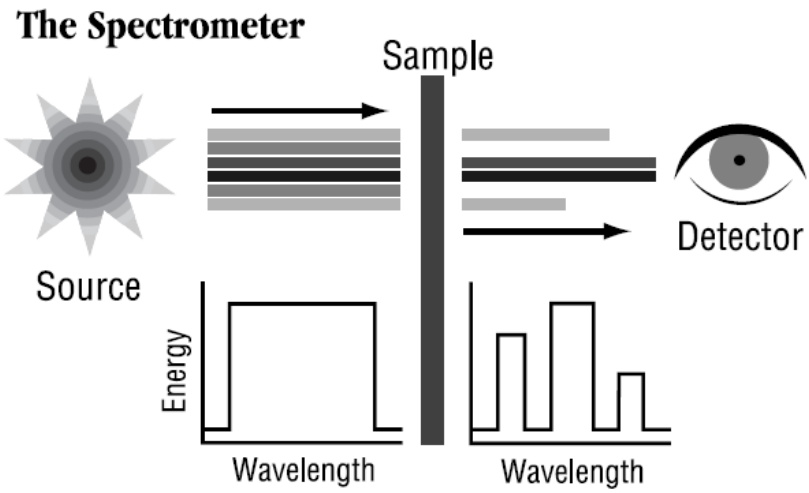


Fig. 5. Schematic diagram of FTIR (Ellis and Goodacre, 2006).

2.4 *In vitro* release studies and *in vivo* skin permeation studies.

2.4.1 *In vitro* methods

Significant amounts of studies are conducted using various *in vitro* techniques. Before these are presented, a distinction must be made between *in vitro* studies used to assess dissolution or release from a transdermal device, and those used to assess absorption across the skin. The former is very similar to techniques used in assessing dissolution of oral drug tablets and measuring the rate of drug release from the formulation. Unlike compendia oral dissolution methods, there are standards developed for transdermal systems such as those described in the US Pharmacopeia (The United States Pharmacopeial Convention, 2007). However, it must be

stressed that they do not assess the absorption of drug across the stratum corneum barrier (Ghosh et al., 1997)

The primary connotation of the term *in vitro* in transdermal studies is the use of skin sections removed from an animal to assess transport in diffusion cell systems. These have been extensively described elsewhere (Kemppainen and Reifenrath, 1990). In these systems, a section of skin is obtained from an animal donor either using a dermatome, which is a slicing apparatus that cuts sections of skin parallel to the surface, to produce a split-thickness preparation or by simply dissection to obtain a full-thickness preparation. This membrane is then mounted in a two chambered diffusion cell apparatus.

Receptor solutions generally consist of saline in pharmaceutical studies assessing hydrophilic drug absorption. Some investigators have added other solvents to the receptor phase to promote solubility of lipophilic penetrates in the perforate. If viability of the skin membranes is desired for cutaneous metabolism studies, an energy source such as dextrose and O_2/CO_2 gas is fed into the perfusate to maintain some degree of metabolism. These systems have a regulated temperature at 32–37°C to mimic skin surface temperature or core-body temperature (Riviere and Papich, 2001).

As can be appreciated, *in vitro* systems are economical to conduct and generate 'clean' data amenable to quantitative analysis since the confounding variability inherent to the *in vivo* setting is absent. If absorption through the stratum corneum is the rate-limiting step in percutaneous absorption, *in vitro* method is appropriate. However, if the drug's pattern of

absorption is dependent upon other biological functions such as blood flow, dermal distribution, etc., then *in vitro* systems may generate misleading results. Another concern is that in full thickness and split-thickness preparations, significant dermis remains that may be a barrier to lipophilic drugs and even serve as an artificial reservoir. To overcome this, some investigators have used techniques that split the epidermal–dermal junction by heat separation or enzyme digestion. However, if the skin being studied has significant hair density, then the resulting epidermal membrane may have holes from where the hair once exited to the surface. Another limitation is that prolonged perfusion may result in membrane degradation due to cellular death. This generally does not occur until after 24 h. However, histological examination of *in vitro* skin sections demonstrates epidermal changes as early as 8 h (Monteiro-Riviere, 1987).

Recent developments in this field have been the use of artificial skin membranes to assess absorption across human skin. Such systems are organ cultures which possess intact stratum corneum but no appendages (Monteiro-Riviere, 1997). Studies conducted to date suggest that permeability through these systems compared to split-thickness human skin may be ten or more fold greater. However, they may be useful to assess the ability of human cells to metabolize applied drugs.

In vitro techniques are amenable to study absorption from transdermal patches. In these configurations, a finite dose flow-through diffusion cell system is used and the patch is sandwiched against the epidermis before mounting in a diffusion cell. Drug flux is then monitored as described above.

The advantages of using *in vitro* experiments include the ability in controlling the experimental conditions and the simplicity when compared with *in vivo* experiments. Sampling and analysis of a relatively clean and small volume of receptor fluid obtained from *in vitro* experiments are much easier than from the chemically complex blood-urine-fecal specimens provided *in vivo* experiments. Additionally, *in vitro* experiments greatly consume lower cost.

The disadvantages of *in vitro* experiments are that the experiments for not precisely replicate the behavior of living subjects such as blood supply and metabolism, and that the *in vitro* results do not provide pharmacokinetic data.

The experimental design of an *in vitro* study requires considerable care since it is easy to produce erroneous results. However, when a well-designed *in vitro* methodology has been established, it is possible to obtain a very high correlation between *in vitro* and *in vivo* data (Gummer,1989; El-Kattan et al., 2000).

2.4.2 *In vivo* methods

Although *in vivo* method is necessary to gain a full insight into the skin permeation process of a drug in a living subject, it is often difficult to achieve in practice. Firstly, the experiments in human volunteers or in animal models have to be studied under ethical constraints and consume high cost. Secondly, this approaches involves analysis of body tissues and fluids, elicitation of biological responses and histological studies (El-Kattan et al., 2000).

2.5 Human Skin

The skin, the largest organ in the human body and function as barrier against external environment, is anatomically divided into stratum corneum (10-15 μm), the living epidermis (100 μm) and the underlying dermis (2,000-3,000 μm). Beneath the dermis lies subcutaneous fatty tissue, connecting to the underlying muscular layer. There are also several skin glands and appendages dispersed throughout the skin, i.e. hair follicles, sebaceous glands, eccrine sweat glands and apocrine sweat glands. Fig. 6 illustrates the anatomical structure of human skin. The outermost layer of the skin is the stratum corneum or horny layer, the main element of the skin barrier function. The stratum corneum is a multicellular, metabolically inactive tissue composed of flattened keratinized cells lipids surrounding the keratinized cells. On a dry weight basis, it consists of 75-80% protein, 5-15% complex lipoidal mixture and 5-10% unidentified material. Its structure has been presented in the brick and mortar model, with the corneocytes (dead keratin filled cells) forming the bricks and the intercellular lipid as the mortar as shown in Fig. 7. The intracellular space of completely differentiated horny cell is fully packed with semicrystalline α-keratin and its amorphous keratin counterpart or β-keratin while the intercellular space is composed of lamellar structures with aqueous and lipid layer (Elias et al., 1981; Flynn, 1995; Higaki et al., 2003).

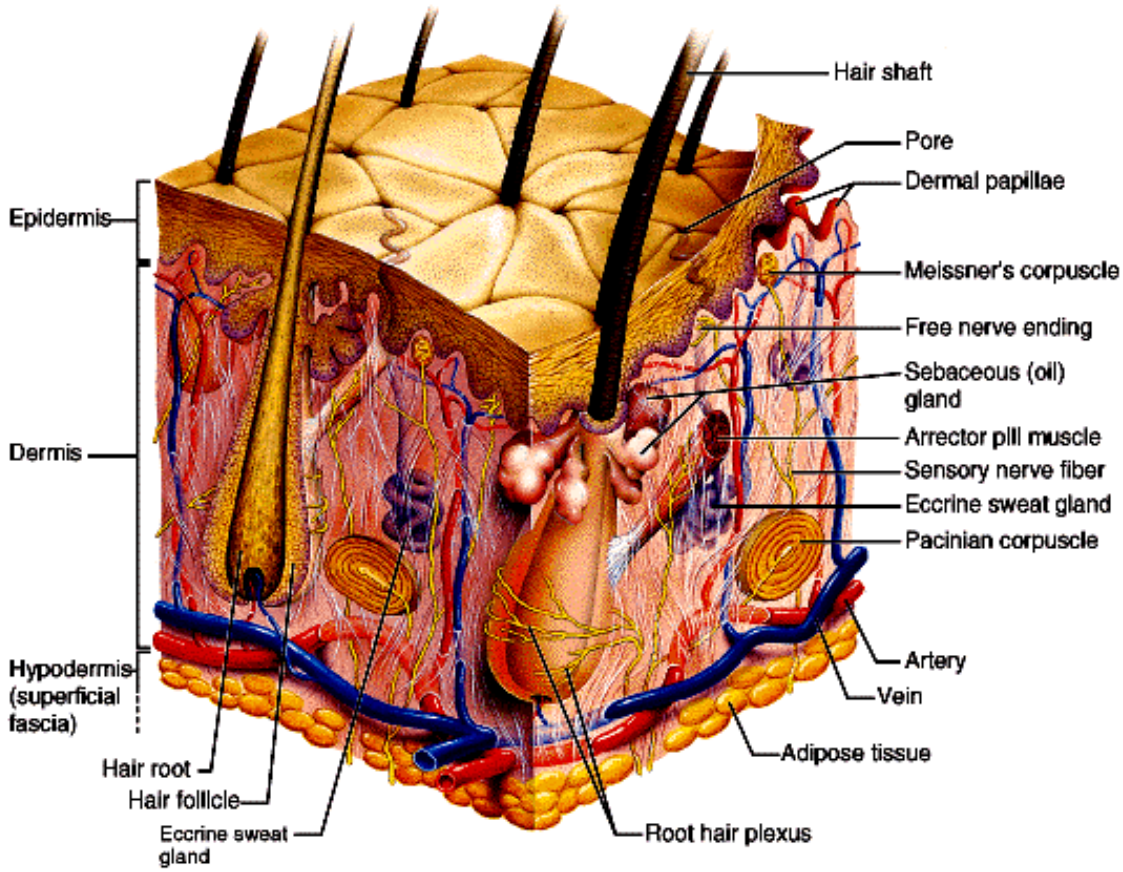


Fig. 6. The anatomical structure of human skin (Addison Wesley Longman Inc., 2002)

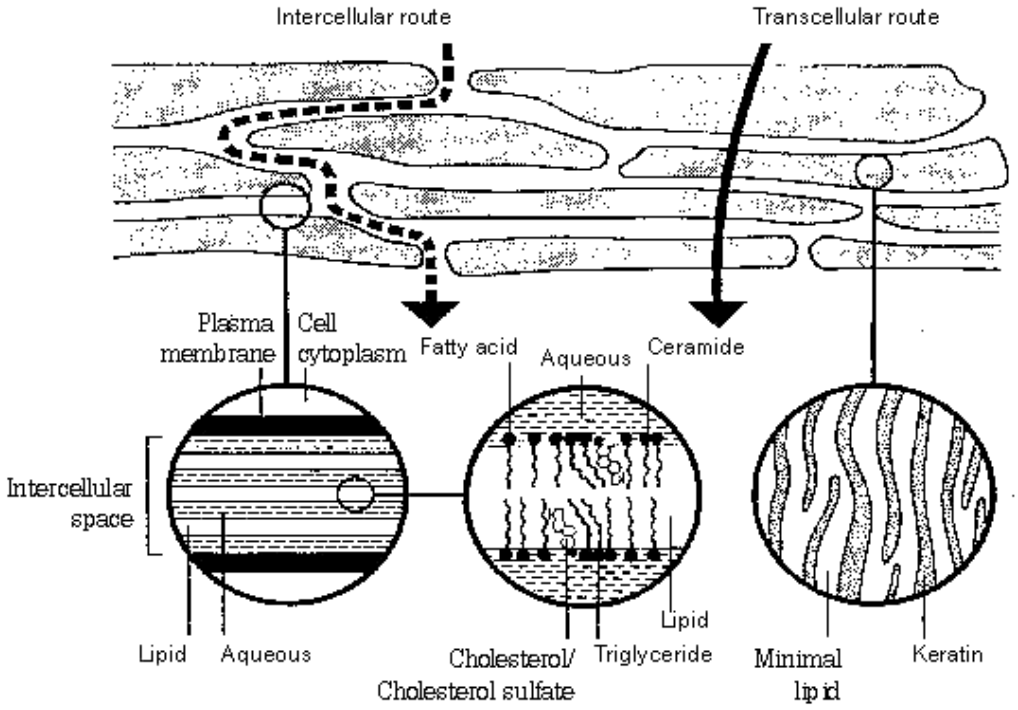


Fig. 7. Brick and motar model of stratum corneum (Research and Development Institute Government Pharmaceutical Organization, 1997)

Possible macro routes for drug penetration across human skin are through the sweat glands, via the intact horney layer (transepidermal route) or through the hair follicles with their associated sebaceous glands. Since the fractional area available for absorption of the sweat glands and the hair follicles with their associated sebaceous glands is small (about 0.1% of the total skin surface area), the transepidermal route is believed to be the most dominant route. For penetrants whose main portal of entry to the viable tissues is via the horny layer, the two micro routes of access are the intercellular (via the lipid domain between the corneocytes) and the

transcellular (across the corneocytes and lipid matrix) paths based on brick-mortar structure of the stratum corneum, as exhibited in Fig. 7 (Barry, 1991; Barry, 2001; Hadgraft, 2001).

2.6 Rubber

Rubber is an elastomer, a polymer having the ability to regain its original shape after being deformed. Rubber is also tough and resistant to weathering and chemical attack. Elastomers can be naturally occurring polymers, such as natural rubber, or they can be synthetically produced substances, such as butyl rubber, thiokol, or neoprene. For a substance to be a useful elastomer, it must possess a high molecular weight and a flexible polymer chain (Yeang et al., 2002).

2.6.1 Natural rubber

Natural rubber is one of nature's unique materials, almost all of it come from one biological source: Para rubber tree (*Hevea brasiliensis*). Crude rubber is primarily hydrocarbon in nature. Its empirical (simplest) formula C_5H_8 was named isoprene. The structure is shown in Fig. 8. In addition, rubber latex composes of 1 to 1.5% protein and 1 to 4% acetone-soluble materials (resins, fatty acids, and sterols) as summarized in Table 1. The molecular weights of rubber molecules range from 50,000 to 3,000,000 g/mol. The 60% of the molecules have molecular

weights of greater than 1,300,000 g/mol. The repeating unit in natural rubber has the cis configuration (with chain extensions on the same side of the ethylene double bond), which is essential for elasticity. If the configuration is trans (with chain extensions on the opposite sides of the ethylene double bond), the polymer is either a hard plastic. Since there are few cross-links in the chains of rubber molecules, natural rubber is thermoplastic. It becomes soft and sticky in summer, hard and brittle in winter. The rubber mixed with sulphur and litharge (lead oxide; PbO) on a hot stove. The operation affected rubber to be heavily cross-linked, and then converted to insoluble and infusible thermosetting polymer or "thermoset" (Kauffman and Seymour, 1990).

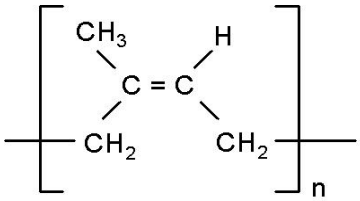


Fig. 8. Cis-1,4 polyisoprene structure (Kauffman and Seymour, 1990)

Table 1. Composition of fresh natural rubber latex (Kauffman and Seymour, 1990)

Constituent	% Composition
Rubber particles (cis-1,4-polyisoprene)	25 - 45
Protein	1 - 1.5
Water	55 - 65
Sterol glycosides	0.1 - 0.5
Resins	1 - 2.5
Ash	0.5 - 1.0
Sugars	1.0 - 2.0

The commercial rubber is supplied either as concentrated latex or in dry rubber form. The fresh latex harvested from the tree is concentrated by centrifuging, removing some of the water and much of the proteins, and is preserved with ammonia. Products such as gloves and condoms are produced by dipping a porcelain or glass mould into the latex. Usually, the latex is prevulcanised by mixing with sulphur and accelerators. In the next steps, the coated former is dipped into a coagulant (typically calcium nitrate) to gel the latex, and then heated in a continuous oven, which dries and vulcanizes the latex films. Most harvested rubber latex is coagulated and converted into dry rubber. Usually small holder rubber has been coagulated prior to sale, already in the tapping cup. The coagulated rubber is milled and washed to remove contaminants introduced by collection and transportation (Kauffman and Seymour, 1990).

Block rubber (Standard Thai Rubber; STR) is one type of dry rubber, prepared by a process of rubber developing from natural to technical specified rubber. It has been introduced in Thailand since 1968. STR is available in five grades i.e. STR-5L, STR5, STR10, STR20 and STR20CV. Only two grades are volume traded in rubber industry i.e. STR-5L and STR20. STR-5L is a light color rubber, processing from quality latex and converting rubber into crumbs and drying rubber at higher temperature of 100°C. The uniform packing is 33.33 kg in pressed bale. STR20 is a large volume grade of technical specified natural rubber. It is processed from field coagulum (cuplump) and mixed with rubber sheet or processed from cuplump only (Kauffman and Seymour, 1990). Therefore, STR-5L had impurity less than STR20 affect to STR-5L rubber was investigated.

2.6.2 Factors affecting on stability of rubber (Gesamtverband der Deutschen Versicherungswirtschaft, 2008)

2.6.2.1 Temperature

Temperatures greater than 30°C and exposure to sunlight, even for periods of a few hours, result in activation of the rot-causing bacteria, especially in conjunction with moisture and proteins. In addition, oxidation and the associated molecular breakdown are encouraged, and the rubber develops soft patches, stickiness and becomes deformed (oxidized rubber).

