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นางสาวศิริพร ง้อเจริญ

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DEVELOPMENT AND EVALUATION OF KETOPROFEN TRANSDERMAL PATCH

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for the Degree of Master of Science in Pharmacy

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ศึกษาการพัฒนาและการประเมินผลยาคีโตโปรเฟนในรูปแบบแผ่นแปะผิวหนัง การตั้งตำรับดำเนินการโดยใช้ยูเดรจิตอี 100 เป็นโพลิเมอร์ชนิดยึดติดในตัว ศึกษาผลของปริมาณยาในสูตรตำรับ ปริมาณของพลาสติไซเซอร์และชนิดของพลาสติไซเซอร์ต่อการปลดปล่อยตัวยาออกจากแผ่นแปะผิวหนัง การประเมินผลในหลอดทดลองพบว่า การปลดปล่อยตัวยาเป็นไปอย่างช้าๆ และใช้เวลานาน สูตรตำรับที่ให้ปริมาณการปลดปล่อยตัวยาคีโตโปรเฟนได้มากที่สุดประกอบด้วยสัดส่วนของตัวยาและพลาสติไซเซอร์ต่อปริมาณของยูเดรจิตอี 100 ที่ใช้ในสูตรตำรับเท่ากับ 35 และ 40 ตามลำดับ พลาสติไซเซอร์ที่ให้ปริมาณการปลดปล่อยตัวยาที่เหมาะสมคือ ไตรบิวทิลซิเตรต โพรพิลีนไกลคอล และกลีเซอริน ซึ่งให้ปริมาณการปลดปล่อยตัวยา 5.27, 5.19 และ 4.34 มิลลิกรัมต่อตารางเซนติเมตร ตามลำดับ ตำรับที่ประกอบด้วยพลาสติไซเซอร์แต่ละชนิดที่ให้ปริมาณการปลดปล่อยที่ดีที่สุดถูกคัดเลือกนำไปศึกษาในสัตว์ทดลองต่อไป

การศึกษาในสัตว์ทดลองดำเนินการในกระต่ายขาวพันธุ์นิวซีแลนด์จำนวน 9 ตัว กระต่ายแต่ละตัวจะได้รับยาแผ่นแปะผิวหนังคีโตโปรเฟน 3 แผ่นของแต่ละตำรับทั้งสามสูตรตามวิธีการทดลองข้ามสลับ เก็บตัวอย่างเลือดตามช่วงเวลาที่กำหนดและนำไปหาปริมาณยาโดยใช้ HPLC พบว่ายาแผ่นแปะผิวหนังคีโตโปรเฟนสามารถปลดปล่อยตัวยาเข้าสู่ระบบการไหลเวียนของโลหิต ผลการทดสอบทางเภสัชจลนศาสตร์ของยาแผ่นแปะผิวหนังคีโตโปรเฟนพบว่า ค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ (AUC_{0-24}, $AUMC_{0-24}$ และ MRT) มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ระดับความเชื่อมั่นร้อยละ 95

ผลการศึกษาสรุปได้ว่า ปริมาณคีโตโปรเฟน ปริมาณของพลาสติไซเซอร์และชนิดของพลาสติไซเซอร์ในสูตรตำรับยาแผ่นแปะผิวหนังมีผลต่อการปลดปล่อยยาและเภสัชจลนศาสตร์ในร่างกาย

ภาควิชา	เภสัชกรรม	ลายมือชื่อนิสิต……..……..
สาขาวิชา	เภสัชกรรม	ลายมือชื่ออาจารย์ที่ปรึกษา……..…
ปีการศึกษา	2544	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..…...……

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KEY WORD: DEVELOPMENT / EVALUATION / KETOPROFEN / TRANSDERMAL PATCH

SIRIPORN NGOHCHAROEN: DEVELOPMENT AND EVALUATION OF KETOPROFEN TRANSDERMAL PATCH. THESIS ADVISOR: ASSOC. PROF. UTHAI SUVANAKOOT, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. POJ KULVANICH, Ph.D. 145 pp. ISBN 974-03-0800-7.

Development and evaluation of ketoprofen transdermal patches were studied. Formulations were prepared using Eudragit® E 100 as a polymer (pressure-sensitive adhesive). The effects of drug loading, amount and type of plasticizer on drug released from transdermal patches were investigated. *In vitro* studies found that ketoprofen transdermal patches gave a slow and prolonged release. The formulations which gave satisfactorily ketoprofen release were those with ketoprofen and plasticizer to amount of Eudragit® E 100 used in formula in the proportion of 35 and 40, respectively. Plasticizer that gave appropriate release was found to be tributyl citrate, propylene glycol and glycerin with the amount of 5.27 mg/cm^2, 5.19 mg/cm^2 and 4.34 mg/cm^2, respectively. Formulations containing each of these three plasticizers that gave the best release were subsequently selected for *in vivo* studies.

In vivo studies were performed using nine white New Zealand rabbits. Each rabbit received three ketoprofen transdermal patches of the individual three formulation in a crossover design. Blood samples were collected at predetermined time intervals and quantitated using HPLC. It was clearly shown that ketoprofen could be released from the transdermal patch into systemic circulation. Pharmacokinetic studies of ketoprofen transdermal patches demonstrated that the pharmacokinetic parameters obtained (AUC_{0-24}, $AUMC_{0-24}$ and MRT) were statistically significant difference ($P<0.05$).

It can be concluded that the amount of ketoprofen loaded, concentration of plasticizer, and type of plasticizer in the patches affected the ketoprofen release and pharmacokinetic of drug.

Department	Pharmacy	Student's signature…….……..
Field of study	Pharmacy	Advisor's signature…….…….
Academic year	2001	Co-advisor's signature…….……

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LIST OF ABBREVIATIONS

ANOVA	=	analysis of variance
AUC	=	area under the plasma concentration - time curve
AUMC	=	area under the moment curve
^{O}C	=	degree of Celcius
cm	=	centimeter
conc.	=	concentration
C.V.	=	coefficient of variation
d.f.	=	degree of freedom
et al.	=	and others
g	=	gram
hr	=	hour
HPLC	=	high performance liquid chromatography
i.e.	=	that is
k	=	release rate constant
L	=	liter
L.A.	=	labeled amount
mg	=	milligram
min	=	minute
mL	=	milliliter
MRT	=	mean residence time
MS	=	mean square
nm	=	nanometer
NSAIDs	=	non-steroidal antiinflammatory drugs
P	=	probability
PAR	=	peak area ratio

pp.	=	pages
PSA	=	pressure-sensitive adhesive
R^2	=	coefficient of determination
rpm	=	revolution per minute
S.D.	=	standard deviation
SS	=	sum of squares
t	=	time
TDDS	=	transdermal drug delivery system
UV	=	ultraviolet
μg	=	microgram
μL	=	microliter
μm	=	micrometer
λmax	=	wavelength of maximum absorption
%w/w	=	pecent weight by weight

CHAPTER I

INTRODUCTION

Previously, dispensing drug-products to treat the disease for the patients, the popular dosage forms are tablets, capsules, pills, cream, ointments, liquids, aerosols, injections and suppositories. Such dosage forms have long been using until now. However, if the desired plasma drug level for the therapeutic efficacy has to be constantly maintained, repeated administration of these conventional dosage forms has to be given.

Presently, the advancement of technologies for manufacturing the new and modern dosage form have been developed. Bioavailability as well as longer duration of action of certain drugs can be controlled. One of the popular dosage form with wide interest is a transdermal drug delivery system. It is anticipated that the transdermal drug delivery system can be designed to give the drug input at an appropriate rate to achieve and maintain suitable plasma drug levels for optimum therapeutic efficacy. Moreover, patient compliance and acceptance would be enhanced because of the convenience of administration of these preparations.

Ketoprofen is a nonsteroidal antiinflammatory drug of the propionic acid derivative. It is an analgesic and antipyretic drug which has been used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosis spondylitis, gout, primary dysmenorrhea and other pain (Gurol, 1996). It is administered via several routes. By the oral route, the dose is 100-200 mg per day, 2-4 times in divided dose because its half-life in plasma is only 1-3 hours (Olin, 1995). It is equal to or more potent than the other nonsteroidal antiinflammatory drugs (NSAIDs) with respect to some effects such as anti-inflammatory and analgesic activities (Saxena, 1978). Although ketoprofen is rapidly absorbed,

metabolized and excreted, it still causes some gastrointestinal complaints such as nausea, dyspepsia, diarrhea, constipation and some renal side effects like other NSAIDs (Fossgreen, 1976). Therefore, there is a great interest for ketoprofen to be developed and formulated in the form of transdermal drug delivery system in order to avoid the GI-side effects and provide relatively consistent plasma drug levels for a long period of time.

All of the transdermal drug delivery systems have basic similarities in device components (Baker and Heller, 1989). They contain backing membrane, peel strip and adhesive. In recent years, various pressure-sensitive adhesives were considered for fabricating transdermal delivery system. To fabricate such a transdermal device, the drug was either dissolved or dispersed in a polymeric solution, and a thin film of desired thickness was then prepared by the solvent-cast method (Borodkin, 1974).

There are many reports presenting the investigation about transdermal drug delivery systems. Among these, the methods of preparing are to be concerned. Preparation via adhesive dispersion-type transdermal drug delivery system is well accepted (Samir, 1996) due to ease of preparation with a resulting thinner film and economical benefits.

In this study, ketoprofen transdermal patch was designed as an adhesive dispersion-type transdermal drug delivery system by using Eudragit® E 100 as a polymer adhesive and varying loading dose of drug, type and concentration of plasticizers. The transdermal patches obtained were evaluated for their *in vitro* and *in vivo* properties.

Objective of this study

1. To formulate ketoprofen transdermal patches by adhesive dispersion-type transdermal drug delivery system technique using Eudragit® E 100 as a pressure – sensitive adhesive (PSA).

2. To evaluate *in vitro* release characteristics of ketoprofen transdermal patches.

3. To evaluate the pharmacokinetics of ketoprofen transdermal patches in rabbits.

CHAPTER II

REVIEW OF LITERATURES

1. History of transdermal drug delivery systems

Over the past two decades, there have been significant advances in the science of controlled transdermal systemic drug delivery, but the concept of systemic therapy via the skin is not a recent innovation. Over several centuries attempts have been made to treat both local and systemic ailments by the application of herbal potions and medicaments to the skin (Hutchens, 1973). Several ancient cultures used ointments, poultices, and plasters in the belief that they would alleviate a variety of symptoms. For example, the ancient Greeks used a mixture of water, olive oil, and lead oxide as a balm. The efficacy of this formulation was probably attributable to the astringent properties of lead oxide and the emollient activity of the olive oil, and this cannot, therefore, be considered as a transdermal delivery system because the effect was the result of activity of the formulation on the skin. On the other hand, the use of poultices of crushed and pulped Comfey or Longwort herbs, for the treatment of inflammation and swellings, where the active principle or principles had to cross the skin, may be considered a transdermal delivery system.

Various transdermal drug delivery systems (TDDS) have recently been developed aiming to achieve the objective of systemic medication through topical application on the intact skin surface. It is exemplified first with the development of scopolamine-releasing TDDS (Transderm-Scop® by Ciba Geigy) for 72 hours prophylaxis or treatment of motion-induced nausea (Shaw and Chandreasekaran, 1978), and then by the successful marketing of nitroglycerin – releasing TDDS (Deponit® by Pharma Schwarz/Lohmann,

Nitrodisc® by Searle, Nitro-Dur® by Key, and Transderm-Nitro® by Ciba Geigy) as well as isosorbide dinitrate-releasing TDDS (Frandol® Tape by Toaeiyo, Yomanouchi) for once-a-day medication of angina pectoris, clonidine-releasing TDDS (Catapress-TTS® by Boehringer Ingelheim) for weekly therapy of hypertension, estradiol-releasing TDDS (Estraderm® by Ciba Geigy) containing 17-β-oestradiol for twice-a-week treatment of postmenopausal symptoms associated with the female menopause (Good et al. 1985). The most recently by the regulatory approval of a nicotine releasing transdermal drug delivery system (Habitrol® by Basel Pharmaceutical, Nicoderm® by Marion-Merrell Dow, Nicotrol® by Warner-Lambert Co., Prostep® by Lederle and Nicotinell® by Ciba Geigy) as an aid in reducing the craving for cigarettes have been released. Current commercial transdermal drug delivery is shown in Table 1 (Sifton, 1994; Cocaba and Kin, 1993; Berba and Banakar, 1990; Sugibayashil and Morimoto, 1994).

The use of the skin as a route of delivery into the systemic circulation was not commercially or scientifically exploited until the 1950s. The development of ointments containing agents such as nitroglycerin and salicylate dispelled the notion that the skin was largely impermeable, simply because they were shown to be therapeutically effective. Angina, for example, could be controlled for several hours by applying an ointment containing 2% nitroglycerin (Reichek et al. 1974). Similarly, topical salicylates could be absorbed through the skin into arthritic joints. More recently nonsteroidal anti-inflammatory agents, such as ibuprofen and ketoprofen, estradiol and testosterone have been developed and marketed as semisolid preparations. A major problem with semisolid preparations is, however, that of control drug concentrations in plasma and duration of action are not reliably predictable for several reasons, most of which are patient dependent. Dosage frequency and amount and area of application can affect therapeutic efficacy by the most significant factors are the inter- and intra - individual variation in skin permeability. The seminal work of Scheuplein and Blank

Table 1. Representative of commercial transdermal products (Govil, 1988).

Product name	**Company**	**Type of device**	**Polymer**
Scopalamine (motion sickness)	Alza/Ciba	Reservoir	PIB, EVA
Transderm-Scop	Myun Moon	Reservoir	
Kimete Patch			
Nitroglycerin (angina)			
Transderm-Nitro (Nitroderm TTS)	Alza/Ciba	Reservoir	EVA, Silicone
Nitro-Dur II (Diafusor)	Key/Schering	Matrix	Acrylic type PSA
Nitrodisc	G.D. Searle	Microsealed	Silicone, PEG
Deponit	Lohman/Schwarz/Wyeth	PSA adhesive	
Minitran	3M Riker	PSA	
NTS Patch (Nitrocine)	Hercon/Bolar	Matrix	PVC
Nitrol	Paco/Adria	Matrix	PVC
Herzer (Myristol)	Nichiban/Taiho/Nihon Kayaku	PSA	Acrylic type PSA
Isosorbide dinitrate (angina)			
Frandol Tape-S	Toeiyo/Yamanouchi	PSA	Acrylic type PSA
Apatya Tape	Teikoku	PSA	Acrylic type PSA
Antup Tape	Tisan/Tejin	PSA	Hollow fiber
Isopit Tape	Toko/Mitsui	PSA	Rubber type PSA
Sawadol Tape	Sawai	PSA	Acrylic type PSA
Nitrous Tape	Taikyo	PSA	
Penety ISDN Tape	Sekisui	PSA	Acrylic type PSA
Rifatac	Meiji	PSA	Acrylic type PSA
Clonidine (hypertension)			
Catapress TTS	Alza/Boehringer Ingelhelm	Reservoir	PIB, PP
Estradiol (hormone treatment)			
Estradiol	Ciba	Reservoir	HPC, EVA, PIB
Nicotin (aid to smoking cessation)			
Nicotinell TTS	Lohman/Ciba	Woven	
Niconil (Prostep, Nicolan, Nicotrans)	Elan		
Nicoderm*	Alza		
Habitrol	Ciba		
Nicotrol	Cygnus		
Fentanil (opioid analgesic)			
Duragesic	Alza/Ivers Lee Reservoir		
Mepindodol (hypertension)			
Pharmed*		Bio-TSD	

*FDA or related organization approved.

(Sheuplein and Blank, 1971) opened a floodgate of research into skin permeation which has finally resulted in the development of modern controlled transdermal drug delivery.

2. The skin – site for transdermal drug administration

The skin of an average adult body covers a surface area of approximately 2 square meters and receives about one-third of the blood circulation through the body (Jacob and Francone, 1970). The skin is a multilayered organ that is complex in both structure and function. Macroscopically, two distinct layers are apparent; the outer epidermis and the inner dermis. Microscopically, the skin is a multilayered organ composing of many histological layers: the epidermis, the dermis, and the hypodermis (Figure 1). The epidermis is further divided into five anatomical layers with stratum corneum forming the outermost layer of the epidermis and exposing to the external environment.

The stratum corneum consists of many layers of compacted, flattened, dehydrated and keratinized cells. These cells are physiologically rather inactive and are continuously shed with constant replacement from the underlying viable epidermal tissue (Zanowiak and Jacob, 1982). The stratum corneum has water content of only 20% as compared to the normal physiologic level of 70%.

An average human skin surface is known to contain, on the average, 40-70 hair follicles and 200-250 sweat ducts on every square centimeters of skin area. These skin appendages, however, actually occupy, grossly, only one-tenth of one percent (0.1%) of the total human skin surface. Even though the foreign agents, especially the water-soluble ones, may be able to penetrate into the skin via these skin appendages at a rate which is faster than through the intact area of the stratum corneum, this

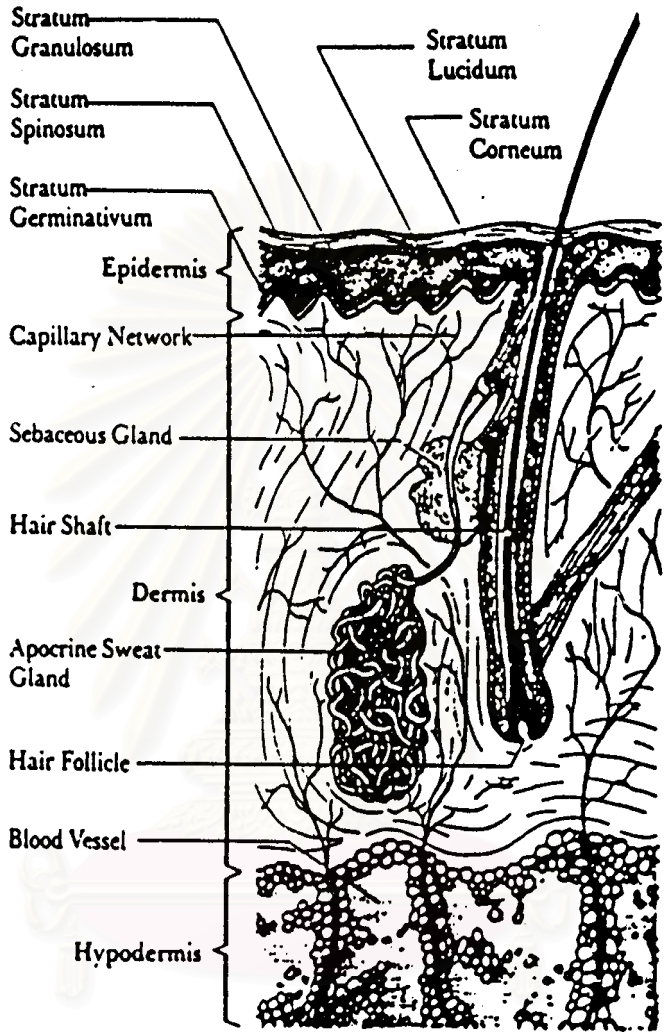


Figure 1. Cross-sectional view of human skin, showing various skin tissue layers and appendages.

transappendageal route of percutaneous absorption has, however, provided a very limited contribution to the overall kinetic profile of transdermal permeation.

The dermis is composed of a network of collagen and elastin fibers embedded in a mucopolysaccharide matrix, that also contains blood vessels, lymphatics, and nerve ending, and which provides physiological support for the epidermis. Because the blood vessels approach the interface of the two layers very closely, the dermis cannot be considered as a significant barrier to inward drug permeation in vivo. The epidermis comprised the viable epidermis and the stratum corneum. The cells of the viable epidermis undergo continuous differentiation to produce the stratum corneum.

In the case that the skin serves as the port of administration for systemically-active drugs, the drug applied topically is distributed, following absorption, first into the systemic circulation and then transported to target tissues, which could be relatively remote from the site of drug application, to achieve its therapeutic action. This new application is exemplified by the transdermal controlled delivery of nitroglycerin to myocardium for the treatment of angina pectoris, of scopolamine to the vomiting center for the prevention of motion-induced sickness, and of estradiol to various estradiol-receptor sited for the relief of postmenopausal syndromes (Shaw et al. 1976).

3. System designs for transdermal drug delivery

Several system designs capable of providing rate-controlled release of drugs have been used in the development and fabrication of TDDS. Each of these designs aims to facilitate the release of drug at a specific rate. The system designs that have been introduced in the market can be classified into the following 4 type:

3.1 Polymer membrane permeation-controlled TDDS

In this system, the drug reservoir is sandwiched between a drug-impermeable metallic plastic laminate and a rate-controlling polymeric membrane. The drug molecules are permitted to release only through the rate-controlling polymeric membrane. In the drug reservoir compartment, the drug solids are either dispersed homogeneously in a solid polymer matrix, suspended in and unleachable, viscous medium to form a paste-like suspension, or dissolved in a releasable solvent to form a clear drug solution. The rate-controlling membrane can be either a microporous or a non-porous polymeric membrane, e.g., silicone or polyisobutylene adhesive, may be applied to provide an intimate contact of the TDDS with the skin surface. The rate of drug release from this TDDS can be tailored by varying the composition of drug reservoir formulation, the permeability coefficient and/or thickness of the rate-controlling membrane. Several TDDS have been successfully developed from this technology and approved by FDA for marketing, such as the Transderm-Nitro® system, for once-a-day medication of angina pectoris, Transderm-Scop® system for 3-day protection of motion sickness, Catapress-TTS® system for weekly therapy of hypertension, and Estraderm® system for twice-a-week treatment of postmenopausal syndromes.

The intrinsic rate of drug release for this type of drug delivery system is defined by:

$$\frac{dQ}{dt} = \frac{K_{m/r}K_{a/m}D_aD_m}{K_{m/r}D_mh_a + K_{a/m}D_ah_m} \cdot C_r \quad \text{.....(1)}$$

where : C_r = drug concentration in the reservoir compartment.

$K_{m/r}$ = partition coeffificients for the interfacial partitioning of drug from the reservoir to the membrane.

$K_{a/m}$ = partition coeffificients for the interfacial partitioning of drug from the membrane to the adhesive.

D_m = diffusion coefficients in the rate-controlling membrane.

D_a = diffusion coefficients in the adhesive layer.

H_m = thickness of the rate-controlling membrane.

H_a = thickness of the adhesive layer.

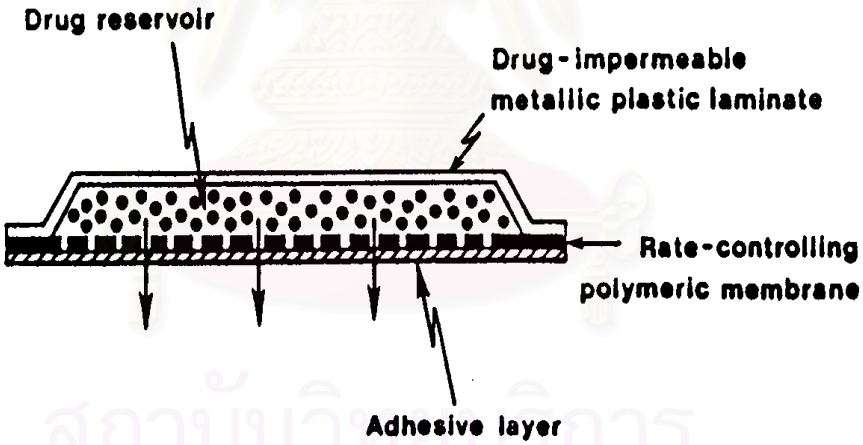


Figure 2. Cross-sectional view of a polymer membrane permeation-controlled TDDS.

3.2 Polymer matrix diffusion-controlled TDDS

In this system, the drug reservoir is formed by homogeneously dispersing the drug solids in a hydrophilic or lipophilic polymer matrix and the medicated polymer formed is then molded into medicated discs with a defined surface area and controlled thickness. This drug reservoir containing polymer disc is then mounted onto an occlusive baseplate in a compartment fabricated from a drug-impermeable plastic backing. Instead of applying the adhesive polymer directly on the surface of the medicated disc, in the present system the adhesive polymer is spread along the circumference of the patch to form a strip of adhesive rim around the medicated disc. The rate of drug release from this matrix diffusion-controlled TDDS is defined as:

$$\frac{dQ}{dt} = \left| \frac{AC_pD_p}{2t} \right|^{1/2} \qquad \text{.....(2)}$$

Where A = drug loading dose initially dispersed in the polymer matrix.

C_p = solubility of the drug in the polymer.

D_p = diffusitivity of the drug in the polymer.

At steady state, a Q vs $t^{1/2}$ drug release profile is obtained as defined by:

$$\frac{Q}{t^{1/2}} = [(2A-C_p)\, C_pD_p]^{1/2} \qquad \text{.....(3)}$$

This type of TDDS is exemplified by the development and marketing of Nitro-Dur® system and NTS® system, which have been approved by FDA for once-a-day medication of angina pectoris.

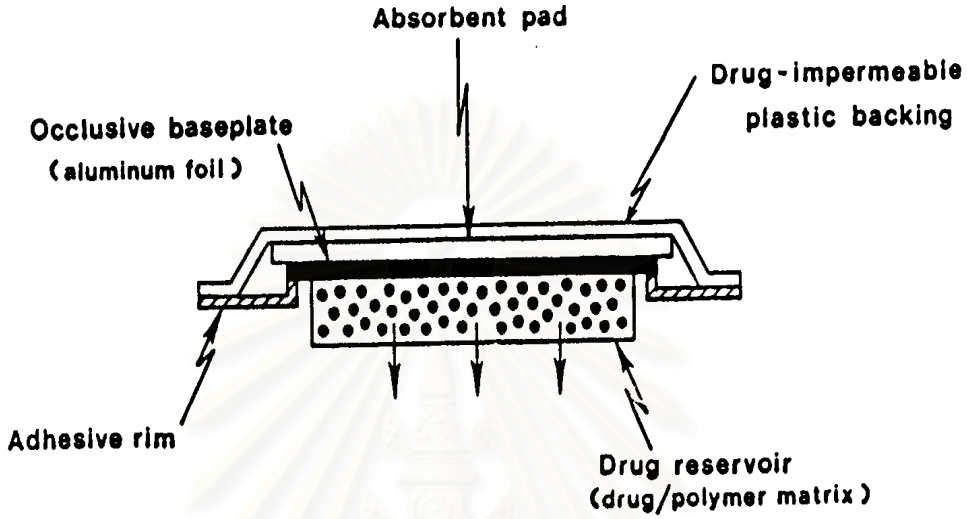


Figure 3. Cross-sectional view of a polymer matrix diffusion-controlled TDDS.

3.3 Adhesive dispersion-type TDDS

This type of drug delivery system can be viewed as a simplified version of the membrane-moderated drug delivery system, in which the release of drug molecules from the drug-dispersing reservoir is meterd by permeation through a rate-controlling membrane. In this system, the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer, e.g., polyisobutylene or polyacrylate, and then spreading the medicated adhesive, by solvent casting or hot melt, onto a flat sheet of drug-impermeable backing support to form a single or multiple layers of drug reservoir. The

released profiles of drug from this type of TDDS will not be constant, as expected from the matrix diffusion process.

This type of TDDS is best illustrated by the development and marketing of the isosorbide dinitrate-releasing TDDS, named Frandol® system and Nitro-Dur® system for once-a-day medication of angina pectoris.

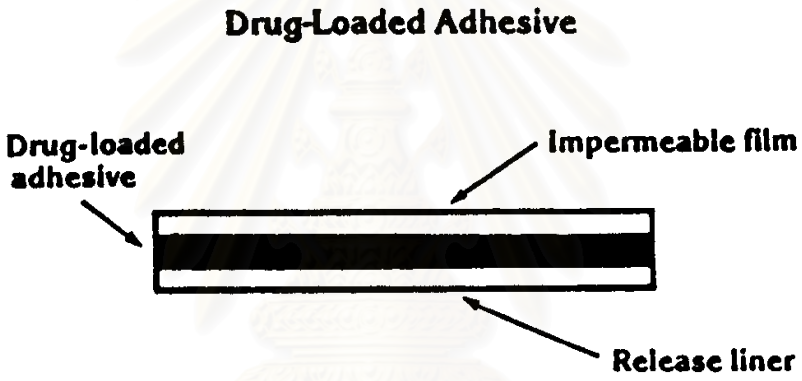


Figure 4. Cross-sectional view of an adhesive dispersion-type TDDS showing various major structural components.

To overcome the non-constant drug release profiles, this type of TDDS can be modified to have the drug loading level varied at increment manner to form a gradient of drug reservoir along the multilaminate adhesive layers. The rate of drug release from this drug reservoir gradient-controlled TDDS can be expressed by:

$$\frac{dQ}{dt} = \left| \frac{K_{a/r} D_a}{h_a (t)} \right| . A (h_a) \qquad \text{..... (4)}$$

where $K_{a/r}$ = partition coefficient for the interfacial partitioning of drug from the reservoir layer to the rate - controlling adhesive layer.

In equation (4), the thickness of adhesive layer for drug molecules to diffuse through is increasing with time [$h_a(t)$]. To compensate this time-dependent increase in diffusional path as a result of drug depletion by release, the drug loading level is also increased proportionally [$A(h_a)$]. A constant drug release profile is thus produced. This type of TDDS is best illustrated by the development of a nitroglycerin-releasing TDDS, named Deponit® system.

3.4 Microreservoir dissolution-controlled TDDS

This type of drug delivery system can be considered as a hybrid of the reservoir-type and matrix dispersion-type drug delivery system. In this system, the drug reservoir is formed by first suspending the drug solids in the aqueous solution of a water-soluble polymer, e.g., polyethylene glycol, and then dispersing homogeneously the drug suspension in a lipophilic polymer, by high-shear mechanical force, to form thousands of unleachable microscopic drug reservoir. This thermodynamically unstable dispersion is quickly stabilized by immediately cross-linking the polymer chains *in situ*, which produces a medicated polymer disc with a constant surface area and a fixed thickness. A TDDS is then produced by forming the medicated disc at the center of an adhesive pad. This system has been successfully utilized in the development and marketing of Nitrodisc® system, which has been approved by FDA for once-a-day treatment of angina pectoris.

The rate of drug release from the microreservoir-type drug delivery system is defined by:

$$\frac{dQ}{dt} = \frac{D_p D_s \alpha' K_p}{D_p \delta_d + D_s \delta_p \alpha' K_p} \left[\beta S_p - \frac{D_l S_l (1-\beta)}{\delta_l} \left| \frac{1}{K_l} + \frac{1}{K_m} \right| \right] \quad \text{.....(5)}$$

where $\alpha' = \partial' / \beta'$

∂' = ratio of the drug concentration in the bulk of elution solution over the drug solubility in the same medium.

β' = ratio of the drug concentration at the outer edge of the polymer coating membrane over the drug solubility in the same polymer composition.

K_l = partition coefficient for the interfacial partitioning of drug from the liquid compartment to the polymer matrix.

K_m = partition coefficient for the interfacial partitioning of drug from the polymer matrix to the polymer coating membrane.

K_p = partition coefficient for the interfacial partitioning of drug from the polymer coating membrane to the elution solution (or skin).

D_l = drug diffusivities in the liquid compartment.

D_p = drug diffusivities in the polymer coating membrane.

D_s = drug diffusivities in the elution solution (or skin).

S_l = the solubilities of the drug in the liquid compartment.

S_p = the solubilities of the drug in the polymer matrix.

δ_l = the thickness of the liquid layer surrounding the drug particles.

δ_p = the thickness of the polymer coating membrane around the polymer matrix.

δ_d = the thickness of the hydrodynamic diffusion layer surrounding the polymer coating membrane.

β = ratio of the drug concentration at the inner edge of the interfacial barrier over the drug solubility in the polymer matrix.

Release of drugs from the microreservoir-type drug delivery system can follow either a partition-control or matrix diffusion-control process depending upon the relative magnitude of S_l and S_p. So, a Q vs. t or Q vs. $t^{1/2}$ release profile is resulted.

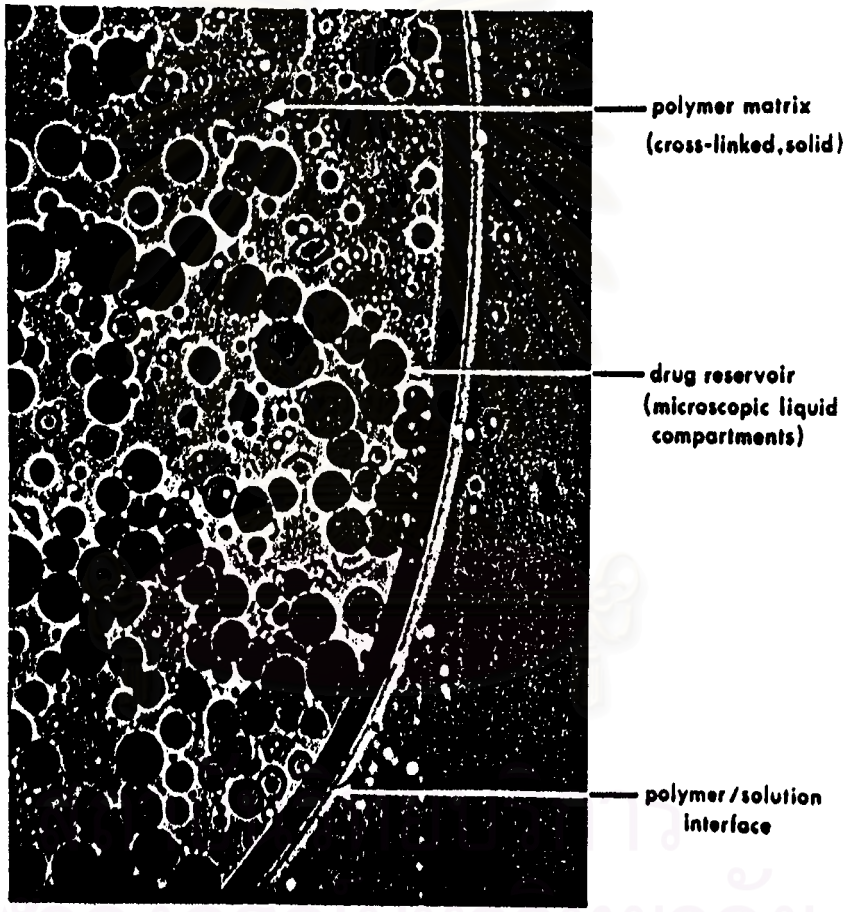


Figure 5. Photomicrograph of a microreservoir dissolution-controlled drug delivery system, showing its microscopic structure.

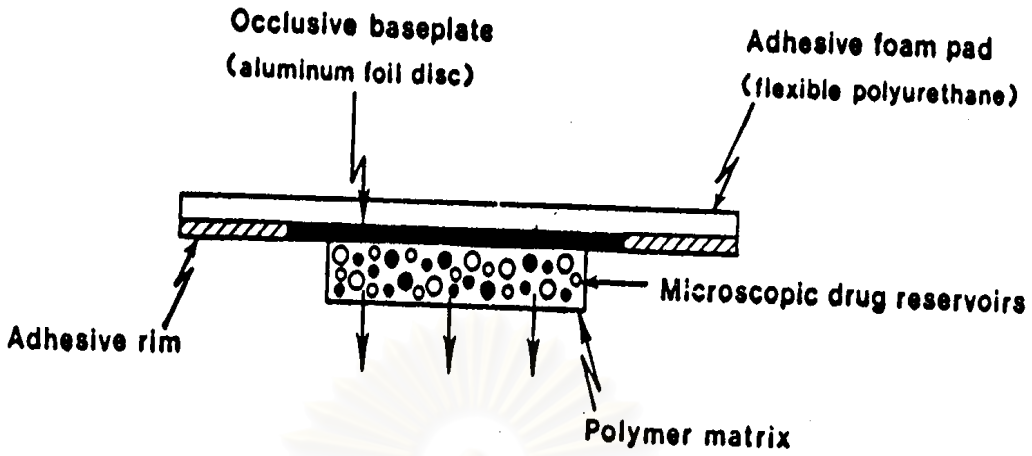


Figure 6. Cross-sectional view of a microreservoir dissolution-controlled transdermal drug delivery system.

Advantages of transdermal drug delivery systems.

A well-designed transdermal controlled-release drug delivery system is expected to provide most of the benefits outlined as follows (Handgraft, 1983; Govil, 1988; Chien, 1983; Berner, 1994; Chien, 1987; and Walters, 1986):

1. Bypass hepatic first-pass metabolism and gastrointestinal incompatibility of drug.
2. Reduce side effects due to the optimization of the blood concentration-time profile.
3. Provide predictable and extended duration of drug action, leading to a lower frequency of administration and total daily dose.
4. Improve patient compliance due to the elimination of multiple dosing schedules.
5. Enhance therapeutic efficacy.
6. Reduce frequency of dosing.
7. Minimize inter- and intra-patient variations.
8. Avoidance of peak and trough plasma concentration profile.