The climate environment of harvesting and travel affects to the properties of rubber. The optimum travel temperature for rubber is 20°C. A temperature of 25°C should be maintained virtually constantly over a long period. Rubber starts to melt at approximately 180°C. The lower limit is 5 - 6°C, as there is a risk of hardening as a result of crystallization. This can cause difficulties unloading the container or means of transport because of the jammed bales tear. Concentrated latex should be protected against extreme temperatures during transport and storage. If it is exposed to direct sunlight in tropical and subtropical climates, the high temperatures can result in a drop in quality. At temperatures below than 4°C, the concentrated latex tends to coagulate, which is an irreversible process. This phenomenon results damage and additional costs.

2.6.2.2 Humidity/Moisture

Natural rubber is not hygroscopic. The water content of rubber is 1.08%. Moisture caused by rain, seawater or condensation water must be strictly avoided, as it can lead to rot, mold, mildew stains and discoloration. Rubber, which has become wet and absorbed too much moisture, is termed bleached rubber**.**

2.6.2.3 Microbial stability

Natural rubber latex can be contaminated with bacteria while dry rubber can be contaminated with mold. This affects to the stability of rubber.

2.6.2.4 Self-heating/Spontaneous combustion

Natural rubber is readily combustible due to its high hydrocarbon content. When natural rubber combusts, it generates considerable heat (temperatures up to 1,200°C) and smoke.

Burning rubber produces sputtering, burning drops of resin, which can cause the fire to spread. If there is a risk of fire, the firefighting personnel should wear automatic breathing apparatus and protective clothing. Foam, CO_2, dry chemicals or sprayed water can be used to extinguish the fire.

2.6.3 Natural rubber latex protein allergy

Hevea latex is regarded as the living cytoplasm of laticiferous cells and, accordingly, it is a rich blend of organic substances including a mélange of proteins. A small number of these proteins has given rise to the problem of latex allergy. Proteins make up about 1–1.5% fresh weight of Hevea latex. Since natural rubber latex is not a homogeneous fluid, latex proteins are not homogeneously dispersed. Latex proteins are found in the latex sera and are also associated with latex organelles that can be separated by high-speed centrifugation. About 70% of latex proteins are soluble, with the remaining being associated with membranes. The purifying of latex allergens have been described below (Yeang et al., 2002).

2.6.3.1 Rubber particle proteins

The Hevea latex allergens— Hev b 1 and Hev b 3 are the two major proteins located on the surface of the rubber particles. Hev b 1 is found mainly on the large rubber particles (generally above 0.4 μm in diameter) whereas Hev b 3 is more abundant in smaller rubber particles.

2.6.3.2 The C-serum proteins

Of the 10 Union of Immunological Societies (IUIS)-recognized latex allergens, for—Hev b 5, 7, 8, and 9—are from the latex C-serum, which is the cytosol of the latex vessel. This number does not include the rubber particle proteins.

2.6.3.3 The B-serum proteins

It is tempting to classify the extracytosolic proteins in the latex B-serum as lutoidic proteins since the B-serum is prepared from the ‘‘bottom fraction’’ which comprises mainly lutoids. Nevertheless, other organelles sedimented by centrifugation are also present in the bottom fraction and the possibility that some proteins in fact originate from a minor organelle cannot be disregarded. All of the recognized allergenic proteins located in the latex bottom fraction—Hev b 2, 4, 6, and 10—are plant defense proteins

2.6.4 Methods for reducing extractable and allergenic proteins (Arnold, 2004).

2.6.4.1 Leaching

Hot- and cold-water washing (leaching) is now used extensively within the dipping industry. The influence of products thickness and the processing conditions have been studied by researchers. Their findings indicate the following:

• There is a need for the protein to migrate to the products surface to facilitate extraction.

• The thinner products, the more effective is protein removal.

• The products produced from latex with a lower rubber content (40%) had a higher level of extractable proteins than products generated from a latex with a higher rubber content (60%). In general, wet gel leaching or leaching before curing the rubber was found to be less effective than dry film leaching. This could be explained that protein had insufficient time to migrate to the film surface during the manufacturing process.

2.6.4.2 Enzyme treatment

The use of proteolytic enzymes to digest latex proteins has been known for many years. However, it is only recently that the using of these enzymes have been shown to reduce allergenic proteins in medical gloves. These enzymes break up the proteins into smaller pieces, which facilitates their removal. Care must be taken to remove residual proteolytic enzymes, which may also give rise to an allergic response.

2.6.4.3 Fumed silica

It has been theorised that fumed silica can be used both in straight and coagulant dipping to reduce extractable protein in surgical gloves. It is claimed that as little as 1-2 part per hundred of dry rubber (phr) may significantly reduce extractable protein from latex gloves without adversely affecting the dipping process. The practical significance of these findings remains to be demonstrated.

2.6.4.4 Ultrasonic leaching

There is some evidence that using ultrasonics in the leaching system can accelerate the removal of latex protein over leaching on its own. The effect seems to be more pronounced in pre-vulcanised films than in post-vulcanised films and there is apparently no detrimental effect on tensile properties.

2.6.4.5 Chlorination

The primary aim of chlorinating medical gloves is to provide the end-user with a means of donning the glove in the absence of lubricating powder. Chlorination is also highly effective in lowering the level of extractable protein. The process does, however, require tight controls, as over-chlorination or inadequate neutralisation of the powerful chemicals used can lead to discolouration and detrimental effects on the ageing properties of the glove.

2.6.4.6 Miscellaneous

There are number of alternative approaches that have received recent press coverage:

- incorporation of water-soluble polymers such as into radiation-vulcanised latex films,
- high-pressure treatment of natural rubber field latex and
- ozone water treatment of latex.

2.7 Lidocaine

A suitable drug for preparation in form of TDDS should possess several physico-chemical prerequisites such as short half-life, small molecular size and low dose (Budhathoki and Thapa, 2005). In this study, lidocaine was used as the model drug.

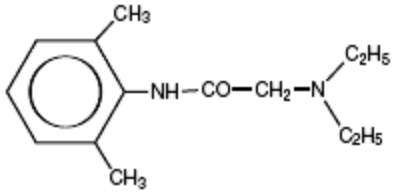
2.7.1 Properties of lidocaine

Lidocaine or lignocaine is a common local anesthetic and antiarrhythmic drug. It is used topically to relieve itching, burning and pain from skin inflammations, injected as a dental anesthetic, and in minor surgery. Lidocaine is the first amino amide-type local anesthetic. Lidocaine has a more rapid onset of action and longer duration of action than amino ester-type local anesthetics such as procaine. It is approximately 90% metabolized in the liver by CYP1A2 (and to a minor extent CYP3A4) to the pharmacologically active metabolites, monoethylglycinexylidide and glycinexylidide. The elimination half-life of lidocaine is approximately 1.5–2 h, as shown in Table 2. This may be prolonged in patients with hepatic impairment (average 343 minutes) or congestive heart failure (average 136 minutes).

Lidocaine alters depolarization in neurons, by blocking the fast sodium channels in the cell membrane. With sufficient blockade, the membrane of the presynaptic neuron will not depolarize and so fail to transmit an action potential, leading to its anesthetic effects. Careful

titration allows for a high degree of selectivity in the blockage of sensory neurons, whereas higher concentrations will also affect other modalities of neuron signaling (Reynolds, 1996; Xu, 2003).

Table 2. Properties of the lidocaine (Reynolds, 1996; Xu, 2003)

Systematic (IUPAC) name	2-(diethylamino)- N-(2,6-dimethylphenyl)acetamide
Structure	
Mol. mass	234.34 g/mol
pH	7.4
Melting point	68 °C (154 °F)
Solubility	Practically insoluble in water; very soluble in alcohol and chloroform; freely soluble in ether; soluble in oils.
Bioavailability	35% (oral), 3% (topical)
Metabolism	Hepatic, 90% CYP1A2-mediated
Half life	1.5–2 h
Excretion	renal

2.7.2 Adverse drug reactions

Adverse drug reactions (ADRs) of lidocaine are rare when it is used as a local anesthetic and is administered correctly. Most ADRs associate with lidocaine for anesthesia relate to administration technique (resulting in systemic exposure) or pharmacological effects of anesthesia; however, allergic reactions can rarely occur.

Systemic exposure to excessive quantities of lidocaine mainly result in central nervous system (CNS) and cardiovascular effects. CNS effects usually occur at lower blood plasma concentrations and additional cardiovascular effects present at higher concentrations while cardiovascular collapse may also occur with low concentrations. CNS effects may include CNS excitation (nervousness, tingling around the mouth, tinnitus, tremor, dizziness, blurred vision, seizures) followed by depression (drowsiness, loss of consciousness, respiratory depression and apnea). Cardiovascular effects include hypotension, bradycardia, arrhythmias, and/or cardiac arrest – some of which may be due to hypoxemia secondary to respiratory depression. (Weinshilboum, 2003)

ADRs associated with the use of intravenous lidocaine are similar to toxic effects from systemic exposure above. These are dose-related and more frequent at high infusion rates ($\geq$3 mg/minute). Common ADRs include headache, dizziness, drowsiness, confusion, visual disturbances, tinnitus, tremor, and/or paraesthesia. Infrequent ADRs assocaited with the

use of lidocaine include hypotension, bradycardia, arrhythmias, cardiac arrest, muscle twitching, seizures, coma, and/or respiratory depression (Weinshilboum, 2003).

2.7.3 Dosage forms (Weinshilboum, 2003)

Lidocaine, usually in the form of lidocaine hydrochloride, is available in various forms including:

- Injected local anesthetic (sometimes combined with epinephine)
- Dermal patch (sometimes combined with prilocaine)
- Intravenous injection (sometimes combined with epinephine)
- Intravenous infusion
- Nasal instillation/spray (combined with phenylephine)
- Oral gel (often referred to as "viscous lidocaine" or abbreviated "lidocaine visc" or "lidocaine hydrochloride visc" in pharmacology; used as teething gel)
- Oral liquid
- Topical gel (as with Aloe Vera gels that include lidocaine)
- Topical liquid
- Topical patch (Lidocaine patch has been marketed as "Lidoderm®" and "Versatis®").

2.7.4 **Commercial lidocaine patch** (Endo Pharmaceuticals, 2006)**.**

Lidoderm® is a patch, was sold in US in 2006, comprised of an adhesive material containing 5% lidocaine, which is applied to a non-woven polyester felt backing and covered with a polyethylene terephthalate film release liner. The release liner is removed prior application to the skin. The size of the patch is 10 cm x 14 cm. Each adhesive patch contains 700 mg of lidocaine (50 mg per gram adhesive) in an aqueous base. It also contains the following inactive ingredients: dihydroxyaluminum aminoacetate, disodium edetate, gelatin, glycerin, kaolin, methylparaben, polyacrylic acid, PVA, propylene glycol, propylparaben, sodium carboxymethylcellulose, sodium polyacrylate, D-sorbitol, tartaric acid, and urea.

2.7.4.1 Clinical pharmacology

2.7.4.1.1 Pharmacodynamics

Lidocaine is suggested to stabilize neuronal membranes by inhibiting the ionic fluxes required for the initiation and conduction of impulses. The penetration of lidocaine into intact skin after application of patch is sufficient to produce an analgesic effect, but less than the amount necessary to produce a complete sensory block.

2.7.4.1.2 Pharmacokinetics

Absorption: The amount of lidocaine systemically absorbed from Lidoderm® is directly related to both the duration of application and the surface area over which it is applied. In a pharmacokinetic study, thee Lidoderm® patches were applied over an area of 420 cm^2 of intact skin on the back of normal volunteers for 12 h. Blood samples were withdrawn for determination of lidocaine concentration during the application and for 12 h after removal of patches. The results are summarized in Table 3.

Table 3. Absorption of lidocaine from Lidoderm® patches normal volunteers (n = 15, 12-hour wearing time)

Lidoderm® Patch	Application Site	Area (cm^2)	Dose Absorbed (mg)	C_{max} (μg/ml)	T_{max} (h)
3 patches (2100 mg)	Back	420	64 ± 32	0.13 ± 0.06	11 h

When Lidoderm® is used according to the recommended dosing instructions, only 3 ± 2% of the dose applied is expected to be absorbed. At least 95% (665 mg) of lidocaine will remain in a used patch. Mean peak blood concentration of lidocaine is about 0.13 μg/ml (about 1/10 of the therapeutic concentration required to treat cardiac arrhythmia). Repeated application of three patches simultaneously for 12 h (recommended maximum daily dose), once per day for three days, indicated that the lidocaine concentration does not increase with daily use.

Distribution: When lidocaine is administered intravenously to healthy volunteers, the volume of distribution is 0.7 to 2.7 l/kg (mean1.5 ± 0.6 SD, n = 15). At concentrations produced by application of Lidoderm®, lidocaine is approximately 70% bound to plasma proteins, primarily alpha-1-acid glycoprotein. At much higher plasma concentrations (1 to 4 μg/ml of free base), the plasma protein binding of lidocaine is concentration dependent. Lidocaine crosses the placental and blood brain barriers, presumably by passive diffusion.

Metabolism: It is not known if lidocaine is metabolized in the skin. Lidocaine is metabolized rapidly by the liver to a number of metabolites, including monoethylglycinexylidide (MEGX) and glycinexylidide (GX), both of which have pharmacologic activity similar to, but less potent than that of lidocaine. A minor metabolite, 2,6-xylidine, has unknown pharmacologic activity but is carcinogenic in rats. The blood concentration of this metabolite is negligible following application of Lidoderm®. Following intravenous administration, MEGX and GX concentrations in serum range from 11 to 36% and from 5 to 11% of lidocaine concentrations, respectively.

Excretion: Lidocaine and its metabolites are excreted by the kidneys. Less than 10% of lidocaine is excreted unchanged. The half life of lidocaine elimination from 81 to 149 minutes (mean 107 ± 22 S.D., n = 15). The systemic clearance is 0.33 to 0.90 l/min (mean 0.64 ± 0.18 S.D., n = 15).

2.7.4.2 Indication and usage

Lidocaine is indicated for relief of pain associated with post-herpetic neuralgia. It should be applied only to intact skin.

2.7.4.3 Overdosage

Lidocaine overdose from cutaneous absorption is rare, but could occur. If there is any suspicion of lidocaine overdose i.e. systems of ADRs is observed, drug blood concentration should be checked. The management of overdose includes close monitoring, supportive care, and symptomatic treatment. Dialysis is of negligible value in the treatment of acute overdose with lidocaine. In the absence of massive topical overdose or oral ingestion, evaluation of symptoms of toxicity should include consideration of other etiologies for the clinical effects, or overdosage from other sources of lidocaine or other local anesthetics.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS

The reagents and substances used in the current study are listed as the following:

1. CNRL (60%DRC) (Lot number CLT2550/03-05, Chalong Latex Industry Co., Ltd., Thailand)
2. Block rubber STR-5L (Lot number 2550/08-01, Chalong Latex Industry Co., Ltd., Thailand)
3. Lidocaine (Lot number 7757-25G, Sigma-Aldrich, Inc., USA)
4. Sodium dodecyl sulfate (SDS; P.T. Kao Indonesia Chemicals., Indonesia)
5. Carboxy methyl cellulose (CMC, MW 250000; Shijiazhuang SimonChem Co., Ltd., China)
6. Petroleum resin (C9 Petro Resin; Hi2000 Infotech Corp., China)
7. Hydrogen peroxide (Arkema Inc., USA)
8. Potassium persulfate (Sinochem Hebei Qinhuangdao And Exp. Corp., China)
9. Ethanol AR grade (Darmstadt, Germany)
10. Methanol AR grade (Lab-Scan Analytical Science, Thailand)
11. Glacial acetic acid (Phillipsburg, USA)

12. Triethylamine (Sigma-Aldrich, Inc., USA)
13. Ammonium acetate (Ajex Finechem, Australia)
14. Disodium hydrogen phosphate (Ajax Finechem, Australia)
15. Sodium dihydrogen orthophosphate (Ajax Finechem, Australia)
16. Sodium chloride USP (S. Tong Chemicals Co., Ltd., Thailand)
17. Potassium bromide (FTIR grade, Aldrich, Germany)
18. Protein standard (BSA; Sigma-Aldrich, Inc., USA)
19. Bicinchoninic acid solution (Sigma-Aldrich, Inc., USA)
20. Copper (II) sulfate solution (Sigma-Aldrich, Inc., USA)
21. Gum fabric (Neoplast. Co., Ltd., Thailand)
22. Artificial skin (silicone rubber) (Polymer Science Program, PSU, Thailand)
23. Commercial patch (Porous capsicum plaster® patch; Dersan pharmaceutical Co., Ltd., Taiwan)
24. Distilled water (Faculty of Pharmaceutical Sciences, PSU, Thailand)
25. Polyamide membrane filter, 0.45 μm (Sartorius AG, Goettingen, Germany)

EQUIPMENT

1. Analytical balance (Model BP210S, K.S.P. Interchem Co., Ltd., Thailand)
2. Test mixing roll machine (Type 191-TM, Yasuda Seiki Seisakusho Ltd., Japan)
3. High performance liquid chromatography (HPLC; Shimadzu Corporation, Kyoto, Japan):
   - Model SIL-10AD, Shimadzu Class VP, two LC-10AT pumps.
   - SPD-10A wavelength programmable UV/Vis detector
   - SCL-10A system controller
4. Chromatographic Column (C18 reverse-phase column, 250 mm × 4.6 mm; particle size 5 µm; Thermo Hypersil-Keystone, Kyoto, Japan)
5. Adjustable micro pipettes, 100-1000 µl (Lot. P3940-1000, Braintree Scientific, Inc., UK)
6. Modified Franz diffusion cells (Model 57-951-016**,** Hanson Research Corporation, USA)
7. Circulating water bath (Model 8005, PolyScience, USA)
8. Magnetic 6-station stirrer (Model 57-951-016, Hanson Research Corporation, USA)
9. Magnetic (stirring) bar (10 x 3 mm; The lab depot, Inc., US)
10. pH meter (Model 20, K.S.P. Interchem Co., Ltd., Thailand)
11. Ultrasonic bath (Molel RK 255H, Bandelin electronic, Germany)
12. Scanning electron microscope (SEM) (XL30 model, Philips, The Netherlands)
13. Tension testing machine (LLOYD Instruments Ltd., UK)
14. X-ray diffractometer (WI-RES-XRD-001, Philips analytical, Eindhoven, Netherlands)

15. Fourier transform infrared (FTIR) spectroscopy (Model EQUINOX 55, Bruker, Billerica, MA)
16. Hot air oven (Medel 100-800, Memmert Co., Ltd., Thailand)
17. Desiccator (TDI International, Inc., USA)
18. Dial thickness gauge (Teclock Corporation, Japan)
19. Capillary Viscometer (Ubbelohde viscometer; Labtex Co., Ltd., UK)
20. Temperature control-Water Bath (Memert Co., Ltd, Germany)
21. Hot plate & stirrer (Model LMS-100, Labtech Co. Ltd., Korea)
22. UV-visible spectrometer (Model Genesys 6, Thermo electron corporation, USA)
23. Ball Mill (Mitsubishi electric corporation, Japan)
24. Mixing roll machine (Type 191-TM, Yasuda Seiki Seisakusho Ltd., Japan)
25. Stirrer machine (Model RM 2076, Bec Thai, Thailand)

METHODS

3.1 Preparation and adherence properties improvement of CNRL patches

3.1.1 Preparation blank patch

In this study, the natural rubber latex used was CNRL having 60% DRC. For each patch, the 5-g CNRL (3-g DRC) was diluted with 2-fold of water and then mixed with 2 phr of 20% SDS. The mixture was stirred for 5 minutes to obtain the homogeneous mixture. The mixture was consequently poured into the discs with 9-cm diameter and oven-dried at 50 $^{\circ}$C for 24 h.