9. Rapid termination of drug input by removal of the system from the surface of the skin.
10. Avoid the risks and inconveniences of intravenous therapy.
11. A relatively large area of application in comparison with the buccal or nasal cavity.
12. The ability to change the site of drug delivery thereby reducing the risks of adverse reactions or toxicity due to repeated exposure at a single site.
13. The ability to avoid a changing physiological environment, and chemical or metabolic degradation (e.g. changing pH, luminal microflora involvement in the gut).
14. Drugs with narrow therapeutic range can be delivered.
15. Drugs with short half-life are utilized.
16. Self administration.
17. The patient can immediately stop the administration by removing the patch.

Device components

All of the transdermal therapeutic systems have basic similarities in device components (Baker and Heller. 1989). They contain backing membrane, adhesive, peel strip and packet.

1. Backing layer

The primary function of the backing layer is to provide support. Typical backing membranes are composed of a pigmented layer, an aluminum-vapor-coated layer, a plastic film (Polyethylene, polyvinyl chloride, or polyester). The backing layer must be impermeable to the drugs and enhancers, have a low moisture vapor transmission rate (MVTR) and have optimal mechanical properties, such as elasticity, flexibility, and tensile strength. Common materials are polyester (Mylar)-polyethylene co-extruded films, polypropylene and ultralow and linear-low density polyethylene

resins. The backing films may be clear (Estraderm®), flesh-colored (Catapres-TTS®), or metalized (Transderm-Scop®). Other nonporous plastics with similar properties could well be used. A new backing material recently marketed by 3M Pharmaceuticals is the CoTran® 9722 polyolefin film. This newer material has been shown to meet the requirements for TDDS integration, patient compliance, and ease of manufacturing. Other backings such as the 3-mil low density polyethylene offer the advantages of transparency and greater flexibility.

2. *Adhesive layer*

The adhesive must hold the device securely in place for periods as long as a week, and removal must not be painful so as to discourage patients from using it. Also, when removed, all the adhesive should remain on the device, leaving no residue on the patient's skin. The adhesive layer can be casted directly onto the skin-facing side of the membrane or monolithic as a thin film. The adhesives commonly used in transdermal delivery devices are pressure-sensitive adhesives.

Pressure-sensitive adhesives

Pressure-sensitive adhesives have been found for application in transdermal drug delivery because of the need to maintain an intimate contact between the transdermal system and the skin surface. Hogson (Hogson, 1978) has defined a pressure-sensitive adhesive as a material that adheres to a substrate when applied with light pressure and that leaves little or no residue when removed. Typically, pressure-sensitive adhesive products consist of webs of paper, plastic films or sheets, or metal foils covered on one side with a coating of a solventless, solid, adhesive composition. The adhesive of the patch must have sufficient tackiness to permit these materials to

adhere to the surface of an object by contacting and momentarily pressing the adhesive layer-carrying side of the web directly against the object surface.

The first adhesive used in a medical application involved tackified natural rubber filled with zinc oxide and used in hospital tape in 1899. During World War II, isobutylene and butyl rubber-based pressure-sensitive adhesives were developed to replace the waning supply of natural rubber. Acrylic adhesives were developed in the post war years. Since 1950, a great number of polymers have been developed and used as pressure-sensitive adhesives, including a relative newcomer, silicone adhesives. Acrylic-based adhesives have traditionally been used for a variety of medical applications. They are usually less irritating than silicone adhesives, and are available in porous grades that air and water vapor are permeable. Migration of plasticizers, drugs, or other components into the adhesive during storage may also be less pronounced with acrylic adhesives than with other types. The choice of adhesive depends on the specific properties desired for the transdermal system. The polyisobutylenes are inherently tacky and flexible. They can be easily blended by varying the polymer composition to obtain a wide range of properties. In contrast to the other adhesives, the polyisobutylenes are the only adhesives that can be formulated in-house. The acrylates and silicones are stable and do not require stabilizing additives. Although better adhesion is generally achieved with natural and synthetic rubber-based formulations, these are more likely to cause skin irritation upon prolonged contact.

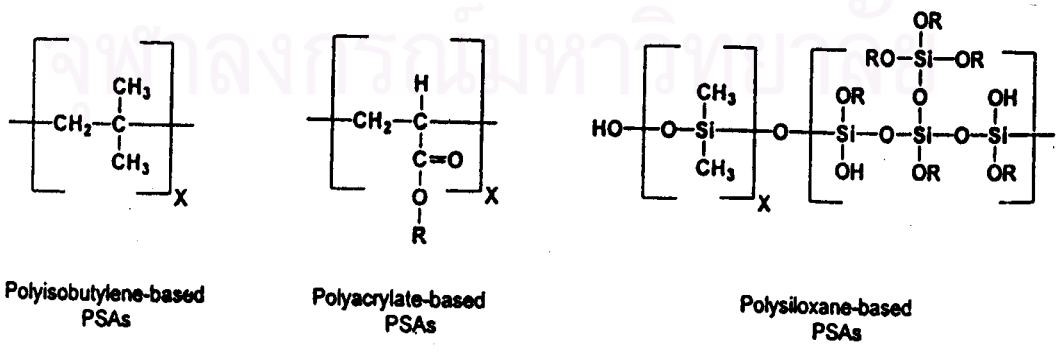


Figure 7. Pressure-sensitive adhesives (PSAs) used in transdermal drug delivery system.

The three major classes of polymers evaluated for potential medical applications in TDDS include polyisobutylene-type pressure-sensitive adhesives, acrylic-type pressure-sensitive adhesives, and silicone-type pressure-sensitive adhesives (Musolf, 1987).

2.1 Polyisobutylene-type pressure-sensitive adhesives.

Polyisobutylenes are homopolymers of isobutylene with a long, straight hydrocarbon chain and a terminal unsaturation. They are available in a range of molecular weights. The low molecular weight polyisobutylenes are soft, tacky, white semisolids which are widely used in FDA-regulated applications. The high molecular weight isobutylenes are used as high strength backbone polymers in pressure-sensitive adhesives. Polyisobutylenes resist the effects of weathering, aging, or heat and have little tendency to crystallize because of the reduced number of double bonds and reactive sites. The amorphous character of these polyisobutylenes imparts an internal mobility resulting in flexibility, permanent tack, and resistance to shock. The lack of polarity of these tacky polymers results in weak adhesion to substrates. This has been overcome by the addition of resins and other tackifiers, which impart a certain degree of polarity to these polymers. Apart from the hydrocarbon resins, hydrogenated esters, and low molecular weight polyisobutylenes which serve as tackifiers, other additives such as waxes, oils, solvents, and other polymers may be added as plasticizers (to modify the tack and cohesive strength), fillers (to reduce cost and improve handling), and antioxidants (to improve adhesive resistance to oxidative degradation).

2.2 Acrylic-type pressure-sensitive adhesives.

Acrylic-type pressure-sensitive adhesives are made by the copolymerization of acrylic esters with acrylic acid and other functional monomers, producing a polymer

with a saturated hydrocarbon backbone. Acrylic esters that contain four or more carbon atoms in the alcohol component are specially suited for use as pressure-sensitive adhesives, where they reduce crystallinity and lower the glass transition temperature, resulting in softer and more flexible polymers with increased tackiness. As these acrylic polymers are saturated, they are resistant to oxidation and do not require stabilizers. An advantage associated with this class of adhesive polymers is the small amount of low molecular weight impurities that could possibly affect adhesion or biocompatibility. The desired tack is usually attained with n-butyl acrylate and/or 2-ethylhexyl acrylate. Modification of the adhesive properties is achieved by copolymerization or cross-linking.

2.3 Silicone-type pressure-sensitive adhesives.

Silicone-type pressure-sensitive adhesives are prepared by the reaction of a linear polydimethylsiloxane fluid with a solvent-soluble, low molecular weight silicate resin. The linear polydimethylsiloxane possesses a backbone of alternating silicon and oxygen bonds (Si-O) and is terminated by silanol groups. The silanol groups in polydimethylsiloxane and silicate resin condense to form a stable siloxane bond (-Si-O-Si-). Each silicon atom in polydimethylsiloxane is attached to two methyl groups. These adhesive have a very low glass transition temperature and hence possess a high degree of flexibility. They are capable of adhesion to both high and low energy surfaces and has a low skin irritation potential. They are resistant to oxidation and do not require stabilizers. Silicone-type pressure-sensitive adhesive compositions for use in TDDS and other medical devices have been patented and are currently used in the Duragesic® (fentanyl) and Transderm-Nitro® (nitroglycerin) systems. The flexibility of silicone pressure-sensitive adhesives offers formulators a variety of alternatives that can be chemically tailored during the formulation process or physically during the coating process to meet the individual requirement of different transdermal devices.

3. Release Liners

The desirable characteristics of release liners are similar to those required for the backing membrane. The release liner has to be removed before the application of the transdermal system, and it prevents the loss of the drug that has migrated into the adhesive layer during storage. It also helps to prevent contamination. The same materials used as backing membranes are used as release liners. Typically, the release liner is composed of a base layer, which may be nonocclusive (e.g., paper or fabric) or occlusive (polyethylene, PVC), and a release coating layer made of silicone or teflon. Other materials commonly used include polyesters, foil, mylar, and other metallized laminates.

4. Evaluation of transdermal drug delivery systems.

Presently, evaluation of transdermal drug delivery systems have been conduted as follows:

4.1 *In Vitro* drug release

4.1.1 Franz diffusion cell

Franz diffusion cell is a finite-dosing vertical-type, developed by Franz and commercialized by Crown Glass has frequently been used for studying the kinetics of percutaneous absorption. Schematic illustration of the commercially available finite-dosing Franz diffusion cell assembly is shown in Figure 8. Each of the diffusion cells consists of two compartments; a donor compartment, which is exposed to an ambient condition, and a receptor compartment which is maintained at 37±1 ^{o}C by circulating a thermostated water jacket. The solution hydrodynamics in the receptor compartment is

kept at constant by a tiny rod-shaped magnetic rotating at 600 rpm by a synchronous motor mounted underneath the cell mounting block (Kehary and Chien, 1984).

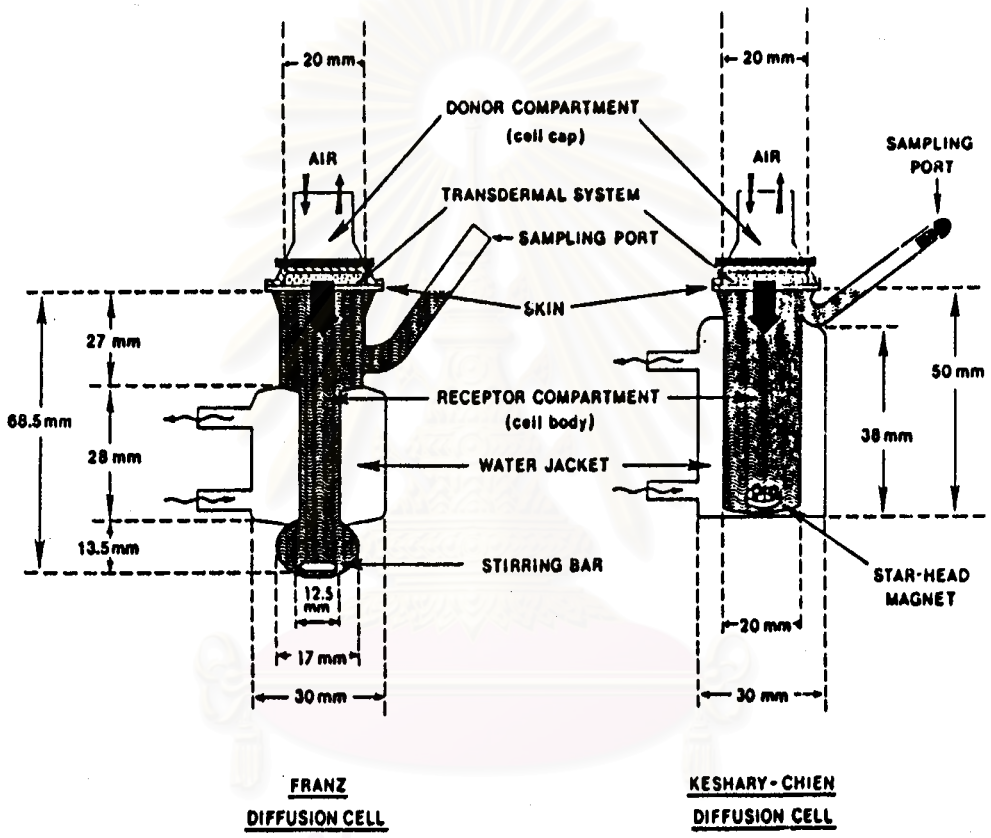


Figure 8. The vertical-type *in vitro* skin permeation system; the Franz diffusion cell is shown along with the Keshary-Chien skin permeation cell.

4.1.2 The paddle over disk (Dissolution Apparatus 5)

This device consists of the paddle and the vessel assembled from USP paddle dissolution apparatus 2 except that the transdermal system is attached to a disk or cell resting at the bottom of the vessel that contains medium.

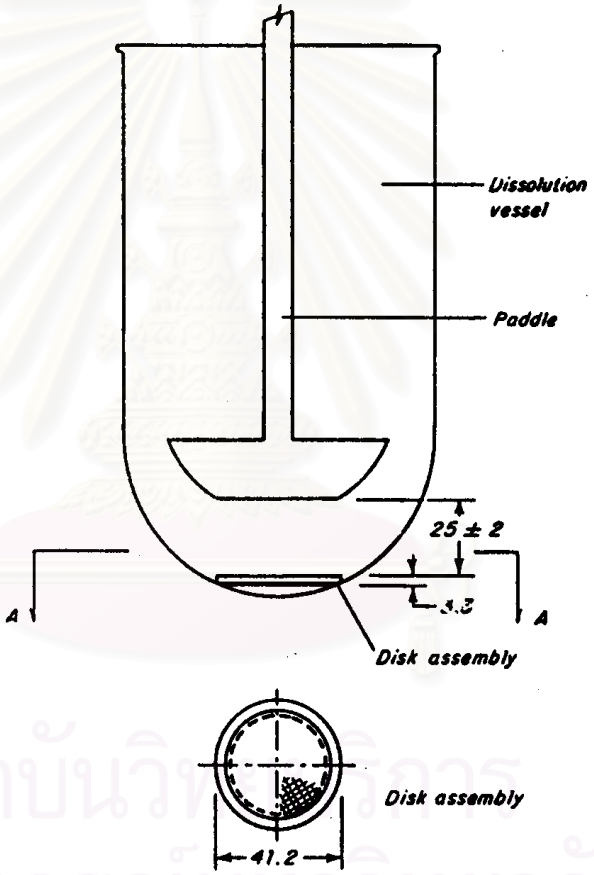


Figure 9. Paddle over disk. (All measurements are expressed in mm.)

4.1.3 The cylinder-modified USP Basket or rotating cylinder. (Dissolution Apparatus 6)

This device consists of the vessel assembled from the USP basket dissolution apparatus 1 except that the system is attached to the surface of a hollow cylinder immersed in medium. This method was used in this study.

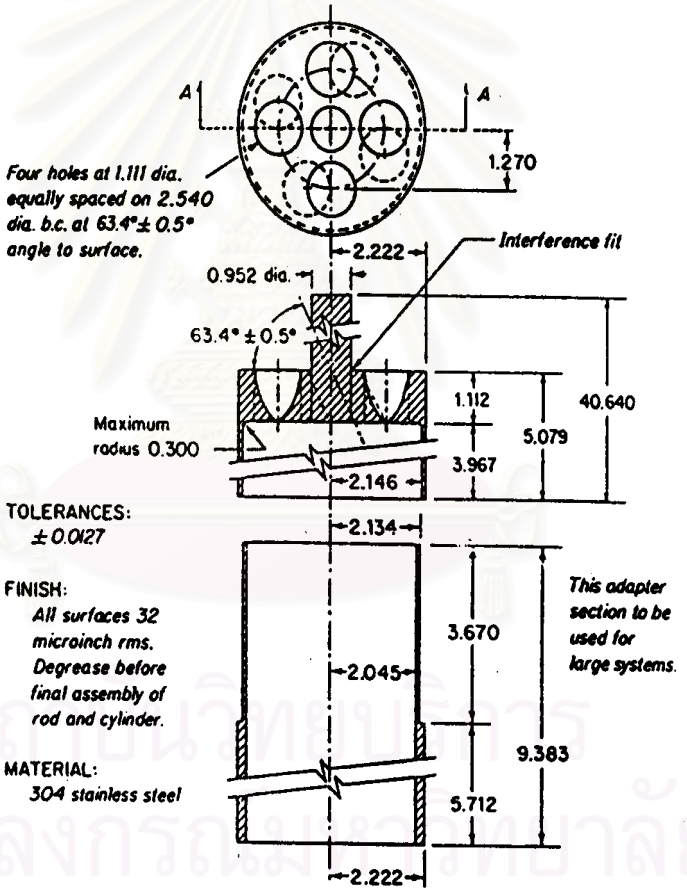


Figure 10. Cylinder-modified USP Basket. (All measurements are expressed in cm.)

4.1.4 The Reciprocating Disk. (Dissolution Apparatus 7)

This apparatus may also be specified for use with variety of dosage form. For transdermal dosage form, patches attached to holders are oscillated in small volumes of medium, allowing the apparatus to be useful for systems delivering low concentrations of drug.

4.1.5 Horizontal diffusion cell.

This cell design has a solution compartment of relatively small volume in each half-cell for maximal analytical sensitivity, and a rather small membrane area to accommodate the skin specimen available. Both the donor and receptor solutions are agitated, under a totally enclosed system, by a matched set of star-head magnets which are rotated at a synchronous speed of 600 rpm at a fixed position in the stirring platforms by a specially designed driving unit positioned directly underneath the cells.

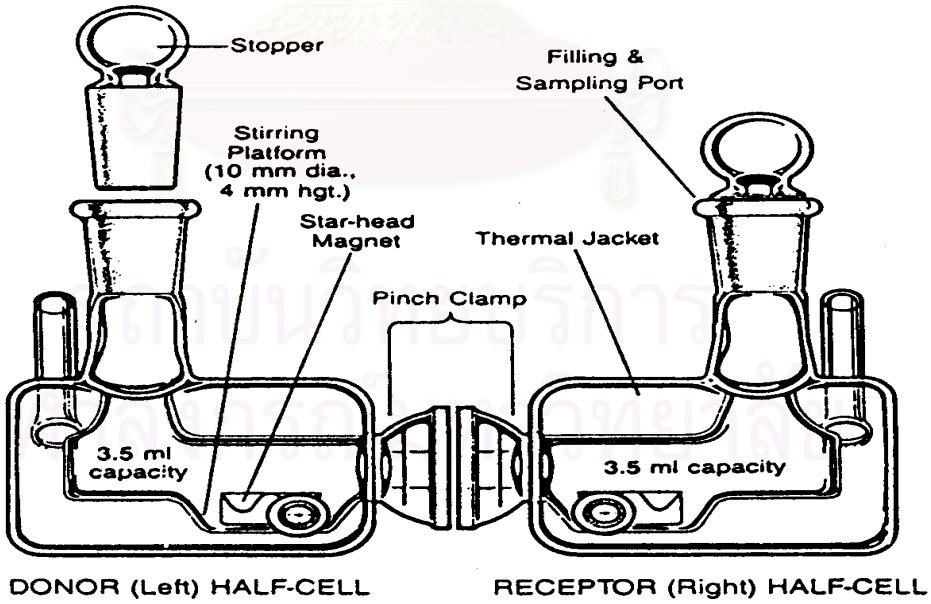


Figure 11. Horizontal-type diffusion cell.

4.2 *In vitro* skin permeation.

The aim of *in vitro* experimentation in TDDS is to understand and/or predict the delivery and penetration of a molecule from the skin surface into the body via the skin of a living animal (Gummer, 1989). The most method that has been used in this study was diffusion cell. Membrane which has been used for this technique is classified into two types: Animal skin model and Human cadaver skin model.

Results of comparative studies indicated that the improved diffusion cell, named Keshary-Chien diffusion cell could provide better results. This is because solution mixing efficiency was substantially improved, so the drug distribution and concentration homogeneity could be achieved in the Keshary-Chien diffusion cell within a duration four times shorter than in the Franz diffusion cell; and a 3-fold reduction in the thickness of the hydrodynamic boundary layer was achieved, so the effect of mass transfer in the hydrodynamic boundary layer on the skin permeation rate profiles was minimized (Monthip, 1995).

4.3 *In vivo* method.

In vivo evaluation of TDDS has been studied using animal models and human experimentation. Although the most relevant data pertaining to TDDS are obtained in humans, this desirable approach is not always possible, since considerable time and resources are required to conduct a safe and meaningful percutaneous absorption study in man. Consequently, one must prepare to use an *in vivo* animal model.

CHAPTER III

MATERIALS AND METHODS

I. Materials

1. Reagents and materials

1.1 Ketoprofen (Donated by Biolab Co.Ltd, Bangkok, Thailand), potency 100.25%, Lot No. 1997 17605A.

1.2 Eudragit® E 100 (Poly (butyl methacrylate, 2-dimethyl aminoethyl methacrylate, methyl methacrylate) = 1:2:1) (Rohm Degussa-Huls Group, Germany), Lot No. 839101009.

1.3 Succinic acid (Carlo Erba Reagenti, Italy), Lot No. 6D523106E.

1.4 Tributyl citrate (Supplied by Department of Manufacturing Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

1.5 Propylene glycol (Srichund United Dispensary, Thailand), Lot No. PL90/9-25.

1.6 Glycerin (Srichund United Dispensary, Thailand), Lot No. PL89/9-16.

1.7 Isopropyl alcohol AR grade (E. Merck, Darmstadt, Germany), Lot No. K2-7290134 002.

1.8 Acetone AR grade (Labscan Ltd, Ireland), Lot No. 97 05 1069.

1.9 Ethanol AR grade (E. Merck, Darmstadt, Germany), Lot No. K268696839-36.

1.10 Methanol AR Grade (E. Merck, Darmstadt, Germany), Lot No. K2782 6509021.

1.11 Sodium hydroxide (Mallinckrodt Baker, Mexico), Lot No. 7708MVKK.

1.12 Potassium dihydrogen phosphate (E. Merck, Darmstadt, Germany), Lot No. A217973 016.

1.13 Sodium acetate trihydrate (Carlo Erba Reagenti, Italy), Lot No. 0C053-310C.

1.14 Diclofenac sodium (Amoli Organic Ltd, Gujarat, India), Lot No. DS/0006/111.

1.15 Acetonitrile HPLC grade (Labscan Ltd, Ireland), Lot No. 2K 07 0122.

1.16 Methanol HPLC grade (Labscan Ltd, Ireland), Lot No. 3J 09 0245.

1.17 Glacial acetic acid AR grade (J.T. Baker, Phillipsburg, USA), Lot No. K44819.

1.18 Heparin 5.000 I.U./mL (Leo Pharmaceutical Products, Ballerup, Denmark) Lot No. E8906B.

1.19 Backing membrane (Silicone-coated polyester film laminate) (Supplied by Neoplast Co.Ltd., Thailand).

1.20 Protective release line (Polyester film) (Supplied by Neoplast Co.Ltd., Thailand).

1.21 Cuprophan® (Regenerated cellulose) (Membrana GmbH, Wuppertal, Germany).

2. Apparatus

2.1 Analytical balance (Sartorius, Germany).

2.2 Magnetic stirrer (Heidolph MR3001, Germany).

2.3 pH meter (Beckman 50, Beckman Instrument, Inc., USA).

2.4 Dissolution apparatus (Sotax AT7, Switzerland).

2.5 Spectrophotometer (UV-160A, Shimadzu, Japan).

2.6 Vortex mixer (Vortex Genies-2, Scientific Industries, Inc., USA).

2.7 Centrifuge (ALC Centrifugette 4206).

2.8 Micropipette (Gilson P55410C, France).

2.9 Ultrasonic bath (Branson 3210).

2.10 High performance liquid chromatography (HPLC) equipped with the following accessories:

2.10.1 An autoinjector (712WISP, Waters, USA).

2.10.2 A constant flow pump (570, Waters, USA).

2.10.3 A turnable UV detector (Waters, USA).

2.10.4 An integretor (745B, Waters, USA).

2.10.5 μ–Bondapak C_{18} stainless steel column (30 cm x 3.9 mm I.D., 10μm) and μ-Bondapak C_{18} guard column (Waters, USA).

3. Animal

3.1 Subjects

White New Zealand rabbits were used as subjects in this study.

II. Methods

A. Formulation of Ketoprofen Transdermal Patch.

1. Preparation of ketoprofen transdermal patch.

The ketoprofen transdermal patch was prepared by dissolving Eudragit® E 100 (used as pressure sensitive adhesive due to ease in preparing) in mixed solvents of isopropyl alcohol, acetone and ethanol using magnetic stirrer for 60 minutes. Plasticizer (tributyl citrate, propylene glycol or glycerin) was added and continued stirring for 30 minutes. Afterward, succinic acid (used as crosslinker) was then incorporated and continued stirring for 60 minutes. Finally, ketoprofen was added and continued stirring for 60 minutes. The compositions of the formulation are shown in Table 2. The prepared mixture (20 g) was poured on the backing membrane (12x15 cm^2) which was fixed on each side over the glass plate. The mixture was then scrapped with a stirring rod in order to control the thickness of the film, and left it at room temperature for 12 hours. The resulting film was covered with a silicone-coated protective release liner. Finally, the films were cut to 4x4 cm^2 (16 cm^2). The cross-sectional view of the components of ketoprofen transdermal patch is shown in Figure 12.

Master formula for preparing ketoprofen transdermal patch (Rohm, 1998):

Eudragit® E 100	43.7 g.
Plasticizer	**20% or 40% of polymer**
Succinic acid	1.6 g.
Ketoprofen	**15% or 25% or 35% of polymer**
Acetone	29.8 g.
Isopropyl alcohol	3.6 g.
Ethyl alcohol	16.6 g.

Table 2. Formulation of ketoprofen transdermal patches.

Formulation	Type of plasticizer	Concentration of plasticizer (%w/w of polymer)	Concentration of ketoprofen (%w/w of polymer)
1	Tributyl citrate	20	15
2		40	
3		20	25
4		40	
5		20	35
6		40	
7	Propylene glycol	20	15
8		40	
9		20	25
10		40	
11		20	35
12		40	
13	Glycerin	20	15
14		40	
15		20	25
16		40	
17		20	35
18		40	

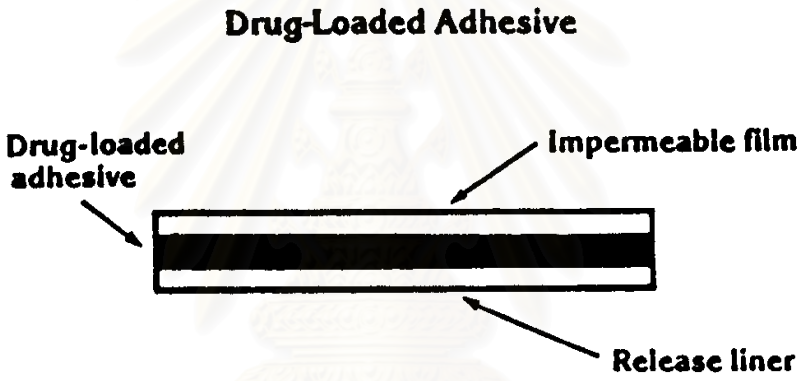


Figure 12. The cross-sectional view of ketoprofen transdermal patches.

2. Evaluation of physical properties of ketoprofen transdermal patches.

2.1 Difficulty of preparing the TDDS

Every formulation was completely prepared within one day. The difficulty of preparing each formula was evaluated according to the time consuming and dissolving to obtain the complete preparation.

2.2 Clarity

Overall clarity and insoluble materials were observed visually.

2.3 Air bubbles

Air bubble of each formulation was detected visually.

2.4 Residue on application.

The residue on human and rabbit skin after applying each preparation of ketoprofen transdermal patch was observed.

B. *In Vitro* Studies

1. Determination of the content of ketoprofen in transdermal patches.

The content of ketoprofen in transdermal patches was determined as follows (Nawarat, 1998): Patches of specified area were cut (4x4 cm^2) and accurately

weighed. The pieces were dissolved in methanol in a 50 mL volumetric flask and diluted to volume with the same solvent. The final solution was assayed by UV spectrophotometry at the maximum wavelength of 255 nm. The absorbances of all samples were converted to ketoprofen concentrations using the calibration curve.

1.1 Preparation of standard solutions for the calibration curve

A 30 mg of ketoprofen was accurately weighed and transferred into a 50 mL volumetric flask. The drug was dissolved and adjusted to volume with methanol to produce the stock solution. Standard solutions with known concentrations of 1.4, 3.6, 4.8, 6.0, 7.2, 9.6, 10.8 and 12.0 μg/mL were then prepared by dilution of the stock solution using methanol. The solutions were assayed by UV spectrophotometry at the maximum wavelength of 255 nm. The absorbances versus known concentrations of ketoprofen were fitted into a straight line using linear regression.

2. Release characteristics of ketoprofen transdermal patches.

In vitro drug release study was performed using the USP 24 rotating-cylinder dissolution apparatus (dissolution apparatus 6). The ketoprofen transdermal patches were gently pressed to a dry, unused square piece of Cuprophan® membrane, an inert porous cellulose material with the adhesive side against the membrane. The transdermal patches was then attached to a stainless steel holder.

Condition of *in vitro* release study:

Instrument : Dissolution apparatus 6 (Rotating cylinder)

Membrane : Cuprophan® (Regenerated cellulose)

Dissolution medium : 900 mL phosphate buffer pH 7.4

Temperature : 37±0.5 °C

Rotating rate : 100 rpm

A 5 mL of sample was withdrawn at 5, 10, 25, 40 minutes, and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 hours after the apparatus was operated and equal volume of warmed dissolution medium at 37 °C was replaced at once to maintain a constant volume. The concentrations of ketoprofen were quantified using a calibration curve. The percent released versus time profiles of ketoprofen from transdermal patches were constructed.

2.1 Preparation of solutions for the calibration curve.

A 30 mg of ketoprofen was accurately weighed and transferred into a 50 mL volumetric flask. The drug was dissolved and adjusted to volume with methanol to produce the stock solution. Standard solutions with known concentrations of 1.4, 3.6, 4.8, 6.0, 7.2, 9.6, 10.8 and 12.0 μg/mL were then prepared by dilution of the stock solution with phosphate buffer pH 7.4. The solutions were assayed using UV spectrophotometry at the maximum wavelength of 260 nm. The absorbance versus known concentrations of ketoprofen were fitted into a straight line using linear regression.

3. Validation of analytical methods for *in vitro* studies.

Validations of analytical method for quantitative determination of ketoprofen from all types of patches were performed. The accuracy in term of percent recovery and precisions in term of percent coefficient of variation of the analytical methods were evaluated (Vanderwielen and Hardwidge, 1982).

3.1 Accuracy

Three sets of standard ketoprofen concentrations (low, medium, high) were analyzed by UV spectrophotometry at specified wavelength. Inversely estimated concentrations were determined and the percent recovery of each concentration was calculated.

3.2 Precision

3.2.1 Within-run precision

Within-run precision was determined by analyzing three sets of standard ketoprofen concentrations (low, medium, high) using spectrophotometer on the same day. Absorbances were compared, and the percent coefficient of variation (%C.V.) of each concentration was calculated.

3.2.2 Between-run precision

Between-run precision was determined by analyzing three sets of standard ketoprofen concentrations (low, medium, high) using spectrophotometer on different days. Absorbances were compared and the percent coefficient of variation (%C.V.) of each concentration was calculated.

4. Selection of the ketoprofen transdermal patches for *in vivo* study.

Only three formulations were selected for further *in vivo* study. Individual formula was selected from the formulas containing each plasticizer and demonstrating best releasing characteristics.

C. *In vivo* studies.

1. Experimental transdermal patches.

Three formulations of ketoprofen transdermal patches with each plasticizer were *in vivo* evaluated. All formulations were freshly prepared and assayed for content of ketoprofen.

2. Subjects and drug administration.

Nine white New Zealand rabbits weighing between 2.8-3.8 kg were used as subjects in this study. They were acclimatized to the research facilities for one week prior to the study. Before the day of experiment, two sides of abdominal skin and right foreleg were carefully clipped using clipper. Then on the experimental day, ketoprofen transdermal patches were gently pressed on the designated area and covered with gauze ($4x4\ cm^2$).

3. Experimental design.

The study was conducted employing a randomized crossover design, with general rule that every subject received all number of formulations. Three patches of each formulation were given to each subject with a washout period of one week between each treatment, as shown in Table 3.

Table 3. A three way crossover design for *in vivo* study.

Sequence	Subject	Treatment in Period		
		I	II	III
I	1,2,3	A	B	C
II	4,5,6	B	C	A
III	7,8,9	C	A	B

Where: A = Tributyl citrate, B = Propylene glycol and C = Glycerin

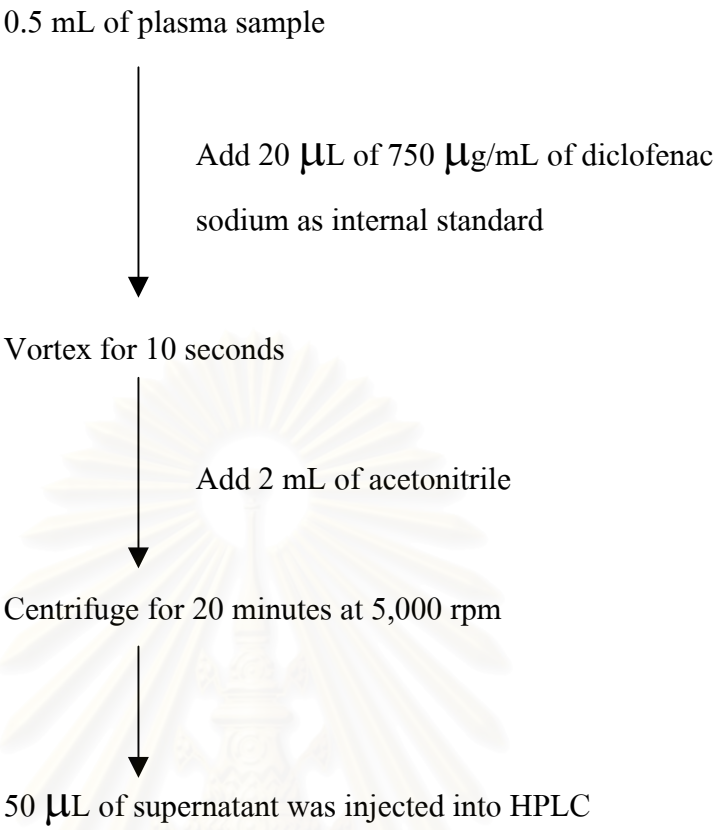
4. Sample collection

A 3 mL of blood sample was collected from a marginal ear vein using a disposable syringe and immediately transferred to tubes containing 20 μL of 5.000 I.U./mL of heparin solution. Blood samples were collected before drug administration and at 1, 2, 3, 4, 6, 8, 10, 12 and 24 hours post dose. They were centrifuged at once at 5,000 rpm for 10 minutes. The plasma was separated and kept at –20^{o}C until subsequent analysis.

5. Determination of ketoprofen in plasma.

Concentrations of ketoprofen in plasma were determined by high performance liquid chromatographic method modified from that described by Panvipa (Panvipa, 1993). The procedure was described as follows.

5.1 Preparation of plasma sample



5.2 Preparation of standard solutions and the calibration curve.

A 500 mg of ketoprofen was accurately weighed and transferred into a 50 mL volumetric flask. The drug was dissolved and adjusted to volume with mixture of acetonitrile and water 1:1. This solution was used as stock solution. A set of standard solution with known concentrations of 14, 30, 40, 70, 90, 120, 160 and 200 μg/mL were then prepared by dilution of the stock solution with the same solvent. An exactly 20 μL of each standard solution was individually added to 0.5 mL of pooled rabbits plasma to produce the plasma concentrations of 0.56, 1.2, 2, 2.8, 3.6, 4.8, 6.4 and 8 μg/mL, respectively. These plasma standard solution were finally clarified and analyzed following the same procedure as mentioned previously. The peak area ratios of ketoprofen to that of diclofenac sodium versus known concentrations of ketoprofen were fitted into a straight line using linear regression.

5.3 Preparation of internal standard solution.

The internal standard, diclofenac sodium 18.75 mg was dissolved in 25 mL of ultrapured water to give the final concentration of 750 μg/mL.

5.4 Preparation of mobile phase.

The mobile phase was prepared by using sodium acetate buffer pH 4.2:acetonitrile in the ratio of 1:1. It was thoroughly mixed, filtered through a cellulose acetate filter paper with a pore size of 0.45 μm and then sonicated for 30 minutes.

5.5 High performance liquid chromatographic conditions.

Column	:	μ-Bondapak C_{18} with particle size of 10 μm,300x3.9 mm. (i.d.)
Mobile phase	:	Sodium acetate buffer pH 4.2 : Acetonitrile = 1:1
Injection volume	:	50 μL
Flow rate	:	1.0 mL/min
Detector	:	UV, 260 nm
Attenuated	:	2^4
Temperature	:	Ambient

The area under the peak of ketoprofen and internal standard were calculated by the integrator. The peak area ratios of ketoprofen to the internal standard were then determined. The concentrations of ketoprofen in plasma samples were quantified using a calibration curve.