3.1.2 Preparation of lidocaine-loaded patches

The 0.15-g lidocaine, equalized to 5% w/w of DRC in each patch, was dissolved in 5.5-ml ethanol and then mixed with the CNRL mixture before pouring into the disc and drying as described above.

3.1.3 Adherence property improvement of patch

3.1.3.1 Adherence property improvement of patches by adding petroleum resin

The 50% petroleum resin dispersion was prepared by mixing the 50 g of petroleum with water using ball mill and then adjusting the mixture to 100 g. The 50% petroleum resin dispersion was added into CNRL as the ratios as shown in Table 4 and then stirred for 10 minutes. The 10 g mixture was poured into 5×5 $inch^2$ mirror plates. The drying methods used were air-drying at ambient temperature. Afterwards, the adherence property was tested via attaching to the human skin.

Table 4. Composition of the mixtures used for patch preparation by adding petroleum resin

Formulation code	CNRL (phr)	petroleum resin (phr)
5P-CNRL	100	5
10P-CNRL	100	10
15P-CNRL	100	15
20P-CNRL	100	20
30P-CNRL	100	30
CNRL	100	-

3.1.3.2 Adherence property improvement of patches from low molecular weight CNRL

Polymer chains in CNRL were cut to obtain low molecular weight CNRL by mixing with the reagents as illustrated in Table 5. The CNRL was stirred slowly in water bath at 45-50°C for 5 minutes. Then, 20% SDS was added and stirred at 45-50°C for 30 minutes. Afterwards, 50% hydrogen peroxide (H_2O_2) was added into the mixture of CNRL and SDS. Subsequently, the mixture was stirred at 45-50°C for 30 minutes. The 10% potassium persulfate ($K_2S_2O_8$) was added into the mixture and stirred at 45-50°C for 24 h (Patarapaiboolchai, 2003).

Table 5. Composition of the mixtures used in low MW CNRL patch preparation

	Solution weight (g)	Dry weight (phr)
CNRL (60%DRC)	167	100
20%SDS solution	40	8
50% H_2O_2 solution	4	2
10% $K_2S_2O_8$ solution	81	8.1

The MW obtained product was measured by viscosity test with a viscometer tube by intrinsic viscosities in relation method. According to the Mark-Houwink-Sakurada relation (Eq. 1), the value of intrinsic viscosity changes with the molecular weight of the polymer in a solvent as:

$$[\eta] = K M^a \quad \text{(Eq. 1)}$$

Where the two parameters *K* and *a* are related to the "stiffness" of the chain and depend on the type of polymer, solvent and temperature.

Viscometric parameters determinations were carried out at 30 ± 0.1 °C, in 0.5% (m/v) toluene solutions, using an Ubbelohde viscometer. By graphic extrapolation to infinite dilution, experimental determination was carried out by counting the efflux time of at least six concentrations of the samples solutions. The efflux time of the solutions was manually

determined by using a chronometer. In single measurements, the lowest value of solutions concentration was chosen for the calculations . For viscosity-average molecular weight $\left(\overline{M}v\right)$ of natural rubber determination the constants K = 5.02×10^{-4} ml g^{-1} and a = 0.67 were employed (Mello et al., 2006).

The patches were prepared by pouring the 10 g low MW CNRL into 5×5 $inch^2$ mirror plates and then left drying at ambient temperature.

3.2 Preparation of DNR (Block Rubber STR-5 L) patches

3.2.1 Preparation blank patch

In this study, the block rubber used composed of 100% DRC. For each patch, the 5-g DNR (5-g DRC) was crushed with mixing roll machine for 15 minutes. The obtained rubber patch, having thickness about 0.5-mm after crushing, was put on elastic fabric as the backing and was covered with grease paper as the liner.

3.2.2 Preparation of lidocaine-loaded patches

The 5-g DNR (5-g of DRC) was crushed with mixing roll machine for 15 minutes. Then, the 0.25-g lidocaine, equalized to 5% w/w of DRC in each patch, was crushed to

combine with DNR for 15 minutes. The obtained rubber mixture patch having thickness about 0.5-mm after crushing was put on elastic fabric as the backing and was covered with grease paper as the liner.

3.3 Evaluation of patches

3.3.1 Physical appearance evaluation of patches

The obtained patches, both blank and lidocaine-loaded rubber patches, were optically observed for their appearances (color, homogeneity and elasticity). Each patch was measured for thickness at different 3 points with a dial thickness gauge. Surface morphology of both sides of blank and lidocaine-loaded CNRL patches was investigated using a SEM. The patches were attached to the slab surfaces with double-sided adhesive tapes and then coated with gold to a thickness of approximately 30 nm under vacuum to make the patches conductive (Ghaffari et al., 2007; Danilatos, 1988).

3.3.2 Peel strength study

This test was performed on the smooth substrates of aluminum. Adhesive sample (patch with the backing) of 25×100 mm was stuck on the substrate. Commonly, the adhesion of the sample was developed. The peel testing accomplished using a tension testing machine. It was performed on a rate of 254 mm/minute dwell time, the T-peel strength testing method followed to ASTM D1876-72 (Race, 1983; Maillard-Salin et al., 2000). The appearance of sample T-peel strength testing was illustrated in Fig. 9.

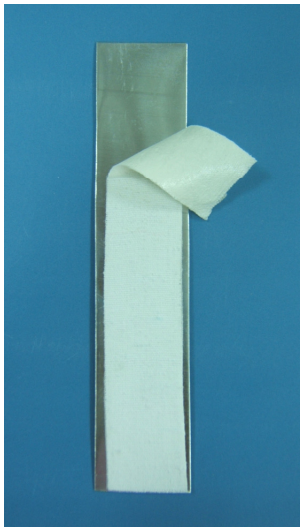


Fig. 9 The appearance of sample T-peel strength testing of a representative of all formulation attached aluminum.

3.3.3 X-ray diffraction study

X-ray diffractometer was employed to study the crystallinity of the pure lidocaine and lidocaine in of lidocaine-loaded rubber patches. The generator operating voltage and current were 40 kV and 40 mA, respectively. The scanning speed was 2°/min, and the 2θ scanning range was 5-50° (Repka et al., 2005). For confirmation, the patches with 10 and 15 %w/w of licocaine were prepared and evaluated with this technique.

3.3.4 Fourier transform infrared (FTIR) spectroscopy

Lidocaine with respect to the potassium bromide (KBr) pellet was prepared by mixing lidocaine with dry KBr. The mixture was ground into a fine powder using an agate mortar before compressing into a disc. Lidocaine-loaded natural rubber patches and blank patches were examined using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) technique. Lidocaine, lidocaine-loaded natural rubber patches and blank patches were scanned at a resolution of 4 cm^{-1} over a wavenumber region of 400–4000 cm^{-1} using a FTIR spectrometer. The characteristic peaks of IR transmission spectra were recorded.

3.3.5 Drug content and drug distribution studies

The 3 circles with 20 mm in diameter were cut from each lidocaine-loaded natural rubber patches. Each circle was measured for weight and thickness and then was dissolved in 20 ml of ethanol under sonication for 3 h. The solutions obtained were analyzed by HPLC as described in item 3.8.3 in order to determine the amount of lidocaine contained in the transdermal patch. As a control, the blank natural rubber patches was analyzed using as the same method to be used as control. The results were expressed as percentage of lidocaine (%w/w) and as mg/cm^2 (Padula et al., 2007).

3.3.6 Moisture content

The patch was weighed and kept in a desiccator containing 0.5 M – sodium chloride (NaCl) solution (75% relative humidity) at 45 $^{\circ}C$ in a oven dried for at least 24 h or more until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight and was reported in terms of percentage (by weight) moisture content (Mukherjee et al., 2005).

3.3.7 Protein assay

3.3.7.1 Protein standard solution preparation

Protein standard (Bovine Serum Albumin – BSA; concentration 1 mg/ml) was diluted with distilled water to 0-1000 μg/ml concentrations. The concentration is within the linear range of 0-1,000 μg/ml. The 2 ml of a working reagent, 50:1 mixture of bicinchoninic acid solution and copper (II) sulfate pentahydrate 4% solution, was added to tube of 0.1 ml BSA protein standard solution with various concentration and then mixed with vortex. The total liquid volume in the test tube was 2.1 ml.

The protein standard solutions were incubated at room temperature for 2 h. Then, the absorbances of the samples were measured by UV-visible spectrometer at 562 nm. Color development continues slowly after incubation at room temperature, but no error is seen if all the tubes are read within 10 minutes of each other. The standard curve was created between absorbance and the BSA protein standard concentration (Smith et al., 1985).

3.3.7.2 Assay of protein in patches

The 3 samples with 0.2 g weight were cut from each lidocaine-loaded natural rubber patches and then incubated in 2 ml of distilled water for 2 h. The 2 ml of a working

reagent was added to tube of 0.1 ml sample solution The protocol after these was as same as that in section 3.3.7.1 Protein concentrations in the samples were determined by comparing the obtained absorbance values with those in the standard curve (Smith et al., 1985).

3.3.8 *In vitro* release study

3.3.8.1 Preparation of isotonic phosphate buffer (IPB) pH 7.4

IPB pH 7.4 was prepared by mixing two stock solutions, 200 ml of a stock solution containing 8.00 g of monobasic sodium phosphate (NaH_2PO_4) per liter and 800 ml of a stock solution containing 9.47 g of dibasic sodium phosphate ($Na_2H\ PO_4$) per liter, the weights being on an anhydrous basic. Then the obtained solution was adjusted with respect to tonicity by adding 4.40 g of sodium chloride (Ansel et al., 1995). The obtained IPB was filtered through a 0.45 µm polyamide membrane (Sartorius AG, Goettingen, Germany) and degassed by sonication before use.

3.3.8.2 *In vitro* release study

In vitro drug release study was performed using modified Franz diffusion cells (Hanson Research Corporation, USA) as exhibited in Fig. 10. The diffusion area of the cells was

1.77 cm^2 and the receptor compartment volume was 12 ml. The diffusion cells were connected with a circulating water bath and the temperature was controlled at 37°C. IPB pH 7.4 was used as receptor fluid and stirred by an externally driven Teflon-coated magnetic bar at 200 rpm.

mj The lidocaine-loaded natural rubber patches (surface area 3.14 cm^2 and thickness 0.05 cm.), which were cut from each patches and was placed on the receptor compartment, then the donor compartment was connected with a clamp. At suitable time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 h), an accurate amount (1.5 ml) of each sample was withdrawn from the center of the receptor compartment using a syringe was connected with a needle. An equal volume of fresh IPB (37°C) was replenished immediately. The amount of the drug in the receptor fluid samples was assayed by HPLC method as described below. The experiment was run in triplicate. The blank patch was used to ensure that no interference from the components of the patch affecting the assay results was found.

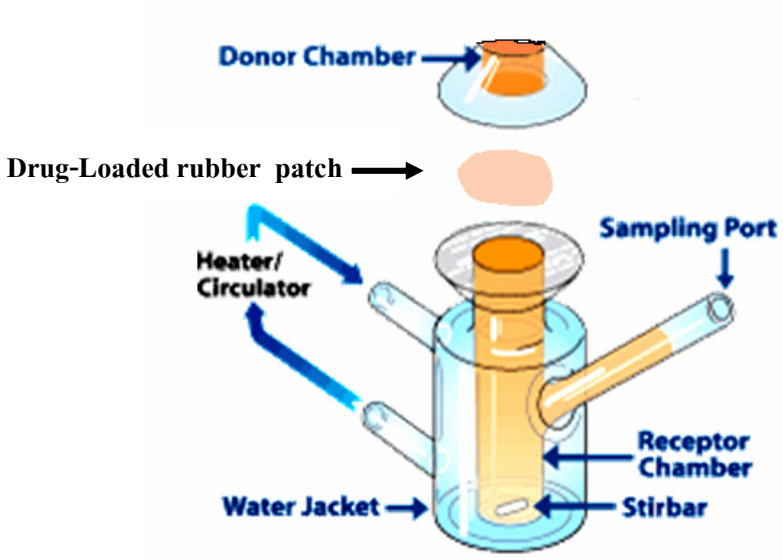


Fig. 10. Modified Franz-diffusion cell (PermeGear Franz Cells, 2005)

3.3.8.3 HPLC assay for lidocaine

The concentrations of lidocaine released to the receptor fluid and drug content in patches were quantitatively analyzed by HPLC.

3.3.8.3.1 HPLC conditions

The HPLC condition use in this study was modified from a guiding article the analysis of local anesthetics (Xu, 2003). The HPLC system connected with a HPLC column, C18 reverse phase, 5 µm particle size, 250 x 4.6 mm (Thermo Hypersil-Keystone, Canada). A mixture of 50 mM ammonium acetate and methanol (60:40% v/v) added with 1% v/v acetic acid of 50 mM ammonium acetate and 0.1% v/v triethylamine of total volume was used as a mobile phase. The injection volume was 50 µl. The samples were detected at 254 nm and integrated with the RF 10A (version 1.1) LC software program. The calibration curve was constructed by running standard solutions of lidocaine in IPB for every series of samples. Validation of the method was performed to ensure that the calibration curve between 5 and 150 µg/ml of lidocaine standard solutions was in the linearity range and coefficient of variation was calculated less than 2%, in both intra-day and inter-day.

3.3.8.3.2 Preparation of standard curve

Standard stock solutions (500 µg/ml) of the lidocaine drugs was prepared by dissolving 0.0250 g of the drug in IPB to make up a solution volume of 50 ml. Then, the standard stock solutions of 100, 200, 400, 600, 800 1,000, 1,500, 2,000, 2,500 and 3,000 µl were separately transferred to 10-ml volumetric flasks and diluted to volume with IPB to obtain the concentrations of 5, 10, 20, 30, 40, 50, 75, 100, 125 and 150 µg/ml, respectively. These standard solutions were analyzed by HPLC in triplicate.

3.3.8.3.3 Validation of HPLC analysis

The HPLC analysis method was validated by selectivity and precision tests before being used in drug analysis (Swartz and Krull,1998). For the selectivity test, the analysis method was tested for the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. In this study, it is important to make sure that the analysis method could separate the peak of the drug from those of the components in the receptor fluid in both cases absence and presence of blank patches.

Precision of the assay procedure was tested by the determination of the coefficient of variation. The drug concentrations used for the precision test were 5, 75 and 150 µg/ml. These solutions were analyzed for the concentration of drug by HPLC on the same day

and at different days to determine intra-day and inter-day variation, respectively. The intra-day precision was determined by assaying 5 replicates of each concentration in one day. The inter-day precision was determined by comparison of one sample of each concentration for five consecutive days. The percentage of coefficient of variation (CV) was calculated using the following equation:

$$\%CV = \frac{S.D. \times 100}{\overline{X}} \qquad \text{(Eq. 2)}$$

Where S.D. is standard deviation and $\overline{X}$ is a mean of drug concentration. The method was acceptable when the coefficient of variation was less than 2%, in both intra-day and inter-day (Swartz and Krull,1998).

The accuracy of the HPLC method was demonstrated by percentage deviation. The calculated concentrations were obtained by re-fitting peak response ratios from standard solutions of known concentrations into a derived regression equation. The conc. found and conc. added was then used to determine the absolute percentage deviation at each concentration of the standard solutions. The accuracy of assay should be more than 90% (Xu, 2005).