6. Validation of analytical methods for *in vivo* studies.

The analytical methods for determination of ketoprofen in rabbit plasma were validated under the following procedures for accuracy and precisions (Shah et al. 1992).

6.1 Accuracy

Accuracy in term of percent recovery was determined by analyzing three sets of standard solutions of ketoprofen (low, medium, high) prepared in rabbit plasma. Percent recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration of ketoprofen multiplied by 100.

6.2 Within run precision

Within run precision was determined by analyzing three sets of standard solutions of ketoprofen (low, medium, high) in rabbit plasma on the same day. The percent coefficient of variation (%C.V.) of the peak area ratios of ketoprofen to the internal standard of each concentration was determined.

6.3 Between run precision

Between run precision was determined by comparing the peak area ratios of ketoprofen to the internal standard of three sets of standard solutions of ketoprofen (low, medium, high) in rabbit plasma on three different days. The percent coefficient of variation (%C.V.) of each concentration was determined.

Acceptance criteria:

For accuracy, the percent recovery should be within ±20 percent meanwhile the percent coefficient of variations for both the within run and between run are not greater than 15.

7. Statistical evaluation.

7.1 Pharmacokinetic parameters.

The relevant pharmacokinetic parameters of ketoprofen from each treatment following administration of ketoprofen transdermal patches were derived from the plasma ketoprofen concentration-time profiles. The area under the plasma ketoprofen concentration-time curve (AUC) and the area under the moment curve (AUMC) from time zero up to 24 hours were calculated by linear trapezoidal rule (Gibaldi and Perrierr, 1982). The Mean residence time (MRT) was also calculated using noncompartmental technique.

7.2 Statistical evaluation of pharmacokinetic parameters.

The comparisons of all three formulations of ketoprofen transdermal patches were established employing the corresponding pharmacokinetic parameters among all formulations by mean of a three way analysis of variance at $\alpha = 0.05$ (Weiner and Yuh, 1994). If the results showed statistically significance difference, the difference of these values between each pair of treatment would be examined by Least Significant Difference test (Steel and Torrie, 1980).

CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of ketoprofen transdermal patches.

All prepared ketoprofen transdermal patches were yellowish and adhesive according to the color of the drug. Each formulation was shaped into a 4x4 cm (16 cm^2) patch.

2. Evaluation of physical properties.

2.1 Difficulty of preparing.

The degree of difficulty for preparation of each ketoprofen transdermal patch was different due to various types and concentrations of plasticizer to be used in individual formulation. Incorporation of Eudragit E® 100 and ketoprofen which are solid form to be completely dissolved in mixed solvents appeared to be more difficult than those with the liquid forms of tributyl citrate, propylene glycol and glycerin. However the time consuming for preparing each formulation was nearly the same. All finished products exhibited equally sticky properties.

2.2 Clarity

All ingredients in the formulation were completely dissolved in the solvent. The appearance of each product was transparent without any insoluble materials remaining in the preparations.

2.3 Air bubbles

Small air bubbles were found in the mixture during preparing process, especially, in those with glycerin and propylene glycol. In the formulation containing tributyl citrate, air bubble was least. This may be related to the low molecular weights of both glycerin and propylene glycol as compared to that almost 4 times higher of tributyl citrate. In low molecular weight compound, the particles may loosely bind together which can be easily occupied by air when stress like stirring was applied. Meanwhile, in case of high molecular weight substance, particles are tightly bound. This impact should be less.

2.4 Residue on the application.

Some residues could be observed on the applied area of the skin.

3. Determination of the content of ketoprofen in transdermal patches.

3.1 Validation of analytical methods for determination of ketoprofen content in the preparations.

Due to the analytical method for ketoprofen content in transdermal patches was not available elsewhere. The spectrophotometry was, therefore, developed to be used in this study. Methanol was used as solvent and assay was conducted at the maximum wavelength of 255 nm. It was validated for accuracy and precision. In most cases, the percent recovery was almost 100 percent and the percent coefficient of variation (%C.V.) for both within run and between run precision of each concentration were less than 5 percent. All standard calibration curves were linear with the coefficient of determinations (R^2) ranged from 0.9985 to 0.9996. All results are accessible in Appendix III.

3.2 Calibration curve

The calibration curve for determination of ketoprofen content in formulations was constructed by fitting the absorbance of standard solutions of ketoprofen versus known ketoprofen concentration using linear regression. The equation obtained is expressed as

$$Y = 0.0673X - 0.0109$$

Where Y = absorbance of ketoprofen solutions

X = concentration of ketoprofen

All calibration curves are shown in Appendix III.

3.3 Analysis of ketoprofen content.

The content of ketoprofen in transdermal patches was determined by UV spectrophotometry using methanol as solvent at the maximum wavelength of 255 nm. The values were expressed as percent labeled amount (%L.A.) as shown in Table 4.

Table 4. Percent labeled amount (%L.A.) of ketoprofen from eighteen formulations of ketoprofen transdermal patches.

Formulation	Amount of ketoprofen added (mg/16cm^2)**	Amount of ketoprofen found (mg/16cm^2) *	%Labeled amount
1	107.2	105.6	98.51
2	97.6	96.1	98.46
3	169.6	166.1	97.94
4	160	157.4	98.38
5	230.4	228.3	99.09
6	212.8	210.2	98.78
7	107.2	104.8	97.76
8	97.6	95.9	98.26
9	169.6	167.3	98.64
10	160	158.2	98.88
11	230.4	227.9	98.91
12	212.8	209.8	98.59
13	107.2	105.4	98.32
14	97.6	96.5	98.87
15	169.6	166.8	98.35
16	160	156.6	97.88
17	230.4	227.5	98.74
18	212.8	210.4	98.87

* n = 6

** Example - Calculated for Rx1:

Amount of ketoprofen = 6.6 g (15% of polymer) / 110.6 g

= 1.2 g/ 20 g = 1.2 g/ 180 cm^2 = 6.7 mg/ cm^2

= 107.2 mg/ 16 cm^2

The percent labeled amount of all formulations were less than 100 percent due to during pouring the mixture on the backing membrane, it was very difficult to spread the liquid over the specific area as needed by hand. Some mixtures might gradually leak out.

4. *In vitro* release study of ketoprofen transdermal patches.

Ketoprofen released data from *in vitro* release study of ketoprofen transdermal patches are shown in Table 5.

4.1 Validation of analytical methods for *in vitro* release study.

Solvent used in this study was phosphate buffer pH 7.4 and the maximum wavelength for spectrophotometry was 260 nm. It was validated for accuracy and precision. In most cases, the percent recovery was closed to 100 percent and the percent coefficient of variation (%C.V.) for both within run and between run precision of each concentration were less than 5 percent. All standard calibration curves were linear with the coefficient of determinations (R^2) ranged from 0.9985 to 0.9996. All results are accessible in Appendix III.

4.2 Calibration curve

The calibration curve for quantitation of ketoprofen released from the patches was constructed by fitting the absorbance of standard solutions of ketoprofen versus known ketoprofen concentration using linear regression. The equation obtained is expressed as

$$Y = 0.0667X - 0.0026$$

Where Y = absorbance of ketoprofen solutions

X = concentration of ketoprofen

All calibration curves are shown in Appendix III.

4.3 Release characteristics of ketoprofen transdermal patches.

To study release profiles of ketoprofen from ketoprofen transdermal patches, dissolution apparatus VI (Rotating cylinder) was employed. Cuprophan was used as a model membrane. The amount of ketoprofen released from ketoprofen transdermal patches of formulations Rx1-Rx18 at each time interval from 0.08 hours upto 24 hours are shown in Table 5. The percent released of ketoprofen computed from the results of Table 5 are presented in Table 6. The plot of percent released of ketoprofen from transdermal patches versus time were constructed and shown in Figures 13-15.

Figures 13-15 clearly show that all formulations (Rx1-Rx18) exhibited sustained release pattern of ketoprofen over 24 hours. The maximum percent ketoprofen released was observed from formulation containing 35% ketoprofen and 40% tributyl citrate or Rx6 with 40.13 percent per 16 cm^2 at 24 hours. Whereas the minimum percent released with 21.80 percent per 16 cm^2 was found from formulation containing 15% ketoprofen and 20% glycerin or Rx13. The percent ketoprofen released at 24 hours may be ranked as Rx6 > Rx12 > Rx10 > Rx2 > Rx4 > Rx8 > Rx18 > Rx11 > Rx16 > Rx5 > Rx9 > Rx3 > Rx17 > Rx7 > Rx15 > Rx1 > Rx14 > Rx13, respectively. However, the patterns of release profile from all formulations were similar regardless of concentrations of individual ingredient used.

Table 5. The average amount of ketoprofen released from ketoprofen transdermal patches using various types and concentrations of plasticizer.

Time	Concentration ($\mu g/cm^2$)																	
(hours)	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6	Rx7	Rx8	Rx9	Rx10	Rx11	Rx12	Rx13	Rx14	Rx15	Rx16	Rx17	Rx18
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.08	27.35	41.13	49.00	47.87	53.21	66.15	43.66	38.74	51.95	56.7279	49.56	93.83	29.60	28.20	57.99	52.65	40.99	51.67
0.17	47.88	75.79	88.62	99.30	124.63	141.29	73.84	102.20	120.97	141.516	132.62	144.53	51.83	55.48	106.67	104.67	98.98	133.76
0.42	109.85	173.76	196.36	247.57	249.71	323.25	152.39	183.45	225.23	247.295	313.97	329.32	120.15	117.21	201.57	230.90	244.30	279.41
0.67	159.51	236.70	278.69	367.57	362.83	460.81	198.07	227.50	297.88	335.807	449.80	482.94	160.45	159.32	285.89	342.24	374.82	417.30
1.00	219.71	316.72	388.87	495.82	494.57	608.69	254.69	288.65	382.87	438.858	601.69	673.95	229.22	215.30	368.71	439.70	515.76	568.45
1.50	310.59	421.49	541.55	749.02	755.63	892.31	335.36	370.95	512.03	610.922	761.30	847.31	284.04	277.34	474.05	569.33	688.25	811.22
2.00	368.94	502.51	627.31	846.39	868.36	1015.62	399.33	448.64	618.01	747.103	990.71	1076.49	338.16	378.66	592.18	711.88	867.03	919.98
2.50	439.00	579.06	721.97	1008.45	1024.21	1211.48	461.67	524.61	723.58	855.77	1117.02	1262.30	389.77	424.59	678.64	820.36	1027.30	1079.83
3.00	498.60	655.45	821.49	1152.41	1172.48	1334.03	526.88	595.55	831.68	1002.46	1251.73	1440.68	434.07	477.79	767.24	923.57	1166.44	1228.39
4.00	575.82	756.00	1077.76	1489.00	1461.39	1646.65	622.65	720.14	1018.29	1213.84	1481.33	1707.15	525.82	560.09	922.51	1128.18	1422.29	1459.97
5.00	672.42	847.96	1288.84	1736.76	1666.13	1864.50	709.53	828.12	1175.63	1371.54	1758.55	1969.44	587.85	642.55	1067.72	1302.29	1679.52	1713.89
6.00	741.29	1010.88	1438.52	2016.08	1953.48	2070.95	793.77	890.60	1327.49	1549.77	1964.17	2198.00	667.36	707.74	1207.04	1473.81	1870.68	1930.52
8.00	848.19	1147.07	1658.56	2090.28	2255.73	2428.33	1012.47	1080.95	1616.50	1856.84	2337.45	2661.08	790.31	853.53	1421.60	1705.98	2270.86	2295.88
10.00	1010.95	1314.91	1911.41	2176.78	2596.86	2814.72	1138.83	1197.84	1798.84	2065.08	2664.24	2962.23	861.77	973.74	1658.40	1993.51	2605.75	2659.70
12.00	1130.27	1467.48	2124.15	2433.76	3018.51	3245.45	1237.76	1332.20	2015.18	2296.2	3001.92	3257.94	1011.24	1056.31	1823.38	2205.99	2873.55	2956.60
24.00	1683.12	2203.47	3080.15	3591.38	4484.37	5272.11	1737.78	2113.83	3206.01	3829.39	4623.96	5193.37	1436.25	1511.07	2663.89	3129.38	4175.85	4342.26

n = 6

Table 6. Percent released of ketoprofen from all formulations of ketoprofen transdermal patches.

Time	%Released																	
(hours)	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6	Rx7	Rx8	Rx9	Rx10	Rx11	Rx12	Rx13	Rx14	Rx15	Rx16	Rx17	Rx18
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.08	0.41	0.68	0.47	0.49	0.37	0.50	0.67	0.65	0.50	0.57	0.35	0.72	0.45	0.47	0.56	0.54	0.29	0.39
0.17	0.73	1.26	0.85	1.01	0.87	1.08	1.13	1.71	1.16	1.43	0.93	1.10	0.79	0.92	1.02	1.07	0.70	1.02
0.42	1.66	2.89	1.89	2.52	1.75	2.46	2.33	3.06	2.15	2.50	2.20	2.51	1.82	1.94	1.93	2.36	1.72	2.12
0.67	2.42	3.94	2.68	3.74	2.54	3.51	3.02	3.80	2.85	3.40	3.16	3.68	2.44	2.64	2.74	3.50	2.64	3.17
1.00	3.33	5.27	3.75	5.04	3.47	4.63	3.89	4.82	3.66	4.44	4.22	5.14	3.48	3.57	3.54	4.49	3.63	4.32
1.50	4.71	7.02	5.22	7.61	5.30	6.79	5.12	6.19	4.90	6.18	5.34	6.46	4.31	4.60	4.55	5.82	4.84	6.17
2.00	5.59	8.37	6.04	8.60	6.09	7.73	6.10	7.49	5.91	7.56	6.96	8.21	5.13	6.28	5.68	7.27	6.10	7.00
2.50	6.65	9.64	6.95	10.25	7.18	9.22	7.05	8.75	6.92	8.66	7.84	9.63	5.92	7.04	6.51	8.38	7.22	8.21
3.00	7.55	10.91	7.91	11.71	8.22	10.15	8.04	9.94	7.95	10.14	8.79	10.99	6.59	7.92	7.36	9.44	8.20	9.34
4.00	8.72	12.59	10.38	15.14	10.24	12.53	9.51	12.01	9.74	12.28	10.40	13.02	7.98	9.29	8.85	11.53	10.00	11.10
5.00	10.19	14.12	12.42	17.65	11.68	14.19	10.83	13.82	11.24	13.87	12.35	15.02	8.92	10.65	10.24	13.31	11.81	13.03
6.00	11.23	16.83	13.86	20.49	13.69	15.76	12.12	14.85	12.70	15.67	13.79	16.76	10.13	11.73	11.58	15.06	13.16	14.68
8.00	12.85	19.10	15.98	21.25	15.81	18.48	15.46	18.03	15.46	18.78	16.41	20.29	12.00	14.15	13.64	17.43	15.97	17.46
10.00	15.32	21.89	18.41	22.13	18.20	21.43	17.39	19.98	17.20	20.89	18.70	22.59	13.08	16.14	15.91	20.37	18.33	20.23
12.00	17.13	24.43	20.46	24.74	21.15	24.70	18.89	22.26	19.27	23.22	21.08	24.85	15.35	17.51	17.49	22.54	20.21	22.48
24.00	25.50	36.69	29.67	36.51	31.43	40.13	26.53	35.27	30.66	38.73	32.46	39.61	21.80	25.05	25.55	31.97	29.37	33.02

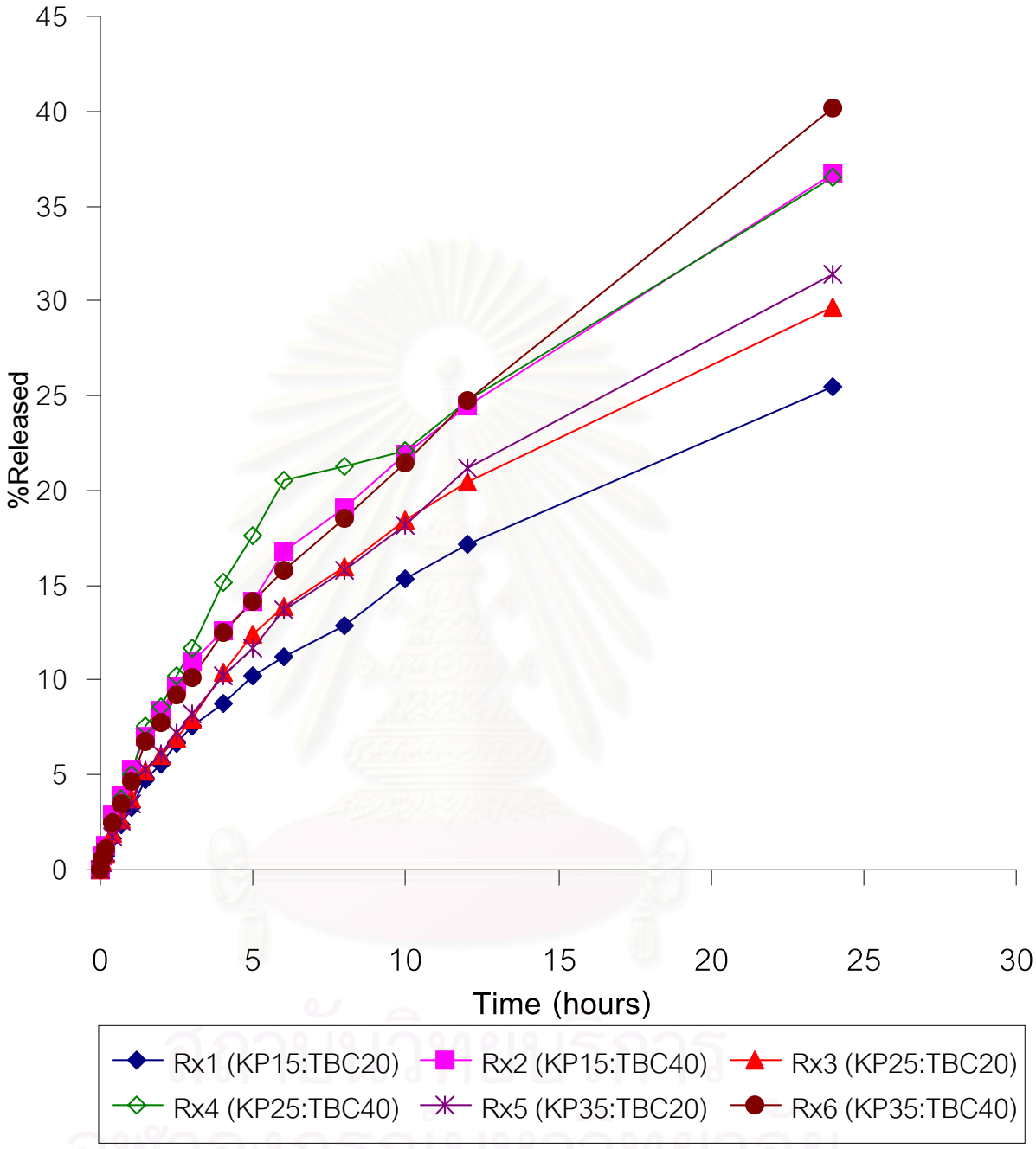


Figure 13. Percent released of ketoprofen from six formulations of ketoprofen transdermal patches using tributyl citrate as plasticizer (n=6).

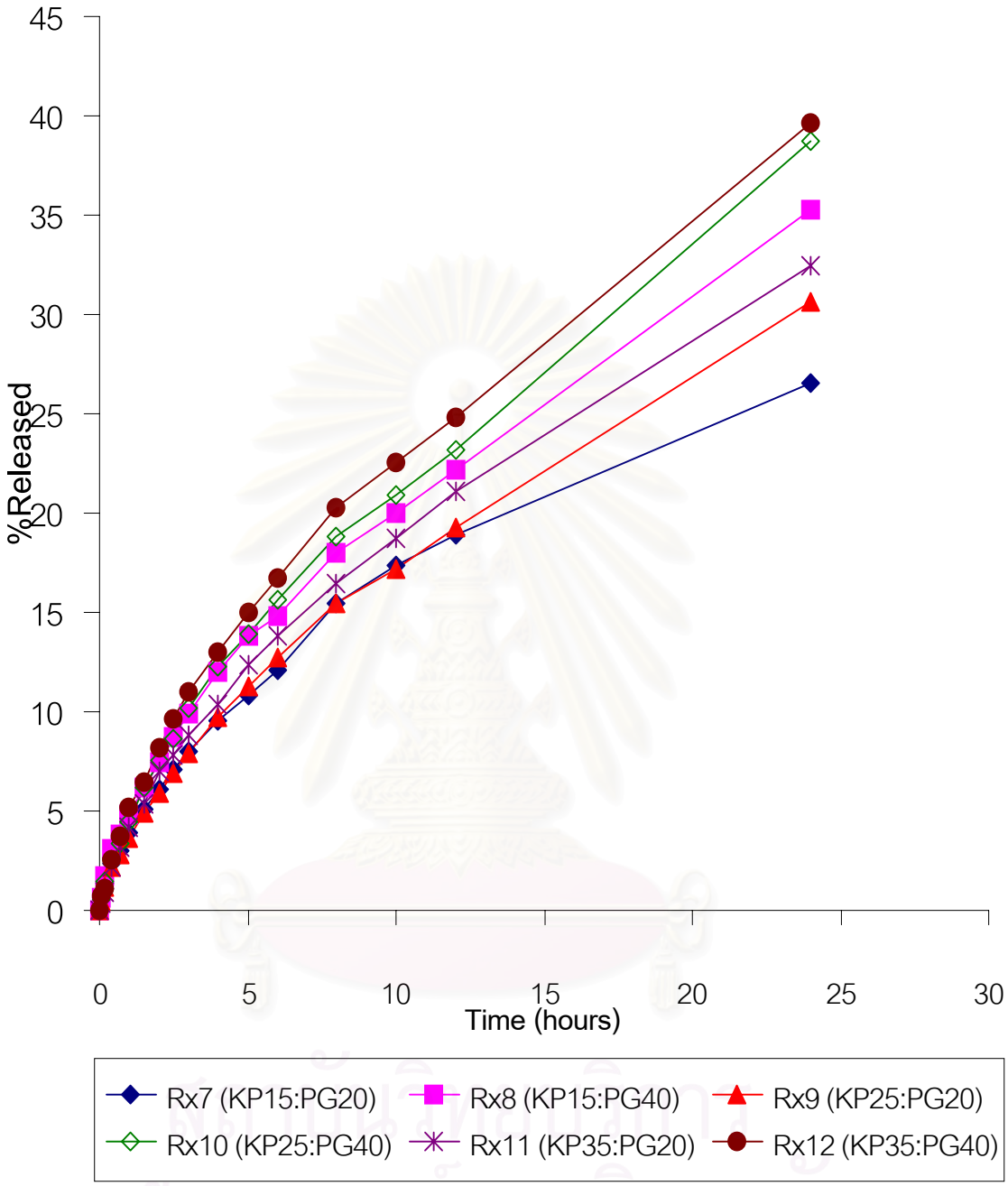


Figure 14. Percent released of ketoprofen from six formulations of ketoprofen transdermal patches using propylene glycol as plasticizer (n=6).

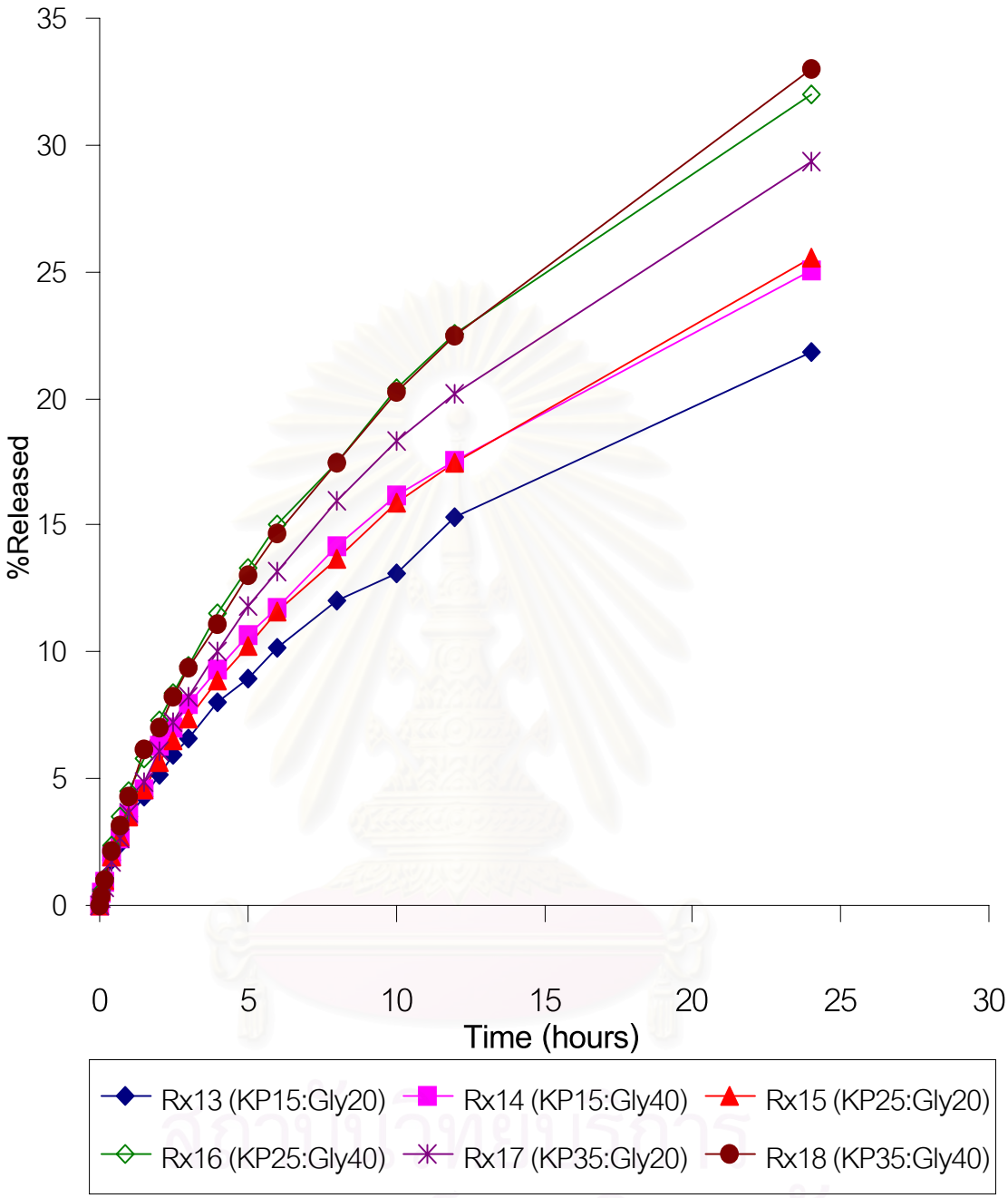


Figure 15. Percent released of ketoprofen from six formulations of ketoprofen transdermal patches using glycerin as plasticizer (n=6).

Effect of ketoprofen concentration on release profiles.

The effect of ketoprofen concentrations on percent released of itself from formulations were investigated by comparing the percent released-time profile from formulations containing 15% ketoprofen (Rx1), 25% ketoprofen (Rx3), and 35% ketoprofen (Rx5), respectively. Results represented all formulations are shown in Figure 16. The percent released of ketoprofen was increased in proportion to the increase of its concentrations (35% > 25% > 15%). The increase of the drug content may cause high concentration gradient affecting thermodynamic activity of the system resulted in increase of drug released (Gurol et al. 1996). These results agree with previous reports (Cho and Choi, 1998; and Valenta and Almazi-Szabo, 1995).

Effect of plasticizer concentration on release profiles.

The effect of plasticizer concentrations on percent released of ketoprofen from transdermal patches were investigated by comparing the percent released-time profile from all formulations containing 15% ketoprofen of polymer : Rx1 (20% tributyl citrate), Rx2 (40% tributyl citrate), Rx7 (20% propylene glycol), Rx8 (40% propylene glycol), Rx13 (20% glycerin), and Rx14 (40% glycerin). Results represented all formulations are shown in Figure 17. It was found that the percent ketoprofen released was increased with increasing concentrations of all plasticizers. This effect arises from that when a plasticizer was incorporated in adhesive polymer, the permeability of ketoprofen was further increased due to increase of fluidity within the adhesive matrix (Cho and Choi, 1998).

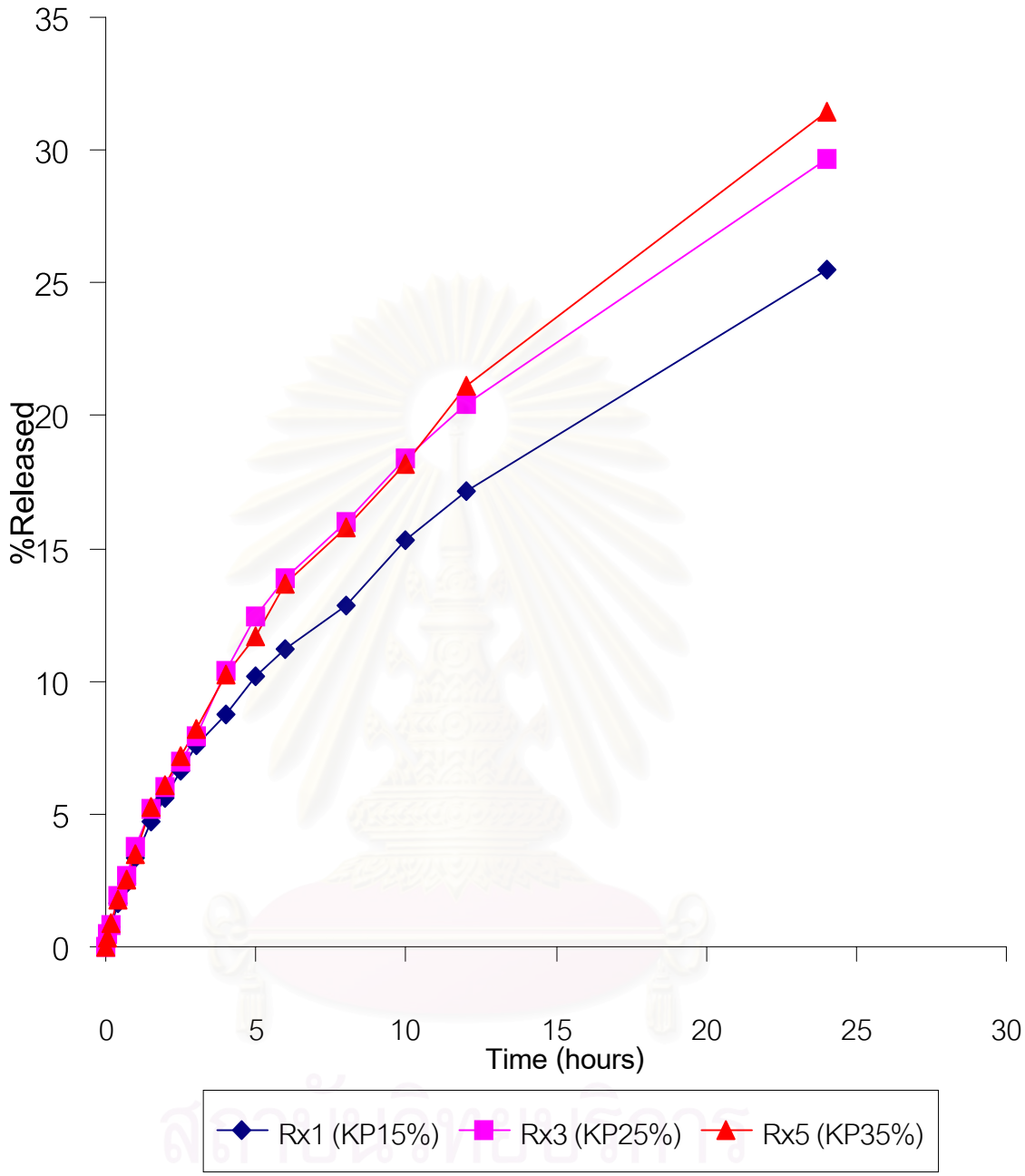


Figure 16. Percent released of ketoprofen from ketoprofen transdermal patches (effect of ketoprofen concentration).

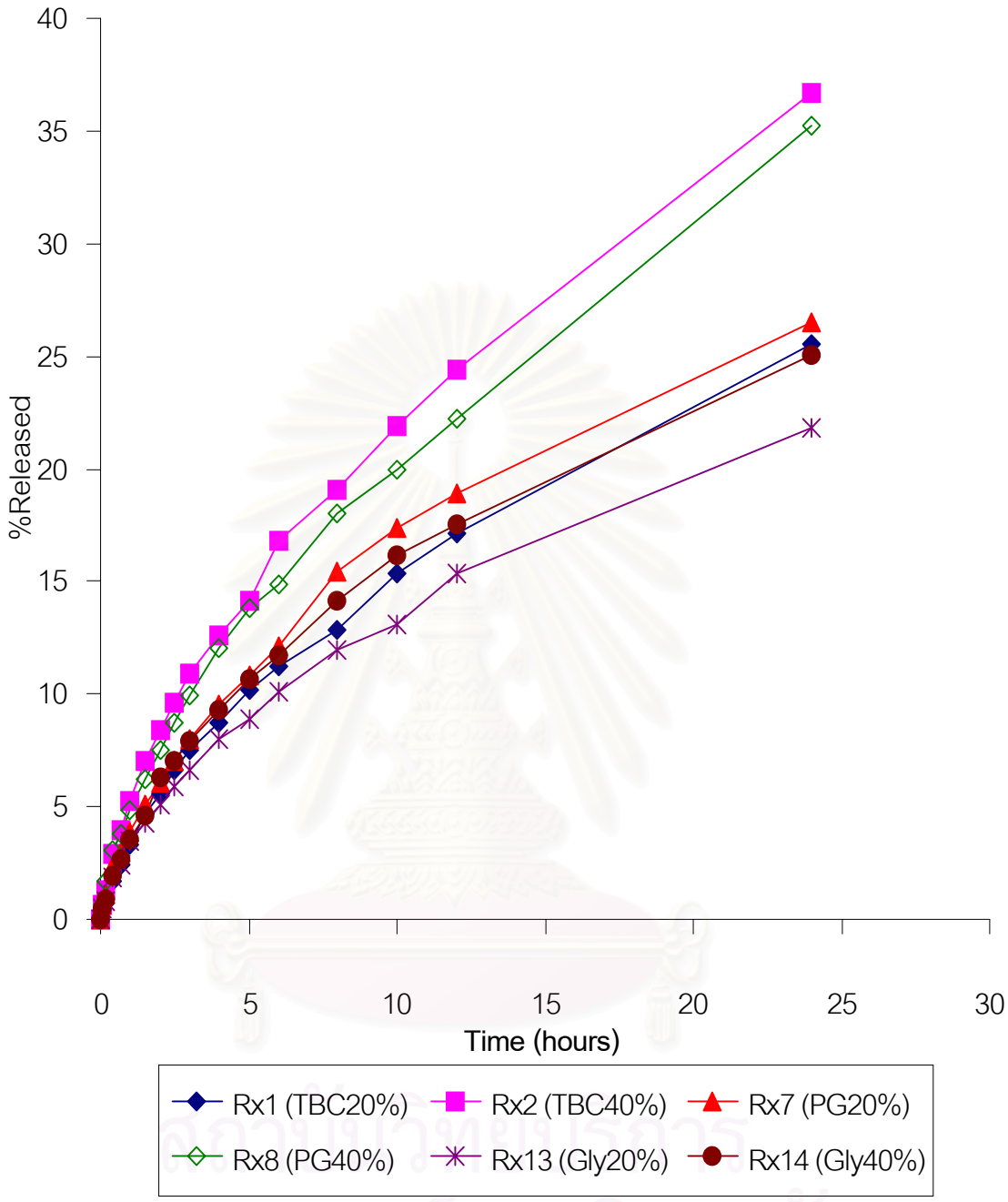


Figure 17. Percent released of ketoprofen from ketoprofen transdermal patches (effect of plasticizer concentration).

Effect of plasticizer types on release profiles.

The effect of plasticizer types on percent released of ketoprofen from transdermal patches were investigated by comparing the percent released-time profile from formulations that gave the best drug released of each plasticizer: Rx6, Rx12, and Rx18. Results are shown in Figure 18.

It was apparent that three plasticizers provided similar profiles, especially for the first 12 hours and thereafter those from tributyl citrate and propylene glycol appeared to be more effective than that from glycerin. The degree of solubility of the drug in vehicle may be responsible for this. Ketoprofen is more soluble in glycerin than that in tributyl citrate and propylene glycol. If solubility of drug in vehicle is high, the amount drug released from the system would be low. This impact is incrementally apparent.

The release patterns of ketoprofen transdermal patches from time 0 upto 24 hours were evaluated by linear correlation test. The relationship between percent released of ketoprofen from transdermal patches versus time and versus square root of time were shown in Table 7. The release profiles were found to be better fitted with a Higuchi's model than that followed a zero order kinetic. This trend of kinetic pattern was decided by the higher coefficient of determination (R^2) as seen in Table 7.