3.3.8.3.4 Data analysis

The amount of released drug from each patch was calculated and compared with that from the other patches. The cumulative drug release (Q_t) was calculated from the following equation:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \qquad \text{(Eq. 3)}$$

where C_t is the drug concentration of the receptor fluid at each sampling time, C_i is the drug concentration of the i^{th} sample, and V_r and V_s are the volumes of the receptor fluid and the sampling volume, respectively.

3.3.4 Stability studies

Lidocaine-loaded natural rubber patches were kept for 3 months at 3 various temperatures i.e. 4, 25 and 45 $^{\circ}$C (Woolfson et al., 1998). They were then evaluated for the possibility of drug content and drug release profile as previously described in items 3.3.5 and 3.3.8 respectively.

3.3.10 Statistical analysis

The analysis of difference between two conditions within one sample was performed using paired t-test. The analysis of difference between each formulations prepared from CNRL and DNR was performed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test and *P* value of less than 0.05 was considered to be significant. The calculation was performed by SPSS 13 software.

CHAPTER 4

RESULTS & DISCUSSION

4.1 Preparation and adherence properties improvement of CNRL patches

The patches prepared from CNRL identically flexible, translucent and brownish. However, it not adherence property. Thus, adherence property was improvement in CNRL.

4.1.1 Adherence property improvement of patches by adding petroleum resin in CNRL

In the industries of adhesive productions, petroleum resin was used as a tackifying agent, respectively (Kajornchaiyakul, 2006). Furthermore, when Paul Beiersdorf received the patent for adhesive bandages in 1982, natural rubber has been one of the most important raw materials for adhesives. Adding resins to make it sticky makes it a low-cost adhesive with a great deal of adhesive force (Tesa AG – A Beiersdorf Company, 1960). The adherence property of patches from CNRL incorporated with petroleum resin at all studied concentrations (5-30 phr) was less than that of CNRL blank patches. The higher concentrations of petroleum resin, the less adherence ability but darker color was observed due to yellow color of

petroleum. Petroleum resin composes of styrene monomer (Fig. 11.) providing hard patches, therefore the obtained patches could not adhere well. However, petroleum resins provide adhesive properties while they are in liquid form (Luvinh et al., 1987). Natural rubber is organic polymers, chemical compounds that consist of chain or branched molecules, can be permanently sticky (Morton, 1987).

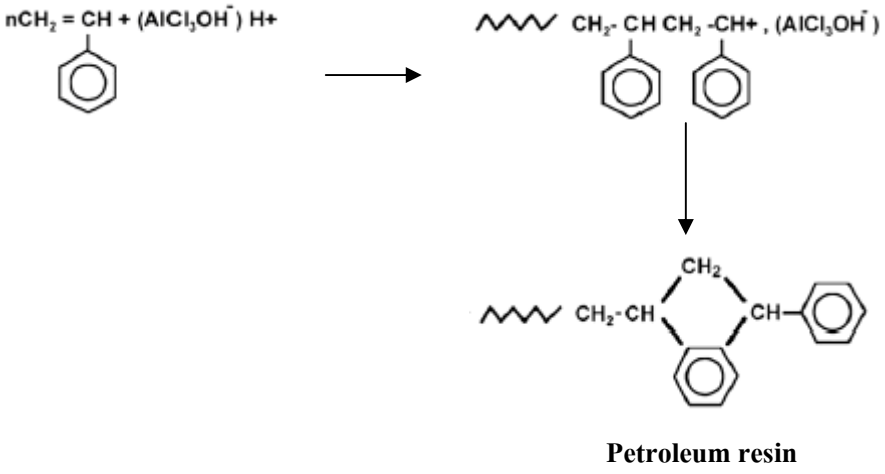


Fig. 11 Synthesis mechanism of petroleum resin (Salari and Jodaei , 2005)

However, the mixtures of CNRL and petroleum resin before dried were viscous fluids which could be applied on the skin directly and left to dry to form peel-off patches.

4.1.2 Adherence property improvement of patches from low MW of CNRL

Generally, CNRL has MW of >1,300,000 g/mol. After reacted with H_2O_2 and $K_2S_2O_8$, the MW was reduced to 150,000 g/mol. The H_2O_2 can break down in reactions giving scission of the polymer chains and producing more free radicals which can initiate further anti oxidation. Thus, ageing could be "autocatalytic" (Patarapaiboolchai, 2003). The patches prepared from low MW CNRL were higher sticky than those prepared from CNRL. In reaction process, some double bonds of polymers chains were cut off resulting in sticky property. However, after drying of film from CNRL and low MW CNRL, no adherence ability on the skin was observed in both the patches. Therefore, CNRL was chosen as raw material for preparation to the patch.

The additives (petroleum resin) could not improve the adherence property of CNRL patches. In addition, using low molecular weight CNRL in patch preparation provide the same adherence the same adherence property compared to using CNRL. Therefore, transdermal patches prepared from CNRL without additives were further investigated as the representative patches from liquid rubber. Moreover, DNR patches were studied as the representative of patches from solid rubber.

4.2 Evaluation of patches prepared from CNRL and DNR

4.2.1 Appearance of patches

The appearance of patches prepared from CNRL and DNR was identically flexible, translucent and brownish. These patches are of the drug-in-adhesive matrix type. The initial of natural rubber was milky white liquid; however, the transforming and storing affected color change due to oxidation reaction at double bond of isoprene molecule (Hofmann, 1980). A patch from DNR had darker color than that from CNRL (Fig. 12) since DNR was treated with chemicals and heat in manufactory process (Morton, 1987). The weight and thickness values of blank and drug-loaded patches were presented in Table 6. The weight and thickness of drug-load patches had nearest of blank patches, since the amount of drug had only 5% of patches that a few.

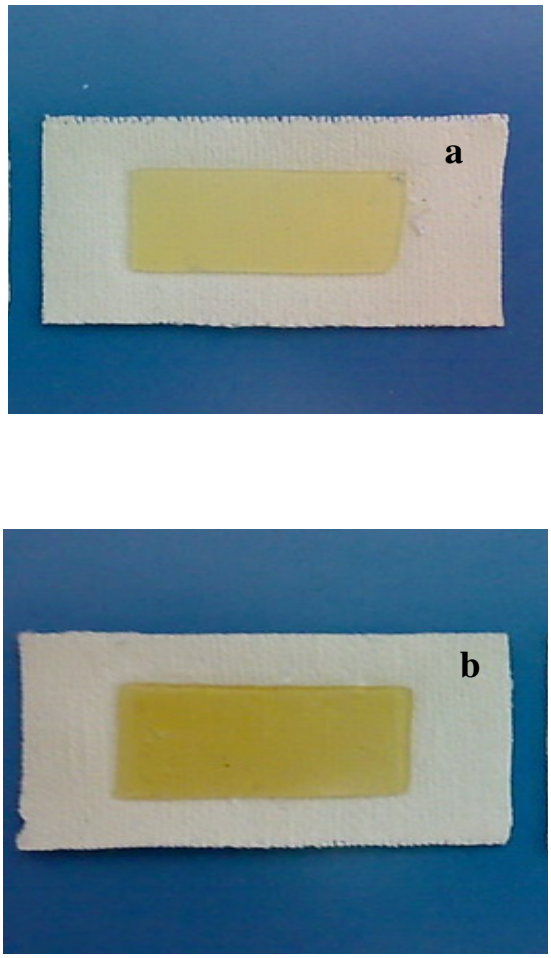


Fig. 12. The appearances of rubber patches: (a) a patch from CNRL and (b) a patch from DNR.

Table 6. Average weight and thickness of the different formulations

Type of patch	Formulation code	Amount of drug* (%)	Weight** (g)	Thickness** (mm)
CNRL blank	CNRL	0	0.1562 ± 0.0138	0.54 ± 0.03
Lidocaine-CNRL	L-CNRL	5	0.1528 ± 0.0114	0.55 ± 0.02
DNR blank	DNR	0	0.1577 ± 0.0129	0.54 ± 0.04
Lidocaine-DNR	L-DNR	5	0.1575 ± 0.0131	0.53 ± 0.02

* Amount of drug was presented in percentage of DRC.

** Reported as mean ± S.D. (n=5).

4.2.2 Peel strength study

The results of peel test obtained according to ASTM D1876-72 (Race, 1983) are shown in Table 7. This test measured the force required to peel away a strip of adhesive from a rigid substrates. Aluminum was used as substrates. The CNRL, DNR and commercial patch(Porous capsicum plaster®) patches required T-peel strength of <0.8, 7.85 ± 0.16 and 4.30 ± 0.66 N/cm, respectively. It could be explained that the long polymer chains must be partially broken by mastication, mechanical shearing forces applied by passing the rubber between rollers or rotating blades resulting in increase of he stickiness of DNR patches (Kajornchaiyakul, 2006).

The DNR formulation needed T-peel strength on aluminum higher than the commercial patch, therefore the DNR patches were sticky and might be attached to human skin as same as commercial patch. CNRL patch were slight sticky or without sticky, while it had sticky as liquid form due to the resin amount 1-2.5 % in CNRL liquid form (Blackley,1997). Therefore, application of CNRL patches on the skin should be used with adhesive backing or could be applied on the skin directly and left to dry to form peel-off patches, while the DNR patches may need no adhesive backing.

Table 7. T-peel strength of the different formulations and commercial patch

Formulation code	Maximum peeling load* (N)	T-peel strength* (N/cm)
CNRL	< 2	< 0.8
DNR	19.64 ± 0.40	7.85 ± 0.16
Commercial patch (Porous capsicum plaster® patch)	10.74 ± 1.64	4.30 ± 0.66

* Reported as mean ± S.D. (n=5).

4.2.3 Surface morphology of patches

SEM photographs (Figs. 13-14) demonstrate the physical property of the blank and drug-loaded patches prepared from two types of natural rubber. The surface morphology of patches from CNRL and DNR as polymer base was different.

The patches from CNRL had crack of surface as shown in Fig. 13(a). The cracking was believed to be caused by the following of events: the surface layer of the matrix lost water rapidly through surface evaporation (Moon and Cooper, 1979). The L-CNRL as initial preparation had decrease of cracking of surface as illustrated Fig. 13(b) since the lidocaine was interfered in polymer matrix influence easier evaporation of water. The L-CNRL as stored at 45°C for 3 months had cracking surface less than L-CNRL as initial preparation as shown in Fig. 13(c). It had shrinkage of polymer matrix after storing at high temperature for long time due to heat during storing of patch (Mizuno et al., 2005).

The DNR blank patches had smooth surface as shown in Fig. 14(a). After lidocaine incorporation, no obvious change was observed in the patches as exhibited in Fig. 14(b). The L-DNR patches as stored at 45°C for 3 months had shrinkage of surface as illustrate Fig. 14(c), the reason was as same as the case of L-CNRL patches as stored at 45°C for 3 months.

The DNR and L-DNR patches had more smooth surface (not cracking) than CNRL and L-CNRL patches, respectively. Since the preparation processes DNR and L-DNR patches had not stage of evaporation of water. However, after storing at 45°C for 3 months, the

L-DNR patches shrinkage more than L-CNRL, since the DNR had shorter molecular chain than CNRL resulting in easier movement of polymer chains (Morton, 1987).

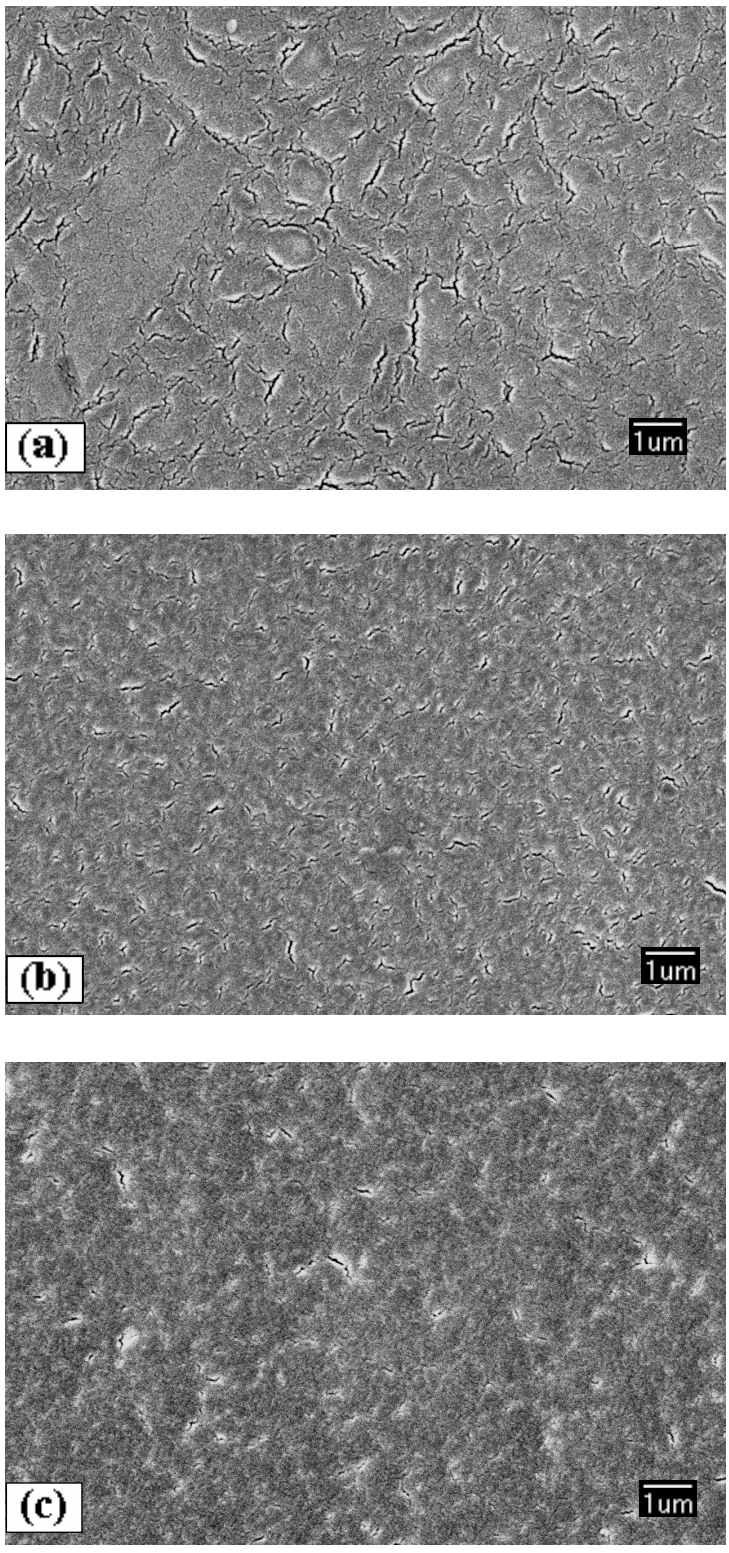


Fig. 13 SEM photographs of (a) CNRL, (b) L-CNRL as initial preparation, (c) L-CNRL as stored at 45°C for 3 months (magnification 10000×).

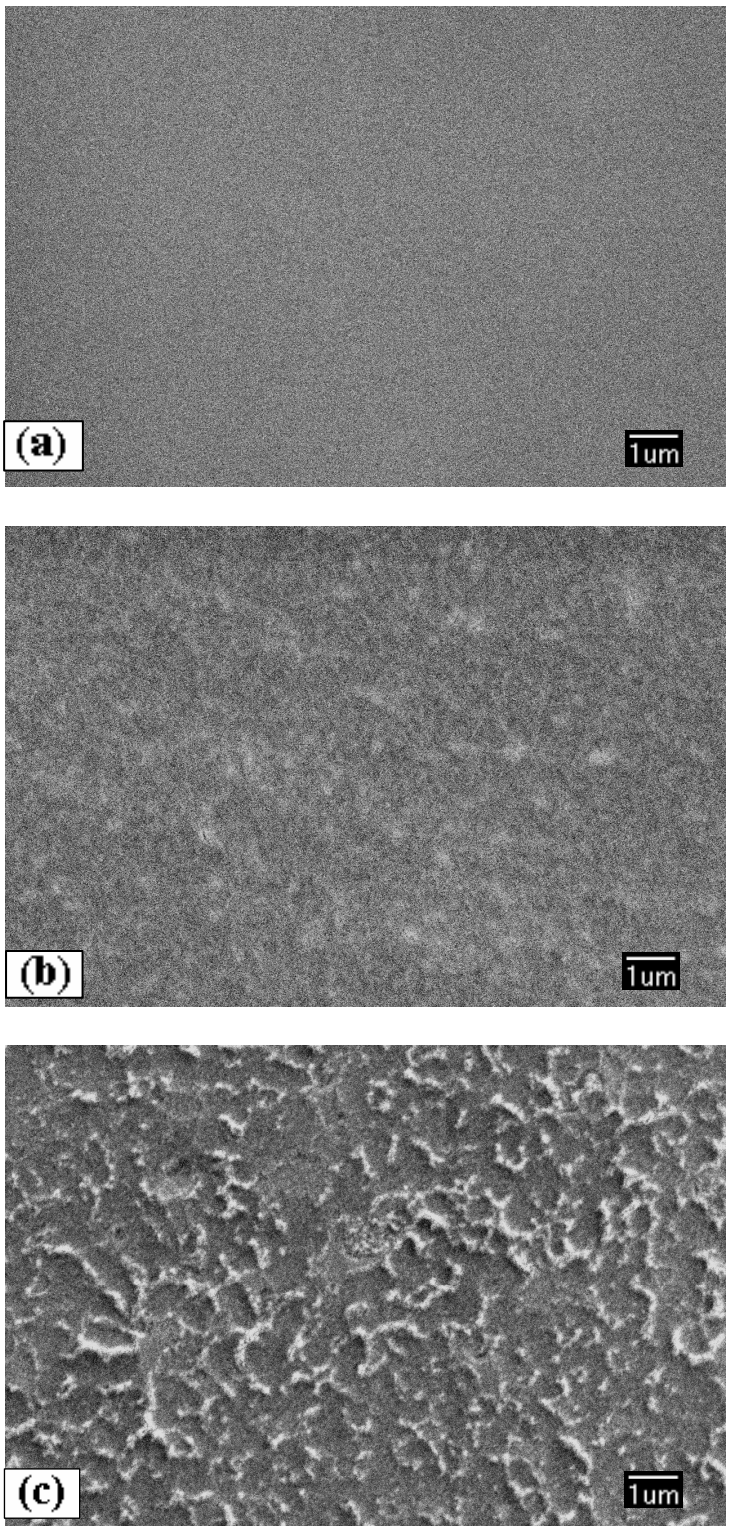


Fig. 14 SEM photographs of (a) DNR, (b) L-DNR as initial preparation and (c) L-DNR as stored at 45°C for 3 months (magnification 10000×).