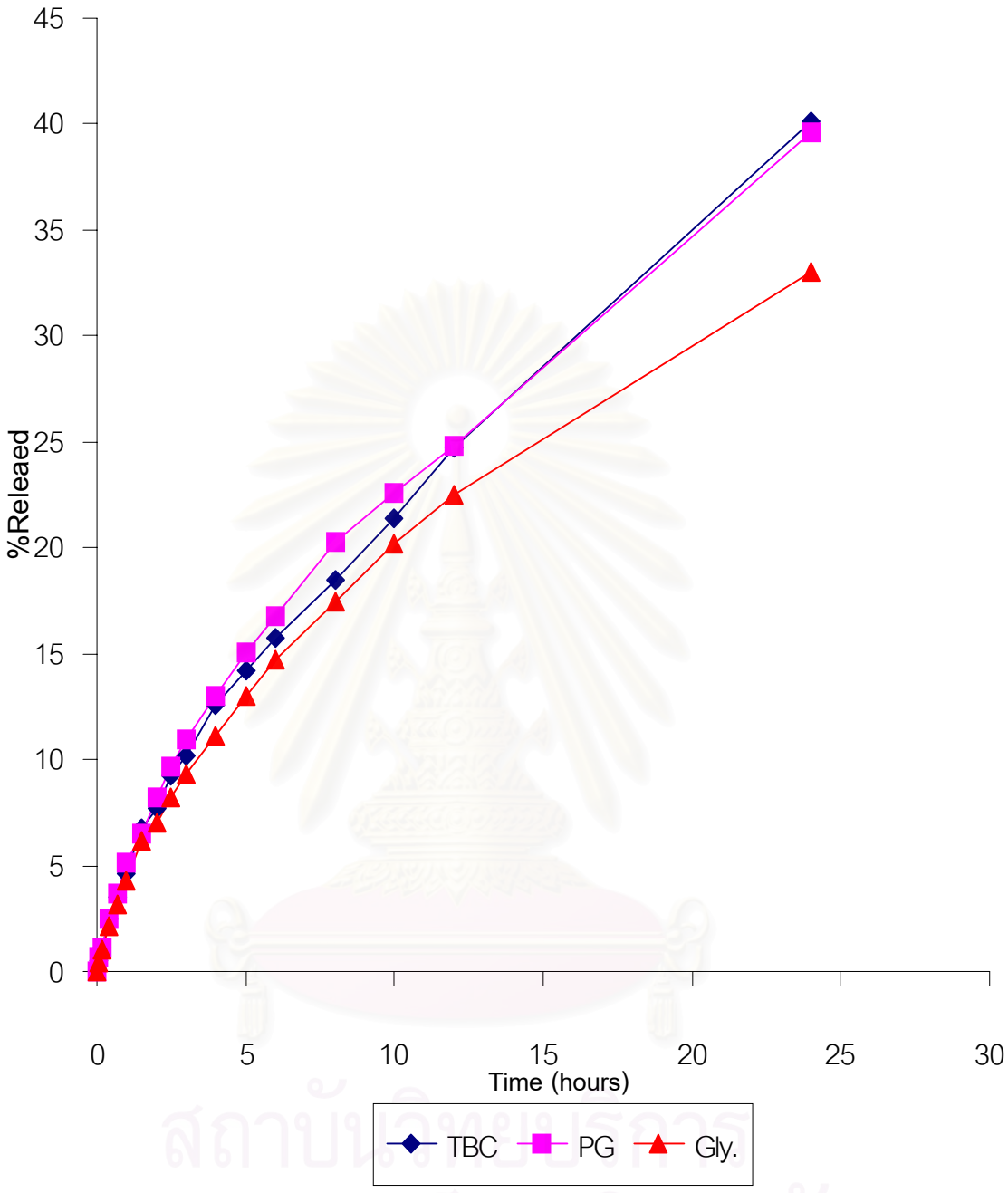


Figure 18. Percent released of ketoprofen from ketoprofen transdermal patches (effect of plasticizer types).

Table 7. The release rate and the coefficient of determination (R^2) of ketoprofen from transdermal patches.

	Zero -order		Higuchi -model	
	R^2	Rate (% /16 $cm^2.t^{1/2}$)	R^2	Rate (% /16 $cm^2.t^{1/2}$)
Rx1	0.9289	1.0867	0.9935	5.3318
Rx2	0.9286	1.5448	0.9946	7.6046
Rx3	0.9211	1.2939	0.9913	6.3680
Rx4	0.8722	1.5669	0.9868	7.9068
Rx5	0.9405	1.3570	0.9884	6.5998
Rx6	0.9581	1.6728	0.9826	8.0370
Rx7	0.9118	1.1495	0.9936	5.6933
Rx8	0.9420	1.4653	0.9908	7.1297
Rx9	0.9509	1.2930	0.9865	6.2482
Rx10	0.9554	1.6119	0.9836	7.7595
Rx11	0.9418	1.3765	0.9907	6.6977
Rx12	0.9415	1.6690	0.9902	8.1198
Rx13	0.9167	0.9279	0.9961	4.5890
Rx14	0.9056	1.0801	0.9960	5.3736
Rx15	0.9261	1.0955	0.9940	5.3844
Rx16	0.9142	1.3850	0.9949	6.8542
Rx17	0.9228	1.2840	0.9921	6.3163
Rx18	0.9280	1.4229	0.9932	6.9835

The coefficient of determinations (R^2) of zero order kinetic ranged from 0.8722-0.9581 meanwhile those of Higuchi's model was ranged from 0.9826-0.9961.

The release rate of ketoprofen for each formulation was calculated from the slope of the plot between percent released of ketoprofen and square root of time. They are also presented in Table 7. As can be seen from Table 7 that the release rate was increased in proportional to the increase of ketoprofen concentration which were found to be ranged from 4.5890-8.1198 percent per 16 cm^2 per square root of time. This indicated that the kinetic of drug released followed Higuchi's model. The formulation that gave the best release rate was Rx12 with 35% ketoprofen and 40% propylene glycol of polymer.

Selection of the best formulation.

From all data presented for *in vitro* studies. Three formulations of ketoprofen transdermal patches were selected for further *in vivo* studies according to the criteria as specified earlier.

They are the formulations Rx6 (35% ketoprofen and 40% tributyl citrate), Rx12 (35% ketoprofen and 40% propylene glycol), and Rx18 (35% ketoprofen and 40% glycerin), respectively.

5. *In vivo* studies of ketoprofen transdermal patches.

All three formulations selected were freshly prepared to be used for *in vivo* studies.

5.1 Analysis of ketoprofen concentration in rabbit plasma.

The high performance liquid chromatographic method for determining ketoprofen in plasma sample was modified from that described by Panvipa (Panvipa, 1993). Figure 19 shows chromatograms of blank rabbit plasma, rabbit plasma spiked with ketoprofen and internal standard, and plasma sample taken at 24 hours postdose from a rabbit following administration of three patches of ketoprofen transdermal patches. Internal standard used in this study was diclofenac sodium.

Ketoprofen and internal standard were eluted with retention times of 6.30 and 9.57 minutes, respectively. No interference peaks were observed in these regions. These supported the specificity of the method used for this analysis.

The validation of analytical method for determination of ketoprofen in rabbit plasma was performed by determining the accuracy, the within run and between run precisions. Results are shown in Appendix V. The accuracy in term of percent recovery at all concentrations was within the range of 94.22-110.53%. The within run and between run precisions expressed as percent coefficient of variations were 0.79-2.63% and 1.23-5.75%, respectively.

The calibration curve of peak area ratio (PAR) of ketoprofen to internal standard versus known plasma ketoprofen concentration was linear covered all concentrations analyzed with the coefficient of determination of 0.9997. The linear regression equation was expressed as

$$Y = 0.1453X + 0.0063$$

Where Y = peak area ratio

X = concentration of ketoprofen

Figure 19. High performance liquid chromatograms of ketoprofen (1) and IS (2).

(A) Blank rabbit plasma.

(B) Spiked rabbit plasma (ketoprofen concentration of 3.6 μg/mL).

(C) Rabbit plasma sample taken after 24 hours of transdermal patch administration.

5.2 *In vivo* evaluation of ketoprofen in rabbit plasma

Plasma ketoprofen concentrations at predetermined sampling time intervals up to 24 hours from nine rabbits following administration of three patches of each formulation of ketoprofen transdermal patches are presented in Tables 8 to 10. Comparisons of the plasma ketoprofen concentration versus time profiles of each rabbit are illustrated in Figures 20 to 28 and the mean profiles of each formulation for nine rabbits are summarized graphically in Figure 29.

The data in Tables 8-10 and their plots as shown in Figures 20-29 indicated that all three formulations had sustained action. This is because the concentrations of ketoprofen were gradually increased up to 24 hours, referring slow absorption of the drug from the sites of application. The maximum plasma drug concentrations as well as the time to peak plasma drug concentrations were not able to detect due to incompletion of early absorption phase. The formulation with propylene glycol as plasticizer appeared to be the fastest release followed by others with glycerin and tributyl citrate as plasticizer, respectively. This is expected because both propylene glycol and glycerin are polar compounds with facilitated action which lead to increment solubility of ketoprofen.

The reasons of small amount of ketoprofen released from the transdermal patch would possibly be due to solubility of ketoprofen in the Eudragit® E 100 was too high. Because if the solubility of a drug in the vehicle is too high, the thermodynamic activity of the drug in the vehicle will be low, leading to low percutaneous absorption rate unless the vehicle alters the barrier property of the skin (Cho and Choi, 1998). An important reason of *in vivo* studies was excellent barrier function of the rabbit skin. The percutaneous absorption is limited by skin (Gurol, 1996), especially stratum corneum, played the role as rate limiting barrier of drug absorption.

Table 8. Plasma ketoprofen concentrations (μg/mL) of nine rabbits after administration of ketoprofen transdermal patches using tributyl citrate as plasticizer.

Time (hours)	Concentration (μg/mL)									Mean concentration (μg/mL)	S.D.
	Rabbit#1	Rabbit#2	Rabbit#3	Rabbit#4	Rabbit#5	Rabbit#6	Rabbit#7	Rabbit#8	Rabbit#9		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
1	0.483	0.479	0.516	0.473	0.452	0.543	0.476	0.470	0.466	0.484	0.03
2	0.498	0.483	0.536	0.481	0.483	0.574	0.495	0.575	0.504	0.514	0.04
3	0.580	0.500	0.630	0.495	0.540	0.645	0.530	0.578	0.536	0.559	0.05
4	0.661	0.603	0.830	0.633	0.603	0.670	0.572	0.609	0.646	0.647	0.08
6	0.675	0.673	0.806	0.867	0.865	0.840	0.692	0.763	0.844	0.781	0.08
8	0.700	0.829	0.938	0.982	0.906	0.973	0.818	0.857	0.897	0.878	0.09
10	0.761	0.927	0.972	0.984	1.012	1.082	1.052	0.991	1.069	0.983	0.10
12	0.819	0.953	1.077	1.071	1.111	1.221	1.246	1.248	1.232	1.109	0.15
24	3.203	2.901	2.979	2.837	3.443	3.425	2.881	3.087	3.288	3.116	0.23

Table 9. Plasma ketoprofen concentrations (μg/mL) of nine rabbits after administration of ketoprofen transdermal patches using propylene glycol as plasticizer.

Time (hours)	Concentration (μg/mL)									Mean concentration (μg/mL)	S.D.
	Rabbit#1	Rabbit#2	Rabbit#3	Rabbit#4	Rabbit#5	Rabbit#6	Rabbit#7	Rabbit#8	Rabbit#9		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
1	0.593	0.583	0.526	0.593	0.781	0.618	0.646	0.634	0.688	0.629	0.07
2	0.611	0.675	0.735	0.644	0.794	0.625	0.793	0.658	0.800	0.704	0.08
3	0.650	0.691	0.779	0.758	0.827	0.690	1.007	0.794	0.978	0.797	0.12
4	0.721	0.717	0.801	0.885	0.877	0.843	1.078	0.819	1.031	0.863	0.12
6	1.043	1.002	1.019	0.994	1.037	0.983	1.102	1.066	1.167	1.046	0.06
8	1.070	1.141	1.060	1.088	1.096	1.089	1.268	1.117	1.213	1.127	0.07
10	1.212	1.425	1.434	1.386	1.182	1.221	1.508	1.218	1.552	1.349	0.14
12	1.395	2.107	1.783	1.896	1.441	1.503	2.049	1.551	1.900	1.736	0.27
24	4.281	4.797	4.598	4.037	4.623	4.682	4.587	4.334	5.938	4.653	0.54

Table 10. Plasma ketoprofen concentrations (μg/mL) of nine rabbits after administration of ketoprofen transdermal patches using glycerin as plasticizer.

Time (hours)	Concentration (μg/mL)									Mean concentration (μg/mL)	S.D.
	Rabbit#1	Rabbit#2	Rabbit#3	Rabbit#4	Rabbit#5	Rabbit#6	Rabbit#7	Rabbit#8	Rabbit#9		
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00
1	0.464	0.644	0.633	0.452	0.441	0.426	0.480	0.476	0.543	0.506	0.08
2	0.695	0.646	0.756	0.693	0.718	0.593	0.610	0.644	0.682	0.671	0.05
3	0.771	0.673	0.784	0.862	0.809	0.646	0.700	0.694	0.825	0.751	0.08
4	0.851	0.875	0.881	0.955	0.847	0.703	0.842	0.761	0.965	0.853	0.08
6	0.977	0.931	0.982	1.140	0.974	0.921	0.941	0.972	1.060	0.989	0.07
8	1.326	1.016	1.017	1.250	1.164	1.067	1.024	1.126	1.138	1.125	0.11
10	1.416	1.257	1.093	1.367	1.472	1.214	1.194	1.421	1.372	1.312	0.13
12	1.471	1.400	1.469	1.519	1.534	1.326	1.544	1.750	1.637	1.517	0.12
24	3.600	3.630	3.474	3.933	4.185	3.718	3.637	3.573	3.765	3.724	0.22

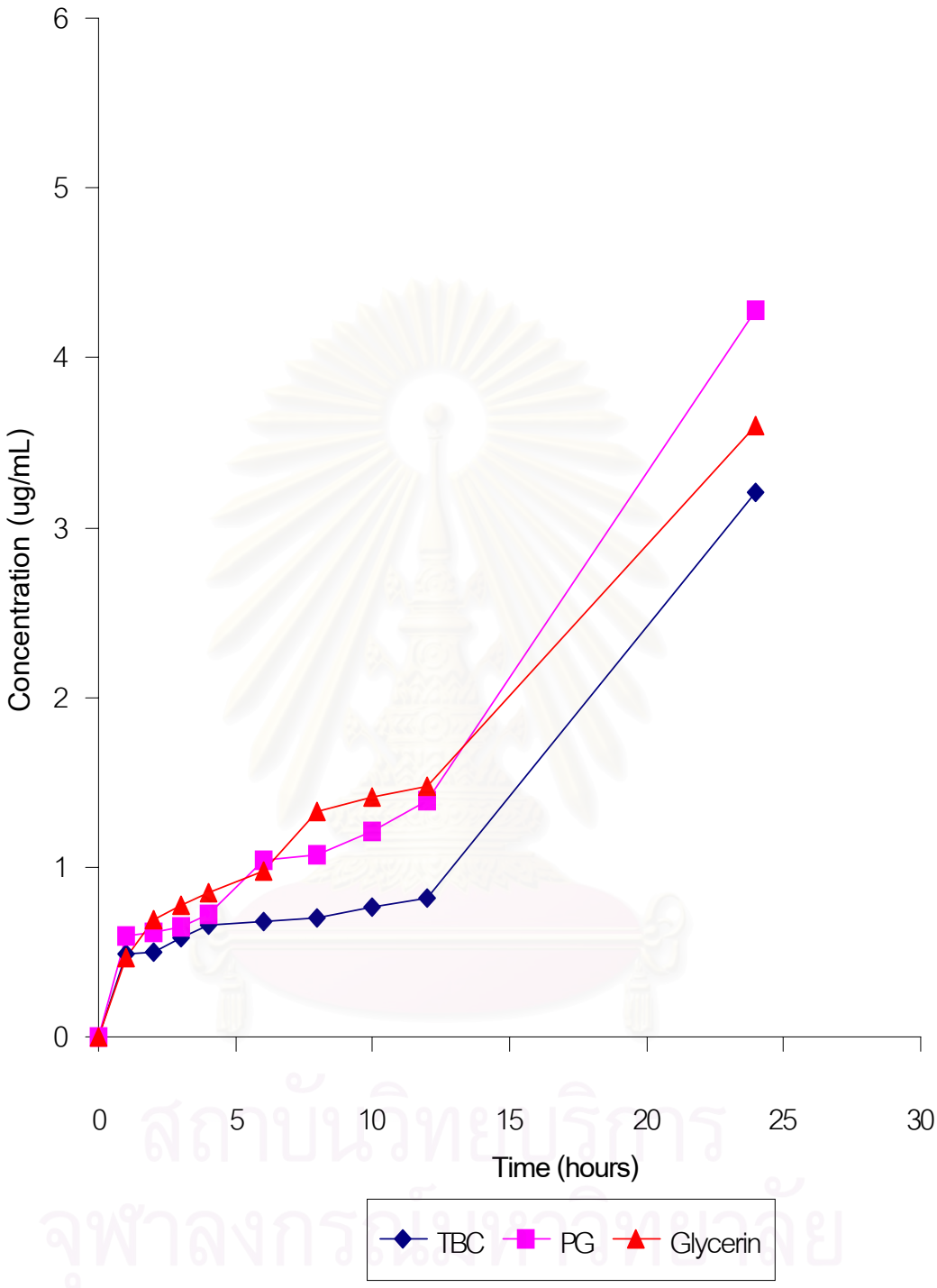


Figure 20. Plasma ketoprofen concentration-time curves of rabbit No.1 after administration of three formulations of ketoprofen transdermal patches.