4.2.4 X-ray diffraction studies

The prepared rubber patches were characterized to determine the crystallinity using XRD. The results are shown in Fig. 15. It was found that blank CNRL and DNR patches presented broad XRD spectra (Fig. 15h and 15i), implying that no crystallinity was observed in both blank patches. The highest peaks of lidocaine (Fig. 15a) were noticed at 2θ of about 10° and 12.5°. This finding is in good agreement with the published literature (Repka et al., 2005). No crystalline peaks were found in 5% lidocaine-loaded CNRL and DNR patches (Fig. 15d and 15g). This result might be caused by too low amount of the drug; therefore 10% and 15% lidocaine-loaded patches were prepared for investigation. The spectrum of 10% lidocaine-loaded CNRL patches (Fig. 15c) could not be seen the crystalline peaks. Thus, the drug could be dissolved in ethanol and not recovered to crystalline after oven-dried at 50°C for 24 h. However, the high and shift crystalline peaks was found in 15% lidocaine-loaded CNRL patches. In the high crystalline peaks, since the concentration of lidocaine in 15% of CNRL might be saturated in ethanol and recrystallization, crystalline peaks could be observed in XRD spectra. However, the shifting of crystalline peaks might be obtained since polymorphism of drug, the conformation of the lidocaine molecule consist of two conformers in the asymmetric unit. The recrystallization mostly induced the less stable polymorph (Schmidt, 2005).

The 10% and 15% lidocaine-loaded DNR patches provided XRD peaks at identical originally positions due to no dissolving in ethanol in the preparation process.

Nevertheless, the peak was less height compared to lidocaine pure drug because of low amount of the drug.

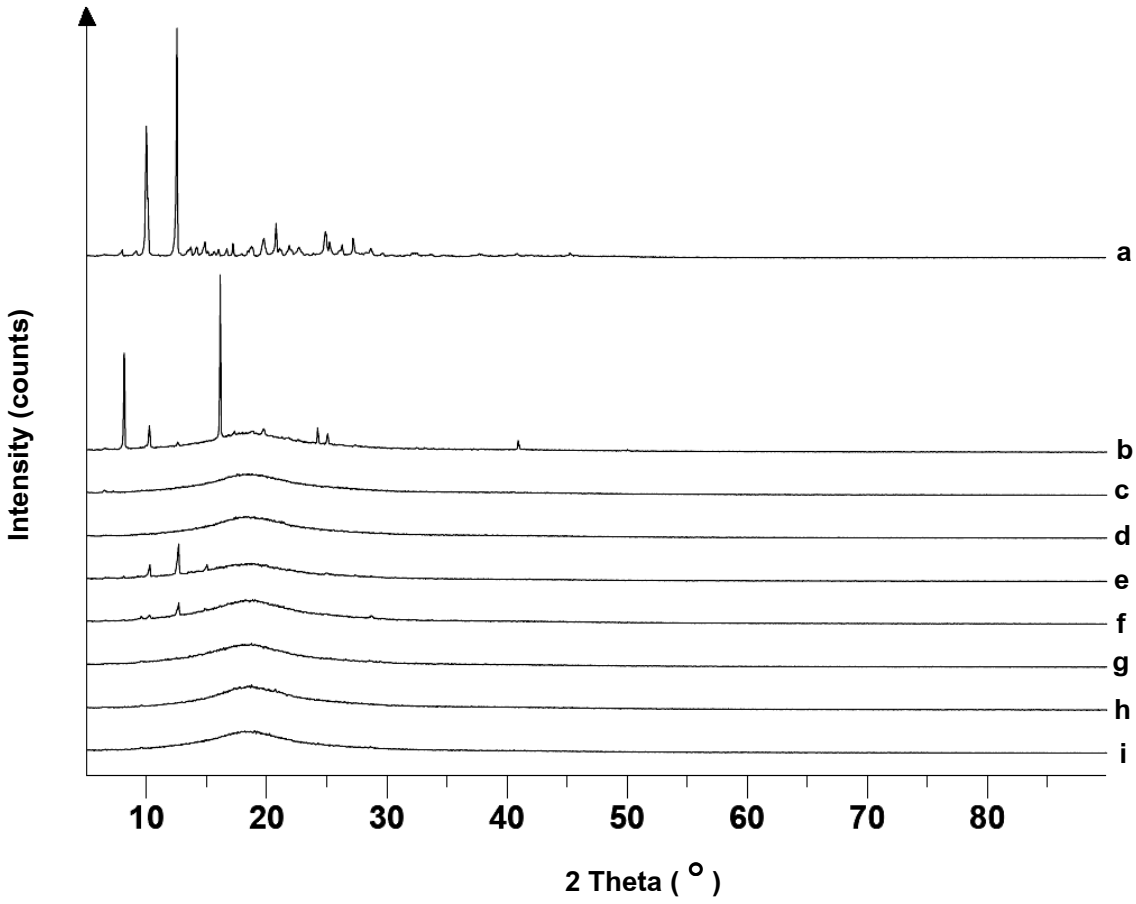


Fig. 15. XRD patterns of (a) lidocaine drug; (b) 15% lidocaine-loaded CNRL; (c) 10% lidocaine-loaded CNRL; (d) 5% lidocaine-loaded CNRL; (e) 15% lidocaine-loaded DNR; (f) 10% lidocaine-loaded DNR; (g) 5% lidocaine-loaded DNR (h) CNRL blank and (i) DNR blank.

4.2.5 FTIR and ATR-FTIR spectroscopies

The FTIR and ATR-FTIR spectra of lidocaine, CNRL, L-CNRL, DNR and L-DNR patches are shown in Figs. 16-17. The ATR-FTIR spectra of CNRL and DNR provided several peaks since natural rubber composes of many kind of chemicals. These peaks were interfered with pure lidocaine. Lidocaine is the amide-type local anesthetic. The peak at about 3500-3100 cm^{-1}, about 1666-1663 cm^{-1}, about 1500 cm^{-1} and 1165-1162 cm^{-1} as peak of N-H stretching (secondary amines), C=O stretching, N-H bending and C-N stretching in Figs. 16c and 17c, respectively, indicated that lidocaine characteristic was incorporated in the patches. No changed characteristic peak of CNRL and DNR was observed when compared to spectra of L-CNRL, L-DNR and lidocaine. These results implied that there was no chemical interaction between the drug and polymer base.

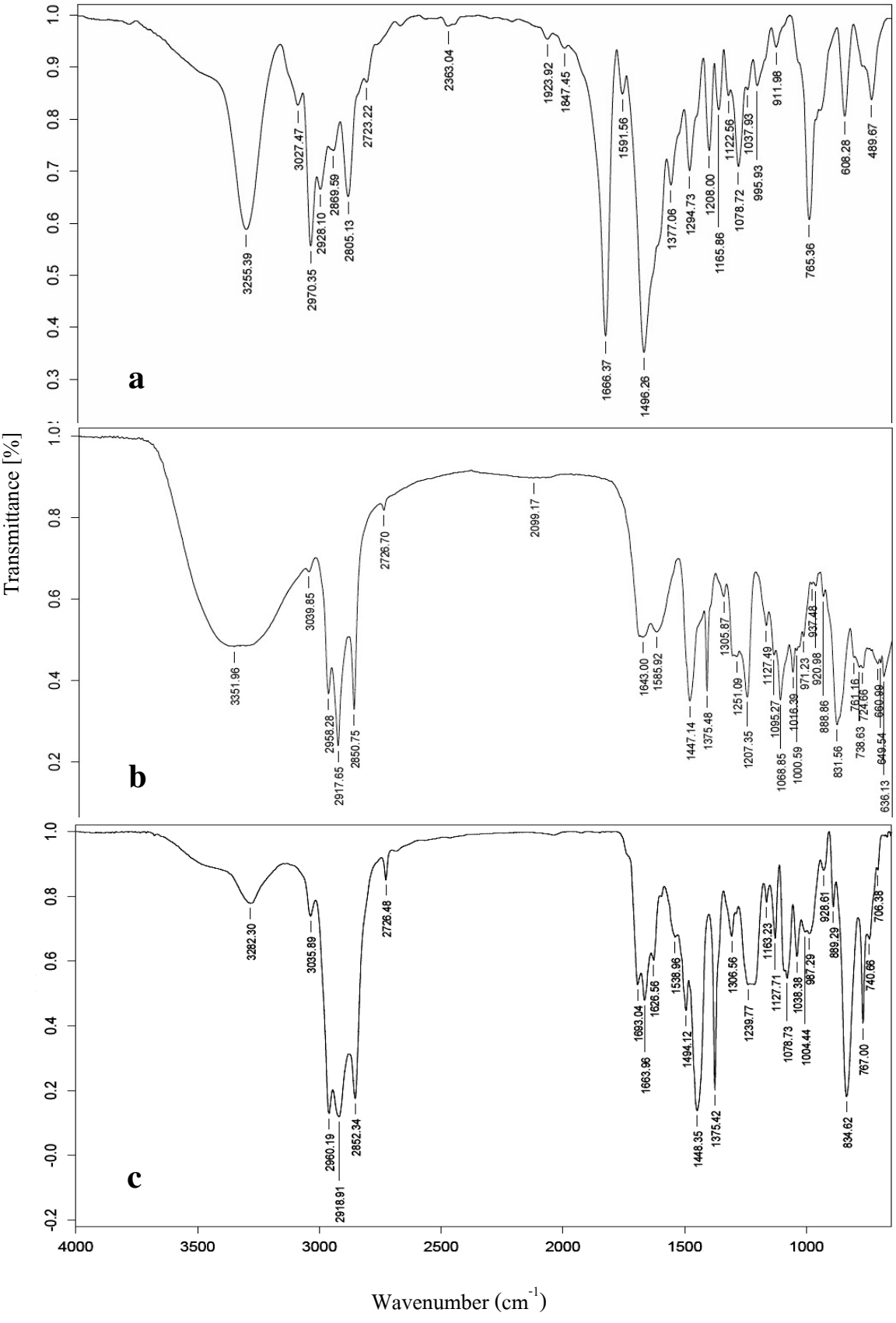


Fig. 16 FTIR spectra of (a) lidocaine, (b) CNRL and (c) 10% Lidocaine-loaded CNRL.

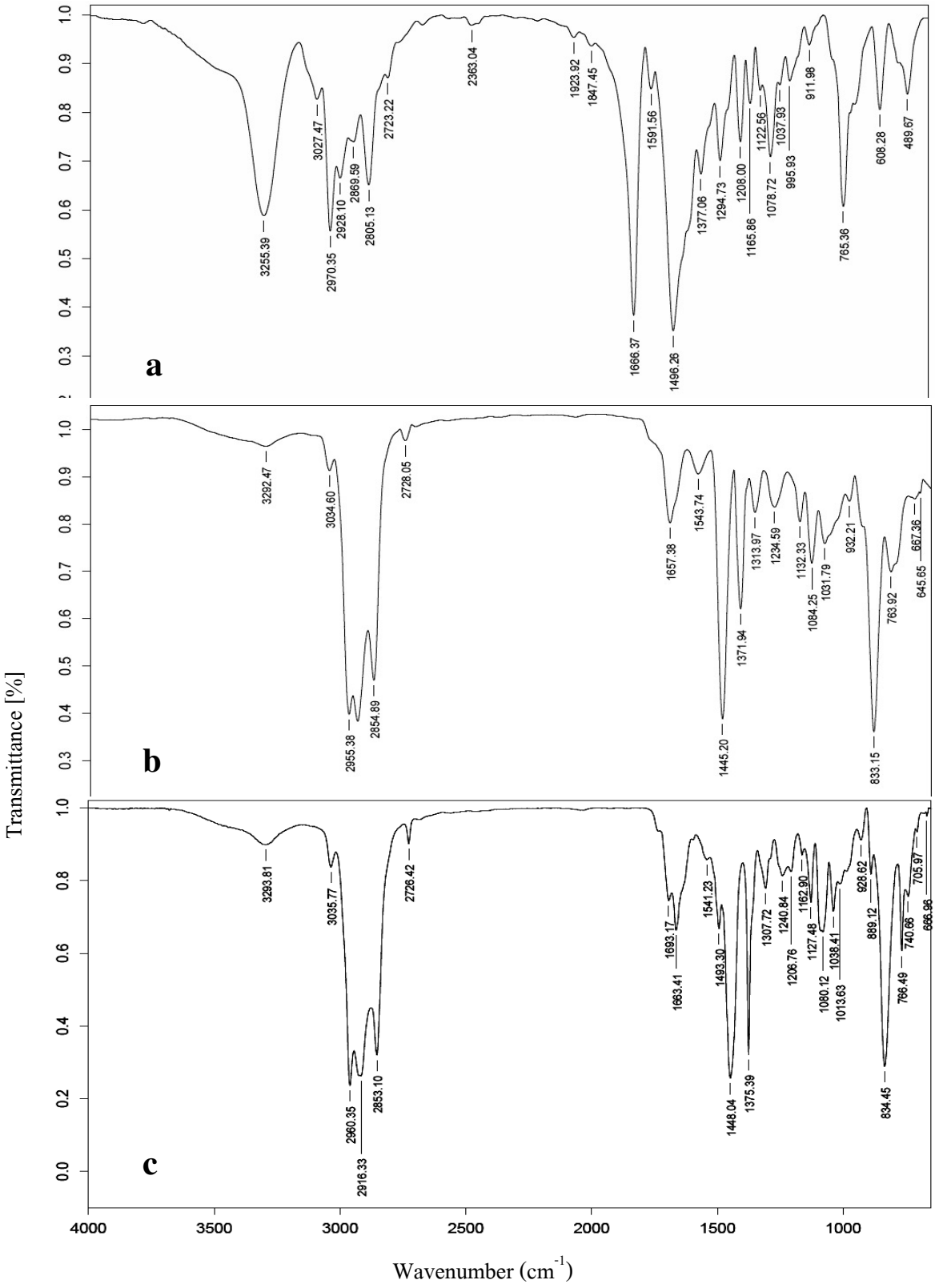


Fig. 17 FTIR spectra of (a) lidocaine, (b) DNR and (c) 10% Lidocaine-loaded DNR

4.2.6 Drug content and efficiency of drug entrapment in patches studies

The drug content of L-CNRL was 1.90±0.09 mg/cm^2, it could be calculated to efficiency of drug entrapment of 80.56±3.80% as shown in Table 8. The drug content and efficiency of drug entrapment of L-DNR were 2.03±0.14 mg/cm^2 and 86.29±5.77%, respectively as shown in Table 9. The results exhibited that the drug could be distributed thoroughly in both types of rubber patches since the drug contents obtained from different three points of each patch were not significantly different (P>0.05). The ideal amount of drug in rubber patches should be 2.35 mg/cm^2 (100% efficiency of drug entrapment). The drug in L-CNRL may remain in matrix of patches since binding of long molecular chain influence drug could not leave to out side. In addition, the L-DNR patches passed crushing process wile preparation resulting in shortening polymer chain and then the drug could easier leave to outside.

Table 8. The drug distribution and drug content in L-CNRL patches.

Patch	Points of cutting in patch	Drug content (mg/cm^2)	% Efficiency of drug entrapment
1	1	2.00	85.03
	2	1.98	84.39
	3	1.85	78.84
2	1	1.90	80.64
	2	1.78	75.90
	3	1.94	82.66
3	1	1.75	74.14
	2	1.97	83.74
	3	1.88	79.73
Average		1.90 ±0.09	80.56 ±3.80

Table 9. The drug distribution and drug content of L-DNR patches.

Patch	Points of cutting in patch	Drug content (mg/cm^2)	% Efficiency of drug entrapment
1	1	2.01	85.15
	2	1.90	80.49
	3	2.19	92.95
2	1	1.89	80.29
	2	1.93	81.72
	3	2.13	90.28
3	1	1.91	81.16
	2	2.11	89.43
	3	2.24	95.14
Average		2.03±0.14	86.29±5.77

4.2.7 Moisture content

The moisture content provided information relating the stability of the studied patches. The moisture content (Fig. 18) varied to a small extent in all the studied formulations in

the range of 0.4 – 0.9%. The moisture content values of CNRL, L-CNRL, DNR and L-DNR were 0.88±0.13, 0.56 ± 0.22, 0.73±0.28, 0.36±0.17 %w/w, respectively. It could be noted that CNRL patch could absorb moisture more than DNR patch since breaking pore occurred from water evaporation in drying stage of patch, confirming with SEM micrographs as described previously. Lidocaine-loaded rubber patches provided lower average moisture absorption than their blank counterparts since lidocaine inserted in breaking pore or between molecular chain of the rubber effecting on high density of matrix and decreasing of moisture absorption.

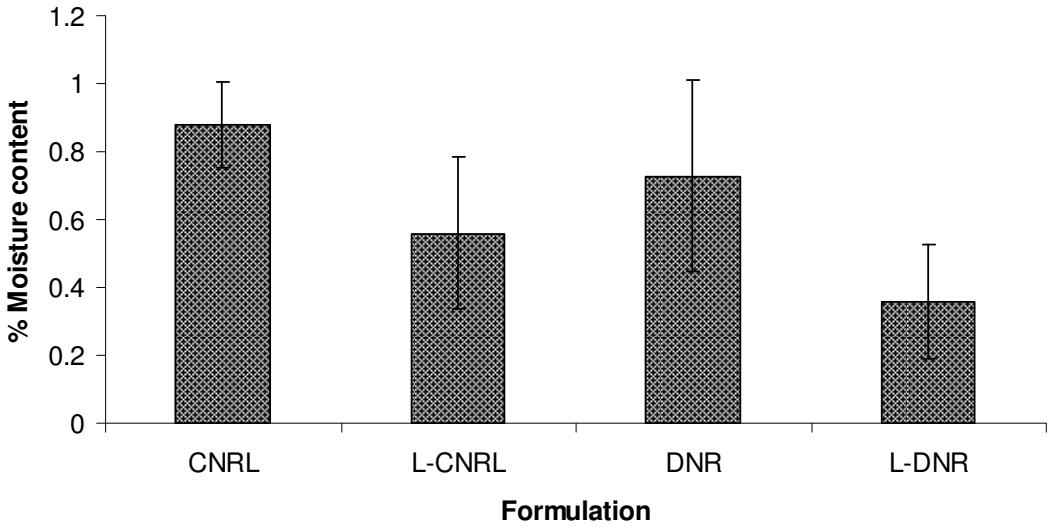


Fig. 18 Moisture content (%) in different studied patches. Reported as mean ± S.D. (n=5)

4.2.8 Protein assay in patch

Protein assay in patch was obtained from comparison with standard BSA protein as standard. Sonication of patch in water could elucidate the protein from patch greater than the soaking in water due to the vibration force. Amount of protein in CNRL and DNR patches extracted by sonication were 1910.00 $\pm$ 441.42 and 1006.25 $\pm$ 365.41$\mu g/dm^2$, respectively as shown in Fig. 19. The US FDA allows manufacturers to make a protein level claim on their products. The ASTM recommends in their standards for medical gloves (D3577 and D3578) that all examination and surgical gloves should contain less than 200 $\mu g/dm^2$ total protein (Beezhold et al., 2002). The both studied formulations could not be accepted by US FDA and ASTM since their amounts of protein were too high. It was found that amount of protein of CNRL was higher than that of DNR since DNR has been washed with water in manufacturing process. Protein in natural rubber was water soluble, thus protein amount of DNR was low when compared to CNRL. In the future suggestion, deproteinized rubber might be used as raw material for preparing transdermal patch.

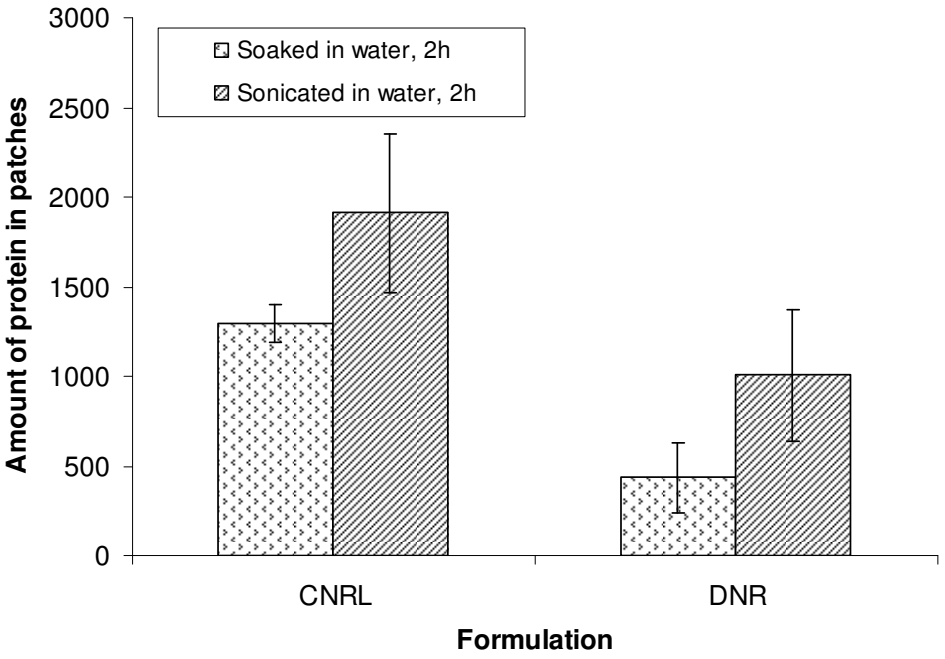


Fig. 19 Amount of protein in patches of different formulations. Reported as mean ± S.D. (n=3)

4.2.9 *In vitro* release studies

The side of L-CNRL patches affected the drug release profile. The drug release profiles from surface contact with air and surface contact with dish of lidocaine-load patches are shown in Fig. 20, kinetic of drug release profiles as higuchi pattern were illustrated in Fig. 21 and release rates were illustrate in Table 10. It was found that surface contact with air provided amount of drug released faster than those for surface contact with dish. Since ethanol was used in the preparation of patch and lidocaine could be easily soluble in alcohol, the lidocaine might be conducted to the up side of the front surface patch in the stage of evaporation. Thus, the evaporation of the solvent the drug concentration within the formulation which increase the

releasing of the drug in the formulation (Schroeder et al., 2007). Furthermore, the cracking of surface from water evaporation might affect to drug releasing, that the patch surface of dish contacting had cracking less than the patch surface of air contacting as different surface shown in Fig. 22 and 23.

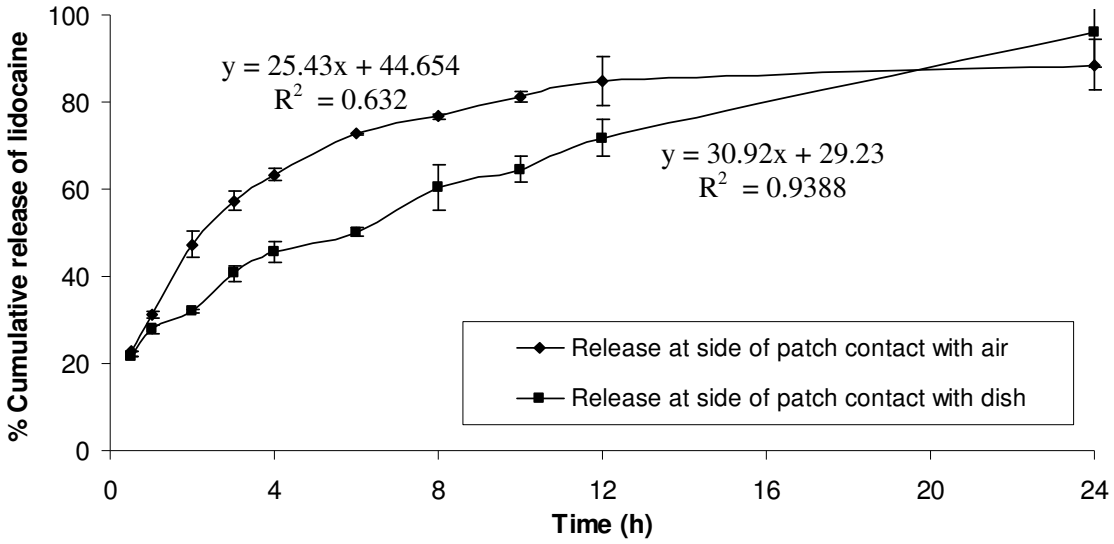


Fig. 20 *In vitro* release of lidocaine from different position of L-CNRL patches.

Each point was the mean ±S.D.(n=3)

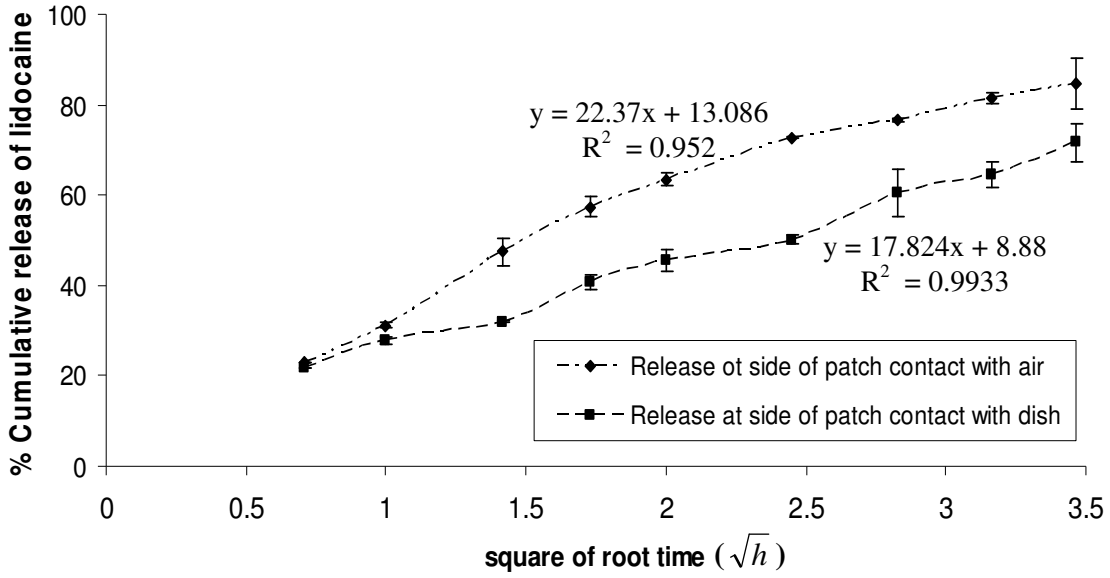


Fig. 21 Higuchi plot of kinetic drug release profile from different position of L-CNRL patches.

Each point was the mean ±S.D.(n=3)

Table 10. Release rate between 0.5 and 12 h of lidocaine from CNRL patch at different of side patch. Each datum points are the mean ±S.D. (n=3)

Patches	**Release rate (%/ $\sqrt{h}$)**
Side of patch contact with air	22.37 ± 0.99
Side of patch contact with dish	17.82 ± 1.44

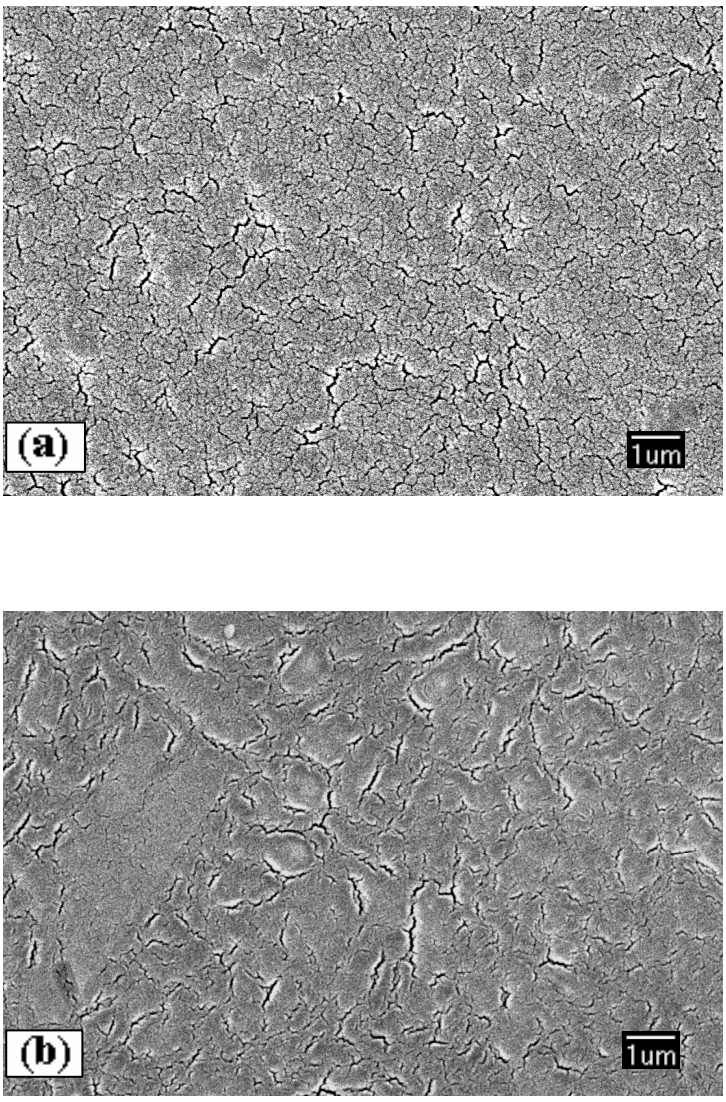


Fig. 22 SEM photographs of CNRL surface (a) dish contact , (b) air contact

(magnification 10000×).

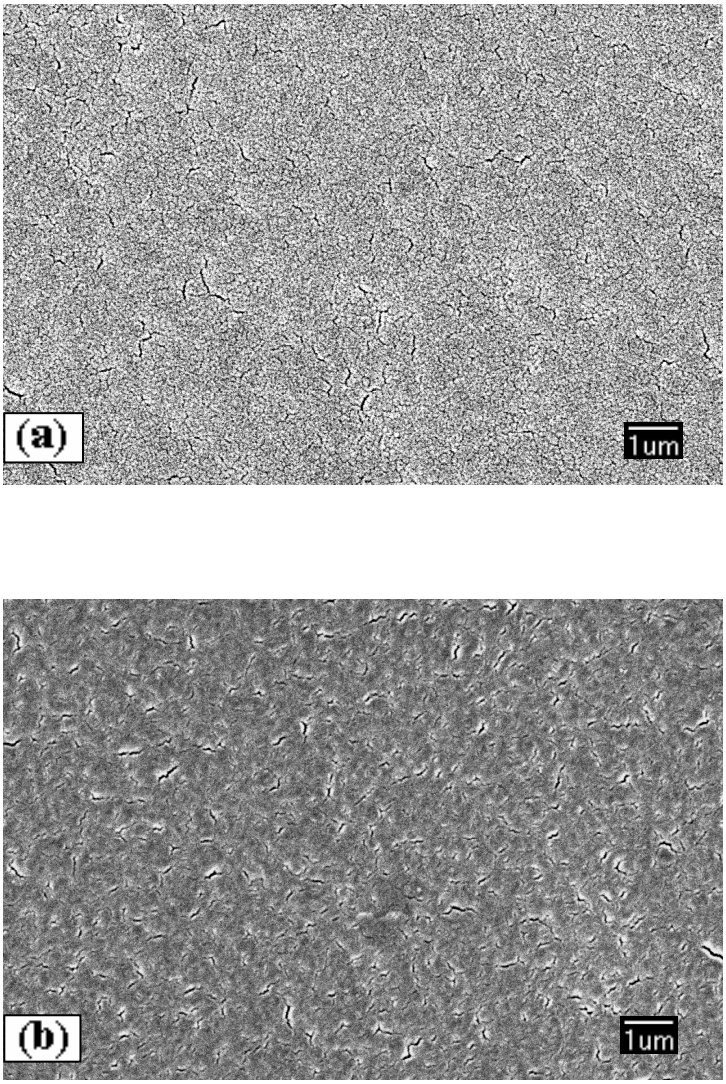


Fig. 23 SEM photographs of L-CNRL surface (a) dish contact , (b) air contact (magnification 10000×).

Fig. 24 shows *in vitro* release of lidocaine from L-CNRL and L-DNR patches, kinetic of drug release profiles as higuchi pattern were illustrated in Fig. 25 and release rates were illustrate in Table 11. Then the declining release rates were found in the late time of release. In

the initial release, L-CNRL provided slightly higher amount of drug release than those of L-DNR due to the deposited drug on the surface of L-CNRL.

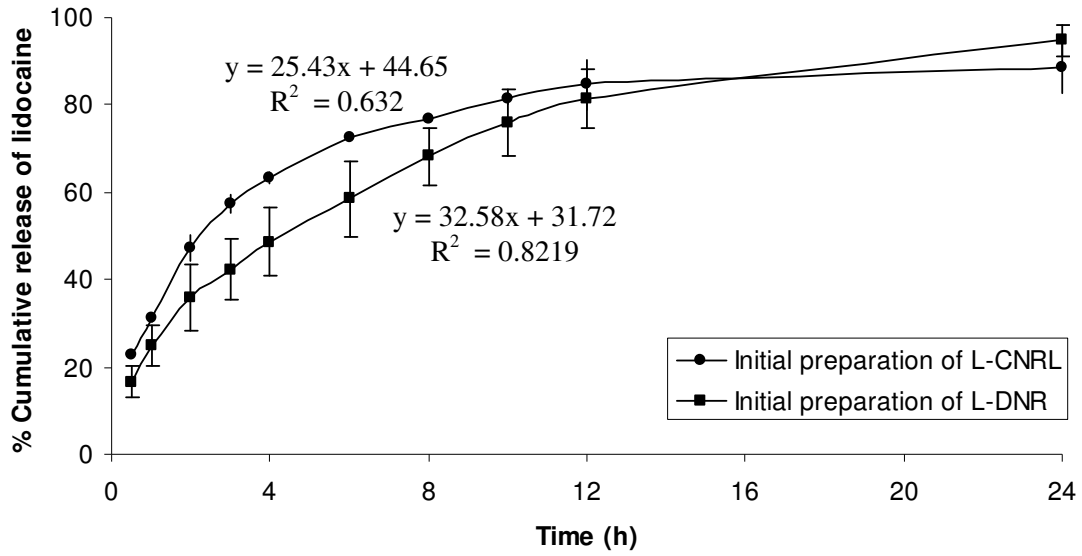


Fig. 24 *In vitro* release of lidocaine from L-CNRL and L-DNR patches.

Each datum point is the mean ±S.D. (n=3)

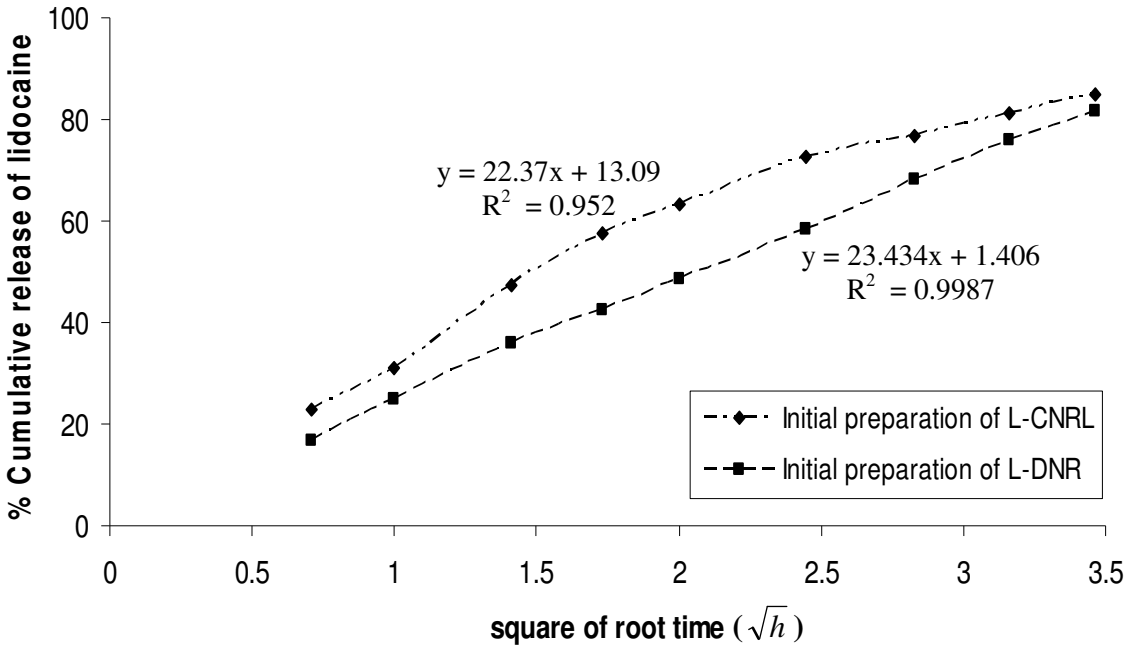


Fig. 25 Higuchi plot of kinetic drug release profile from L-CNRL and L-DNR patches.

Each point was the mean ±S.D.(n=3)

Table 11. Release rate between 0.5 and 12 h of lidocaine from L-CNRL and L-DNR patches.

Each datum points are the mean ±S.D. (n=3)

Patches	**Release rate (%/$\sqrt{h}$)**
Side of patch contact with air	22.37 ± 0.99
Side of patch contact with dish	23.43 ± 1.02

4.2.10 Stability studies

The appearance of L-CNRL and L-DNR patches after storage kept at 4, 25 and 45°C as shown in Fig. 26 and 27, respectively, indicated that color changes of them were in similar tendency. The L-CNRL was noticed that the appearance of samples as kept at 4°C was similar to that of initial preparation patches. However, the color of samples kept at 25°C and 45°C was darker, related to storage temperatures. Temperature affected color changing of patches, due to oxidation reaction (Morton, 1987).

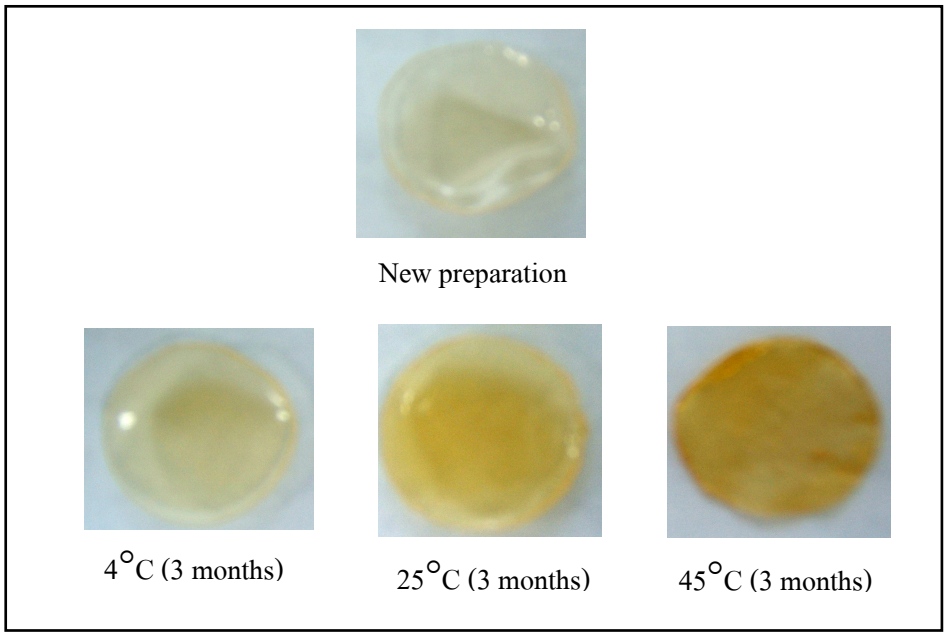


Fig. 26 Appearance of L-CNRL patches after preparing and storage at various temperatures for 3 months.

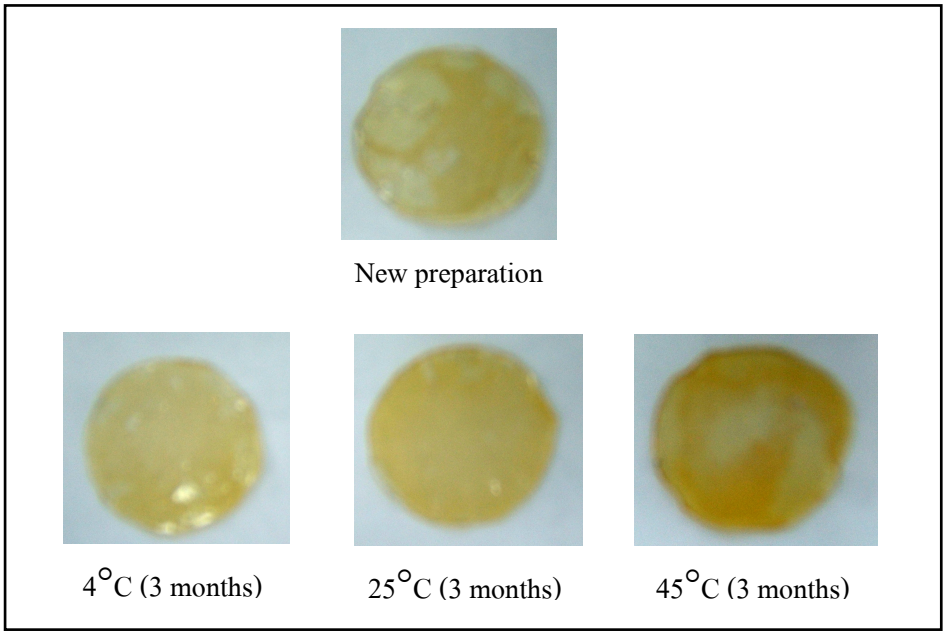


Fig. 27 Appearance of L-DNR patches after preparation and storage at various temperatures for 3 months.

The drug contents of L-CNRL and L-DNR patches stored at 4, 25 and 45°C for 1, 2 and 3 months are shown in Fig. 28 and 29, respectively. The drug contents of both patch types while storage at 4°C for 1, 2 and 3 months significantly decreases along the time. The patches which kept at 25 and 45°C provided decreasing of drug contents along the storage time. Thus, storage for long duration affected decreasing of drug contents. Natural rubber is amorphous polymer influence regulations rearrange of molecular chain when storage for long duration (Morton, 1987). This phenomenon affected difficulty leaving of drug to outside. In addition, it could formed that the drug contents in patches storage at 4, 25 and 45°C in the same duration

decreased as different significantly ($P<0.05$), respectively. Since, the temperatures were activating agent of rearrange of molecular chain to regulation rearrange (Morton, 1987). While property of lidocaine are temperature resistant over an enormous range -20°C to +70°C, it may be not affected to drug content due to stability of lidocaine (Kupper et al., 2006). Thus, decreasing of drug content depended on property of natural rubber.

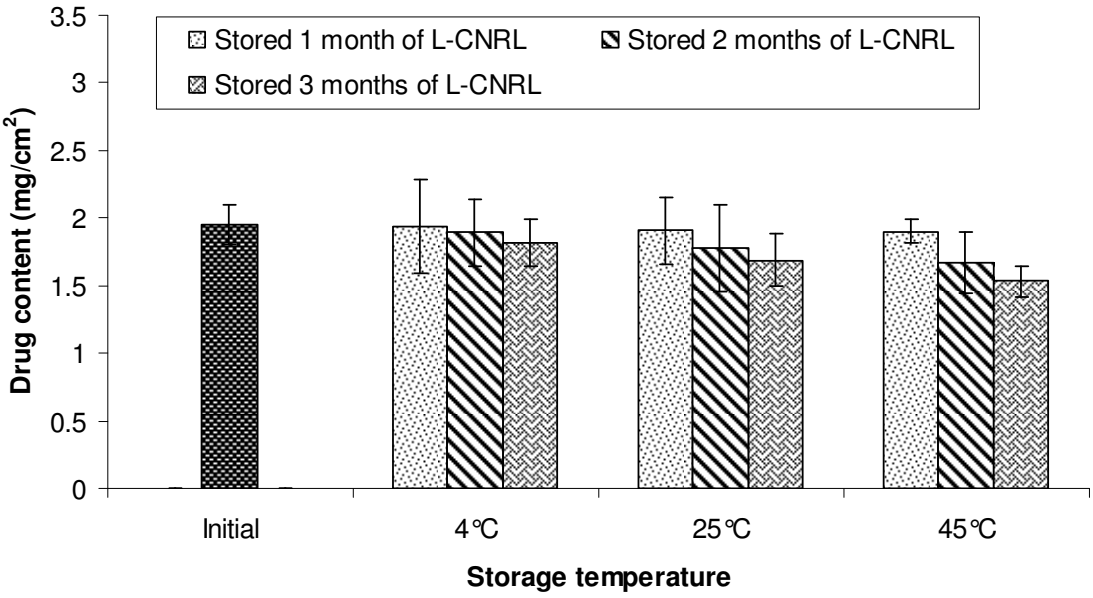


Fig. 28 Drug content in L-CNRL when stored at various temperatures for 3 months.

Reported as mean ± S.D. (n=3)

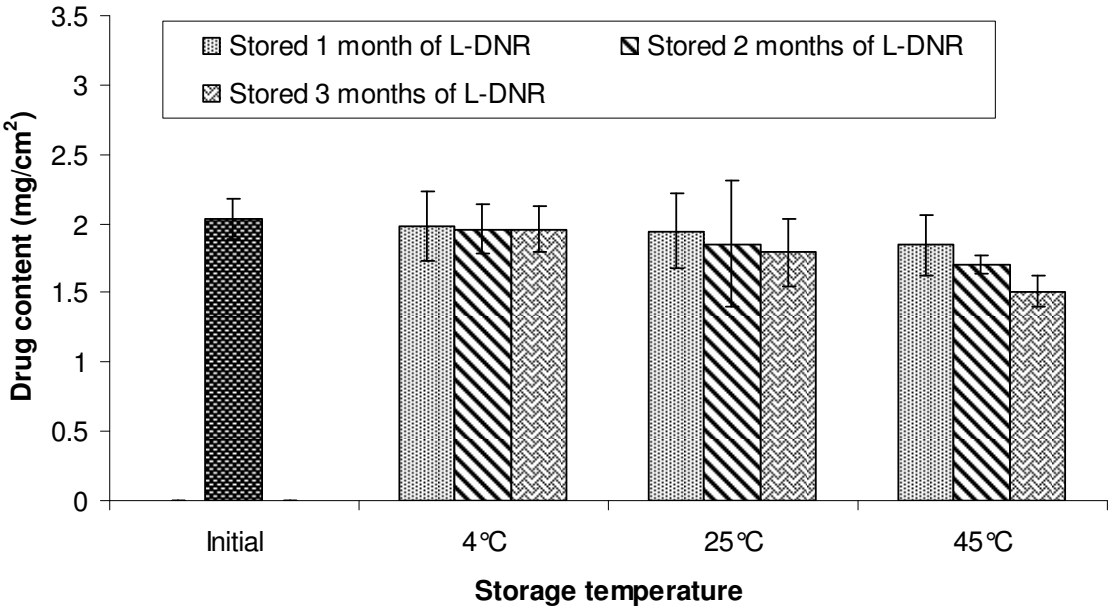


Fig. 29 Drug content in L-DNR when stored at various temperatures for 3 months.

Reported as mean ± S.D. (n=3)

The release profiles of lidocaine from the L-CNRL and L-DNR patches as initial preparation and after storage at 4, 25 and 45 °C for 3 months are shown in Fig. 30 and 31, respectively. These indicated that release amount from L-CNRL at initial preparation the patches and stored at 4, 25 and 45 °C for 3 months influence decreasing release, respectively. Furthermore, the linear regression of initial preparation the patches and stored at 4, 25 and 45 °C for 3 months were $y = 25.43x + 44.65$ ($R^2 = 0.632$), $y = 28.09x + 39.10$ ($R^2 = 0.755$), $y = 27.97x + 35.15$ ($R^2 = 0.792$) and $y = 26.69x + 29.93$ ($R^2 = 0.824$), respectively. Storage for long duration and at high temperature caused increasing rearrange of molecular chain (morton, 1987).

From the release profile of L-DNRL at initial preparation and storage at 4, 25 and 45 °C for 3 months. It could be found that patches at initial preparation and storage at 4 °C

had nearly release profile. Since low temperature to low affect to regulations rearrange of molecular chine (Morton, 1987). Release profile and release rate have agrees due to higher temperature affected decreasing release. Furthermore, the linear regression of initial preparation the patches and stored at 4, 25 and 45 °C for 3 months were y = 32.58x + 31.72 (R^2= 0.822), y = 30.39x + 33.97 (R^2= 0.855), y = 34.08x + 26.61 (R^2= 0.839) and y = 33.68x + 19.06 (R^2= 0.947), respectively.

The kinetic of drug release profile as higuchi pattern in L-CNRL and L-DNR patches was illustrate in Fig. 32 and 33, respectively. The release rates were illustrate in Table 12. The linear regression of initial preparation L-CNRL patches and stored at 4, 25 and 45 °C for 3 months of L-CNRL patches as higuchi pattern were y = 22.37x + 13.09 (R^2= 0.952), y = 21.41x + 10.57 (R^2= 0.971), y = 18.927x + 5.59 (R^2= 0.992) and y = 20.92x + 7.59 (R^2= 0.995), respectively. Furthermore, the linear regression of initial preparation L-DNR patches and stored at 4, 25 and 45 °C for 3 months of L-DNR patches as higuchi pattern were y = 23.43x + 1.41 (R^2=999), y = 20.82x + 7.69 (R^2= 0.995), y = 20.13x + 4.29 (R^2= 0.994) and y = 18.96x + 2.12 (R^2= 0.948), respectively. The release rate of initial preparation patch illustrate release faster than the patches as for long duration storage at different temperature. Since, the molecular chain in initial preparation patch might be not regular rearrange affected to easier drug releasing.

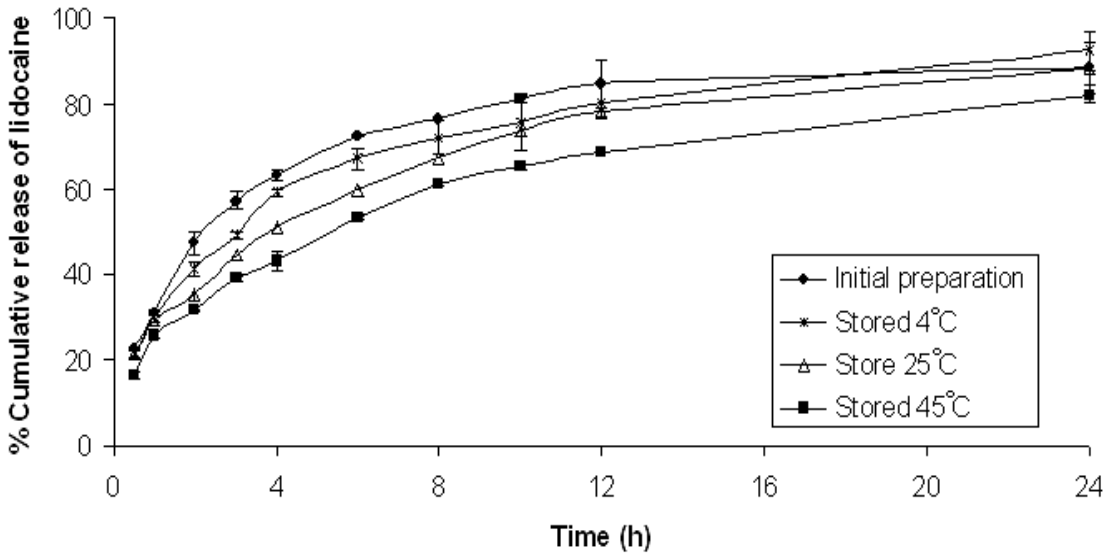


Fig. 30 *In vitro* release of lidocaine from CNRL patches when stored at different temperature for 3 months. Each point was the mean ±S.D. (n=3)

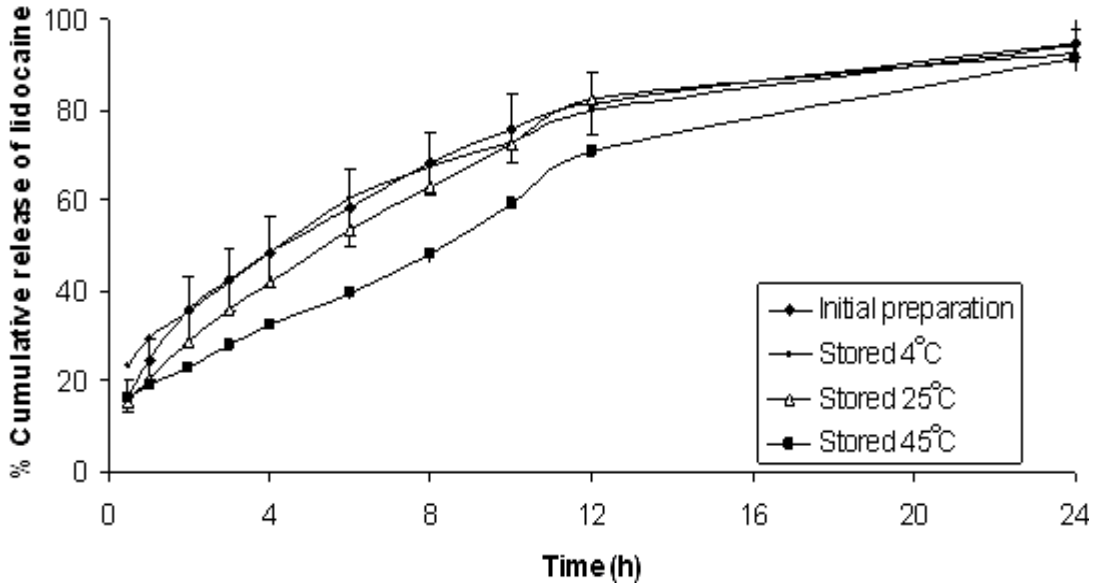


Fig. 31 *In vitro* release of lidocaine from DNR patches when stored at different temperature for 3 months. Each point was the mean ±S.D. (n=3)

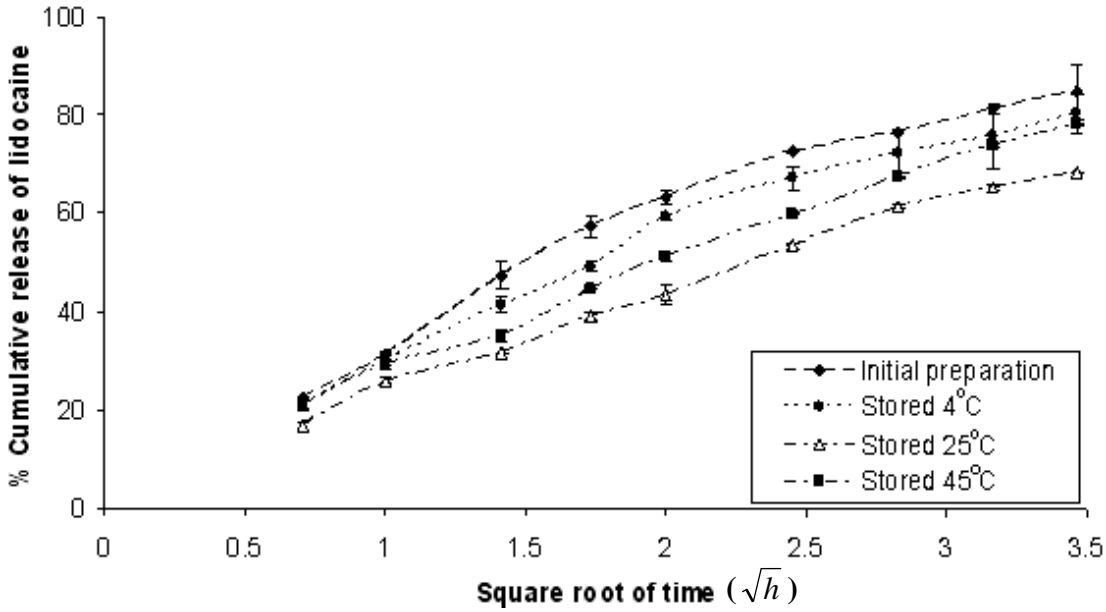


Fig. 32 Higuchi Plot of kinetic drug release profile from L-CNRL patches for 3 months.

Each point was the mean ±S.D.(n=3)

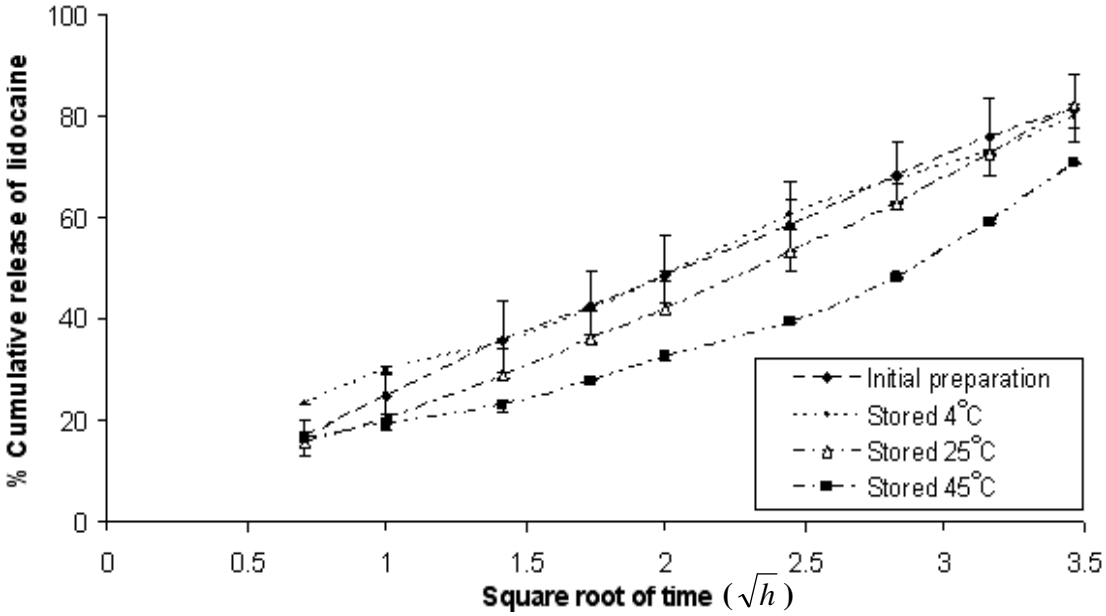


Fig. 33 Higuchi Plot of kinetic drug release profile from L-DNR patches for 3 months.

Each point was the mean ±S.D.(n=3)

Table 12. Release rate between 0.5 and 12 h of lidocaine from CNRL and DNR patches at different temperatures for 3-months storage. Each point was the mean ±S.D. (n=3).

Patches	**Release rate (%/$\sqrt{h}$)**
Initial preparation of L-CNRL	22.37 ± 0.99
Initial preparation of L-DNR	23.43 ± 1.02
Stored 4°C of L-CNRL	21.41 ± 2.77
Stored 4°C of L-DNR	20.82 ± 0.51
Stored 25°C of L-CNRL	18.93 ± 0.63
Stored 25°C of L-DNR	22.13 ± 0.24
Stored 45°C of L-CNRL	20.92 ± 0.35
Stored 45°C of L-DNR	18.96 ± 1.18

CHAPTER 5

CONCLUSIONS

In this study adherence property development of CNRL patches by additive incorporation, molecular chain cutting and studies of physical as well as drug release properties of L-CNRL and L-DNR patches.

Petroleum resin (a tackifying agent) and was used as additives in CNRL-patches. However, it was found that they could not improve the adherence ability of the patches, low molecular weight CNRL-patches were more sticky but not adherent as same as origin CNRL-patches. Therefore, these two methods were not suitable. Although CNRL-patches could not adhere on the skin by themselves, they can be used with adhesive backing. Thus, the investigated CNRL-patches do not be added additives or prepared using low molecular weight CNRL.

The patches were prepared from CNRL and DNR which were the representatives of liquid and solid rubber, respectively. The appearances of patches from both types of rubber were identically by translucent and brownish. However, the DNR patches had darker color than the CNRL-patches according to the color of raw materials.

The adherence ability of DNR patches was higher than CNRL patches, hence application of CNRL patches on the skin should be used with adhesive backing while the DNR patches may need no adhesive backing.

The SEM results indicated that types of rubber and drug-loading affected the surface morphology. The patches from CNRL had surface cracking due to evaporation of water while those from DNR had smmoth surface. From XRD spectrum, the patch preparation methods influenced the crystallinity of the loaded-drug. The FTIR and ATR-FTIR suggested that no chemical interaction of lidocaine and rubber was found.

The drug contents of L-CNRL and L-DNR were 1.90 $\pm$0.09 and 2.03$\pm$0.14 mg/cm^2, respectively. These contents were less than theoretical contents since the drug may inserted in matrix of molecular chain. Incorporation of lidocaine into CNRL and DNR matrix caused in decrease of moisture content since lidocaine molecules inserted in the matrix and resulted in decrease of cracking or pores as confirmed by SEM data.

The protein amounts in both types of patches were over the acceptable amount. Thus, deproteinized rubber should be used in the future studies to avoid allergy effects.

Both L-CNRL and L-DNR provided the initial fast release and constantly slow release in the late time of release. Types of rubber slightly affected the release profile while sides of L-CNRL gave obviously difference in pattern of release lidocaine. The kinetic of drug release profiles are higuchi pattern in L-CNRL and L-DNR patches. The stability data indicated that lidocaine-loaded patches (both L-CNRL and L-DNR) could keep at low temperature (4°C), these temperature less affected rearrange of chain molecule of rubber.

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APPENDIX A

HPLC ASSAY

The example of HPLC chromatogram of lidocaine is shown in Fig. 34. It can be noticed that the analysis method could separate the peaks of the drug and the components of the vehicle (IPB). The retention time of the lidocaine was approximate 9.0 min. The solvent peaks were found between 3.5 and 4.5 min. IPB did not provide any peaks from 4.5 to 12 min. Therefore, the analytical method had high selectivity for the model drug.

Fig. 35 present example of standard curve of lidocaine in IPB. It was found that standard curve gave high linear relationship between drug concentration and peak area (R^2>0.99). Table 13 shown the coefficient of variation of lidocaine at concentrations of 5, 75 and 150 µg/ml in both intra-day and inter-day precision. The results indicated that the analysis method gave acceptable coefficient of variation, i.e. less than 2%. However, % accuracy gave not acceptable in low concentration standard test.

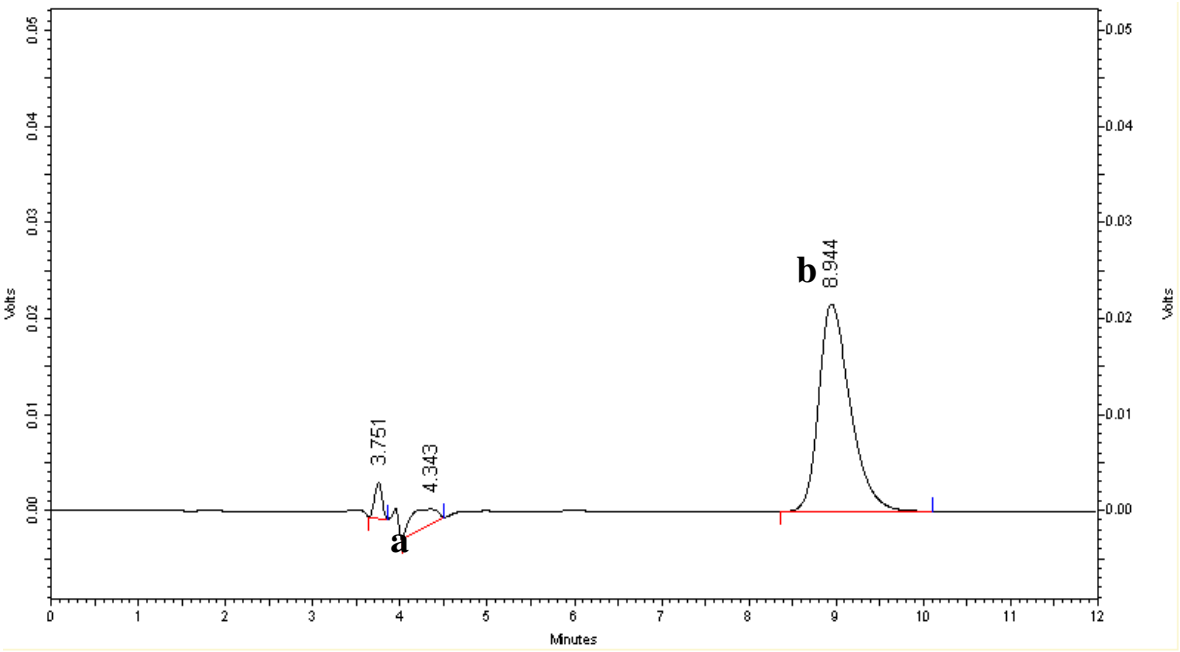

Fig. 34. HPLC chomatogram of (a) mobile phase and (b) lidocaine 100 µg/ml in IPB.

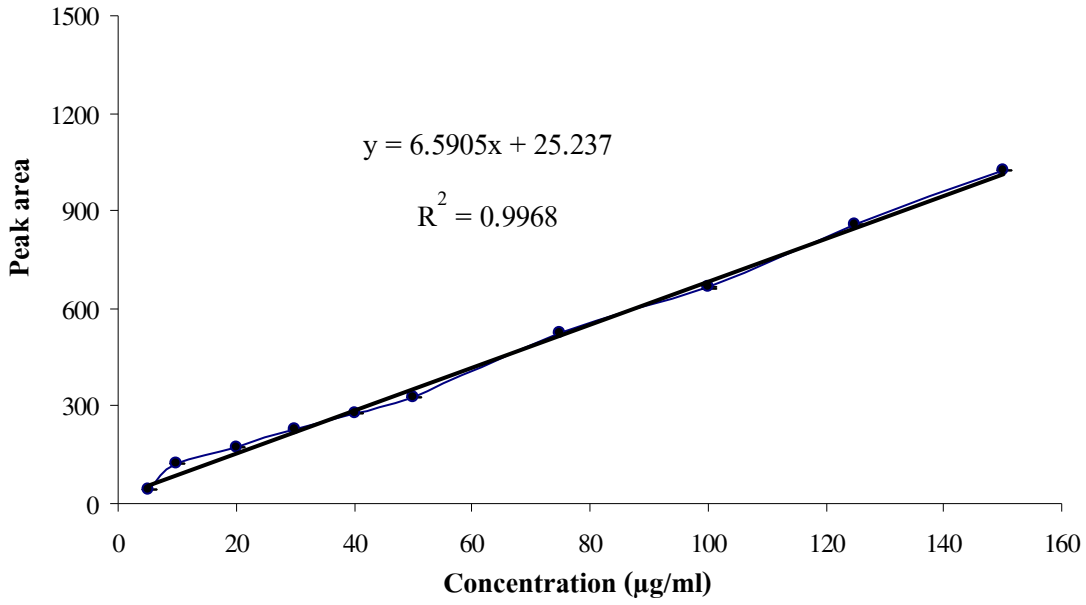

Fig. 35. Standard curve of lidocaine in IPB.

Table 13. Intra-day and inter-day precision of lidocaine at concentrations of 5, 75 and 150 µg/ml

	Coefficient of variation (%)			Accuracy (%)		
	5 µg/ml	75 µg/ml	150 µg/ml	5 µg/ml	75 µg/ml	150 µg/ml
Intra-day	1.14	1.55	0.55	94.49	102.29	99.45
Inter-day	1.80	1.43	1.99	93.31	104.05	99.42

APPENDIX B

STANDARD THAI RUBBER

Table 14. Block rubber comparison chart (Standard Thai Rubber , 1985)

		TSR CV	TSR L		TSR 5	TSR 10		TSR 20	
Parameter	Unit	STR 5 CV	STR XL	STR 5L	STR 5	STR 10	STR 10CV	STR 20	STR 20CV
Dirt (max)	% wt	0.04	0.02	0.04	0.04	0.08	0.08	0.16	0.16
Ash (max)	% wt	0.60	0.40	0.40	0.60	0.60	0.60	0.80	0.80
Nitrogen (max)	% wt	0.60	0.50	0.60	0.60	0.60	0.60	0.60	0.60
Volatile Matter (max)	% wt	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Initial Plasticity (min)		–	35	35	30	30	–	30	–
PRI Index (min)		60	60	60	60	50	50	40	40
Colour Lovibond Scale (individual value, max)		–	4.0	6.0	–	–	–	–	–
Mooney Viscosity** (ML, 1+4, 100°C)		60 +7 / -5	–	–	–	–	60 +7 / -5*	–	65 +7 / -5*

* Not specification status, but are controlled at the producer end.

** Note: Mooney Viscosity limits at time of production. Values will drift higher over time.

VITAE

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List of publication and proceeding

Boontawee H, Taweepreda W, Pichayakorn W, Boonme P. 2007. Formulation development of transdermal patches from natural rubber latex. The 1st Thailand-Japan Rubber Symposium, Thailand, 20-22 August, pp. 190-191.

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