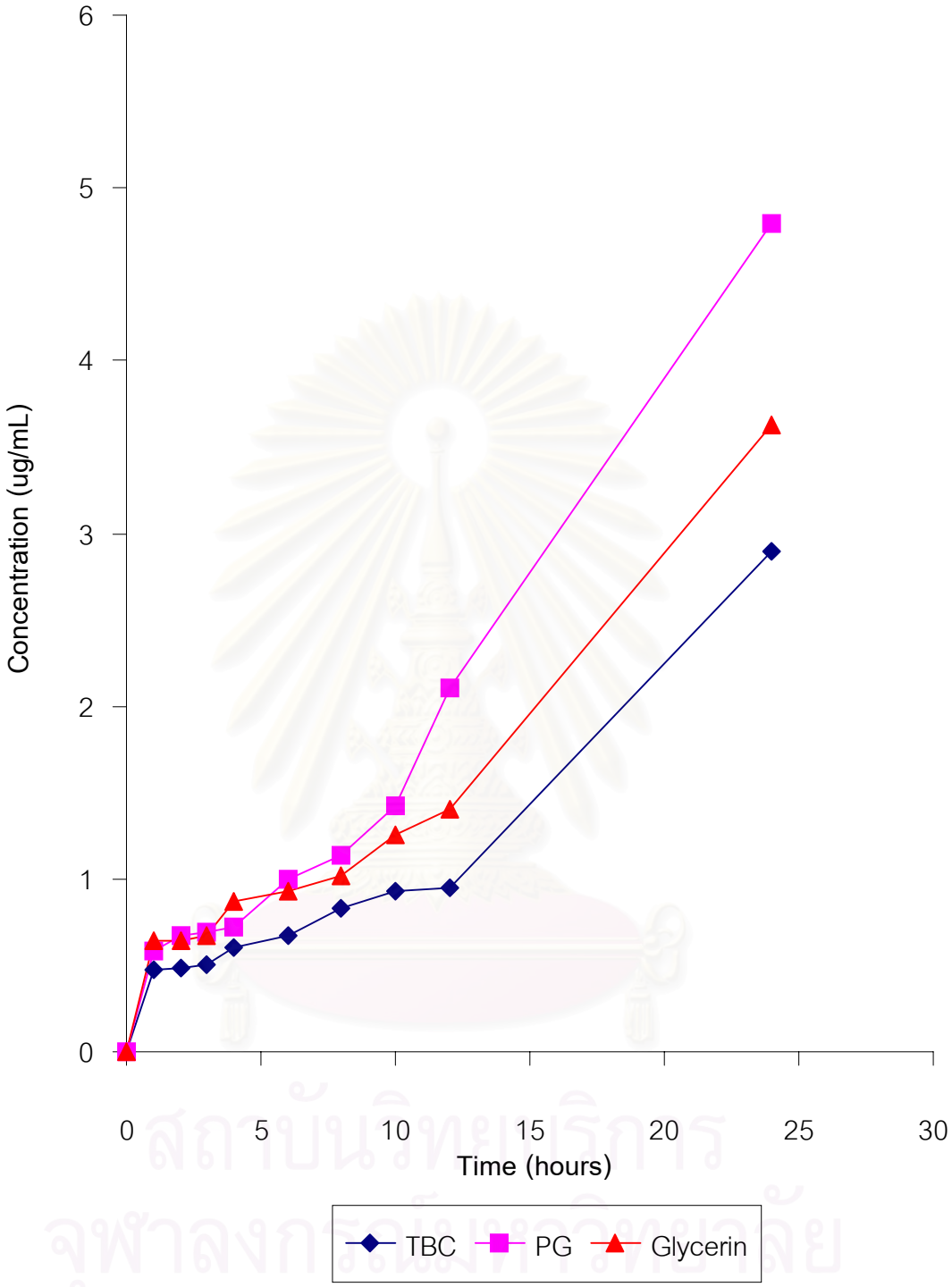


Figure 21. Plasma ketoprofen concentration-time curves of rabbit No.2 after administration of three formulations of ketoprofen transdermal patches.

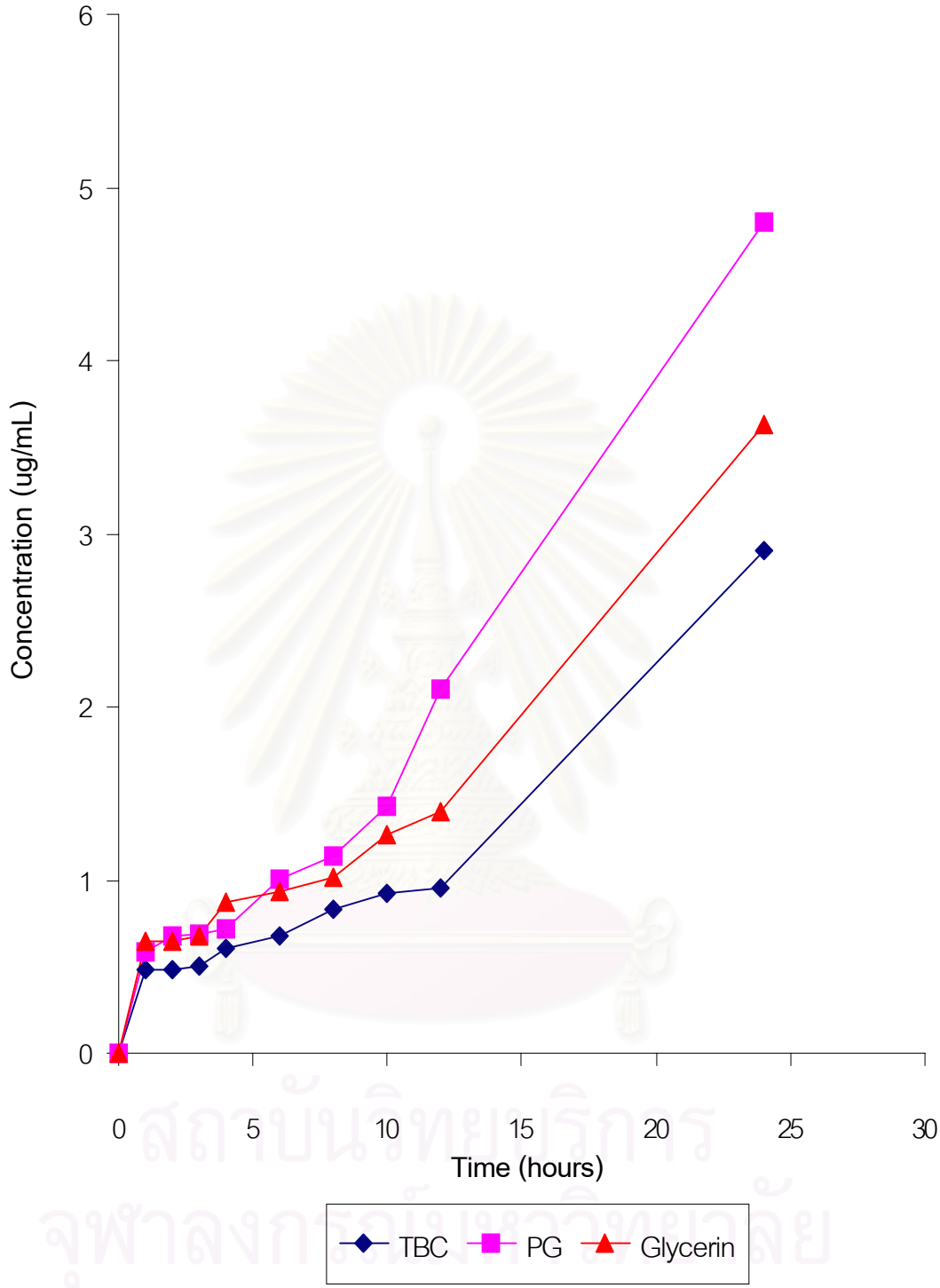


Figure 22. Plasma ketoprofen concentration-time curves of rabbit No.3 after administration of three formulations of ketoprofen transdermal patches.

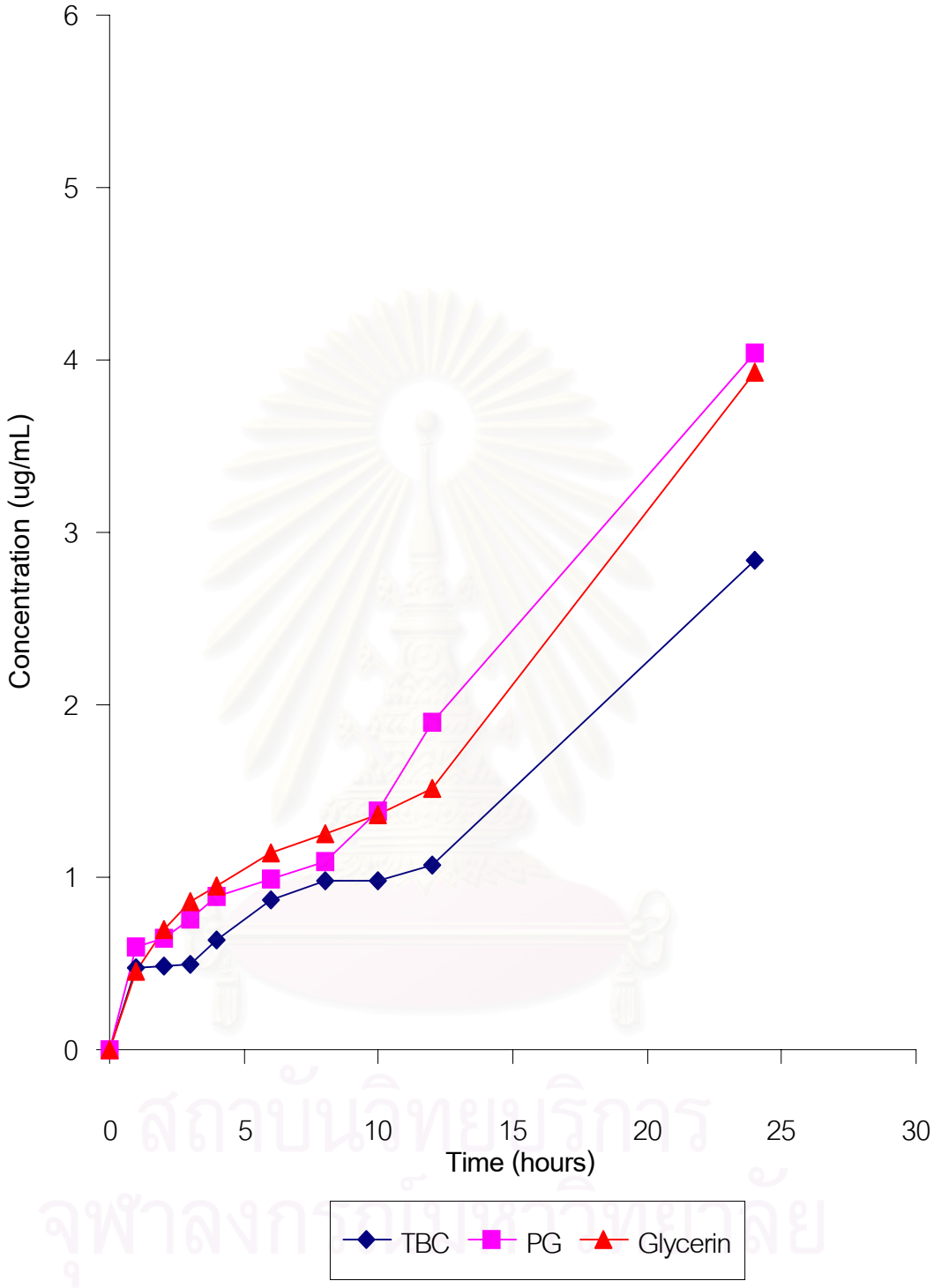


Figure 23. Plasma ketoprofen concentration-time curves of rabbit No.4 after administration of three formulations of ketoprofen transdermal patches.

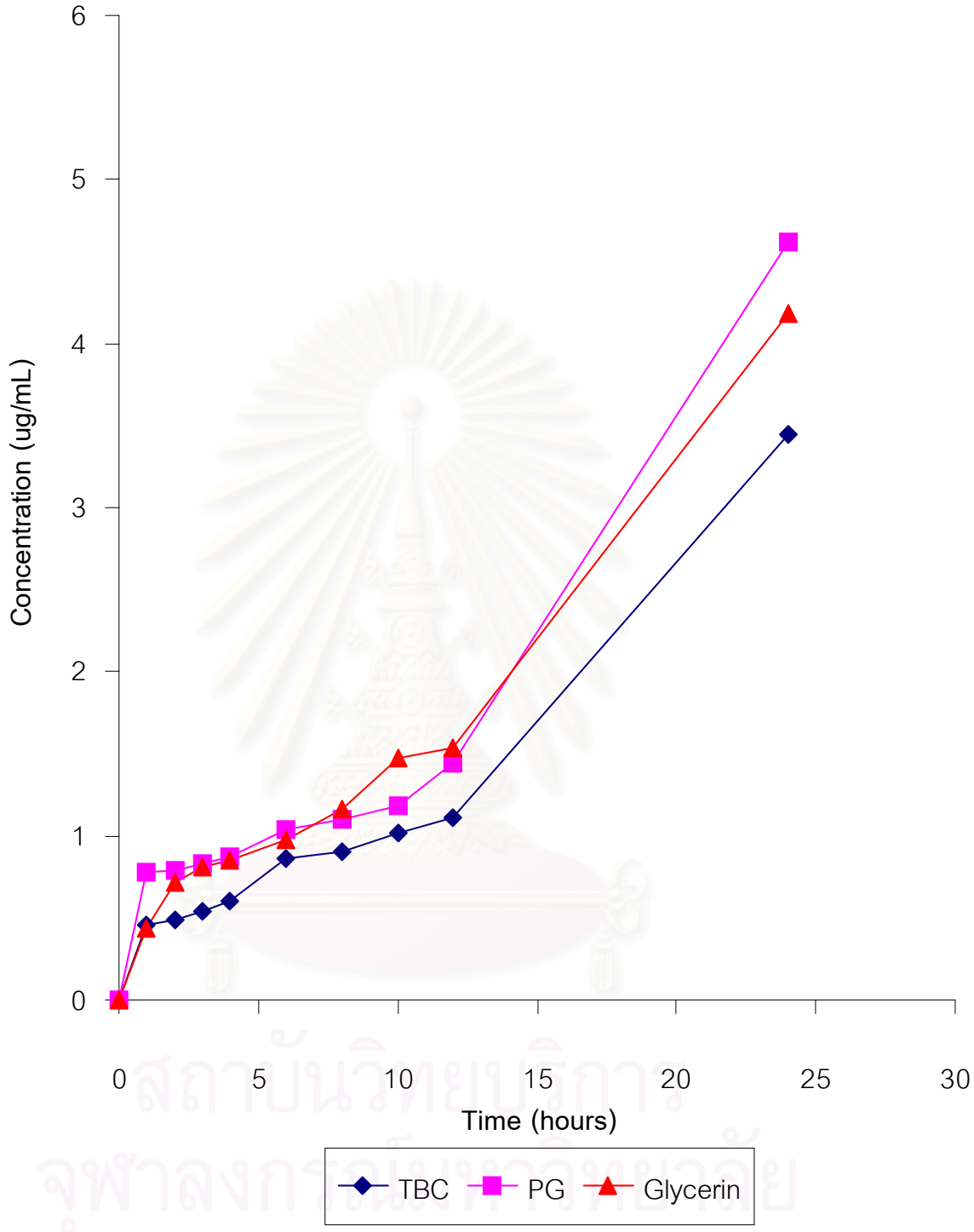


Figure 24. Plasma ketoprofen concentration-time curves of rabbit No.5 after administration of three formulations of ketoprofen transdermal patches.

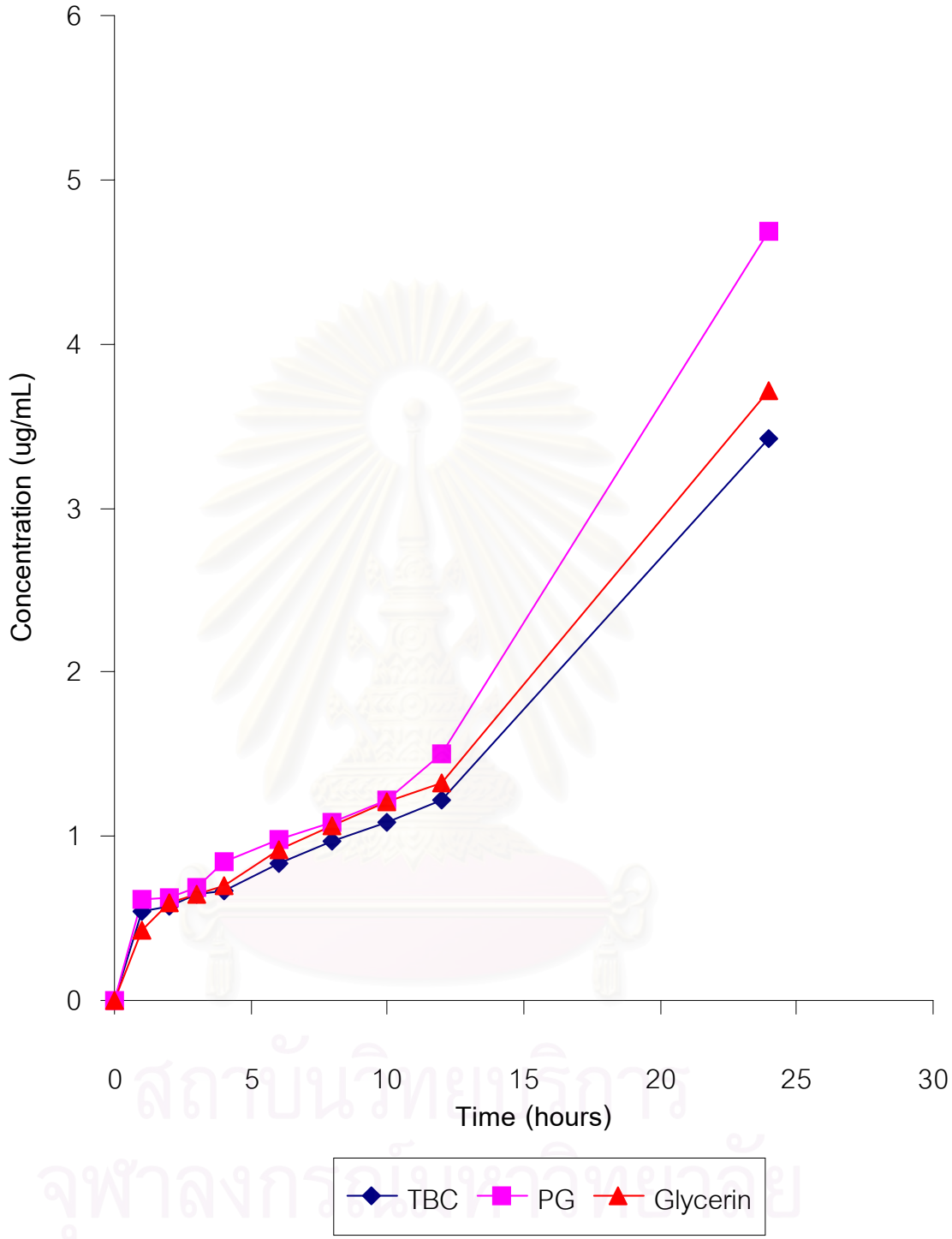


Figure 25. Plasma ketoprofen concentration-time curves of rabbit No.6 after administration of three formulations of ketoprofen transdermal patches.

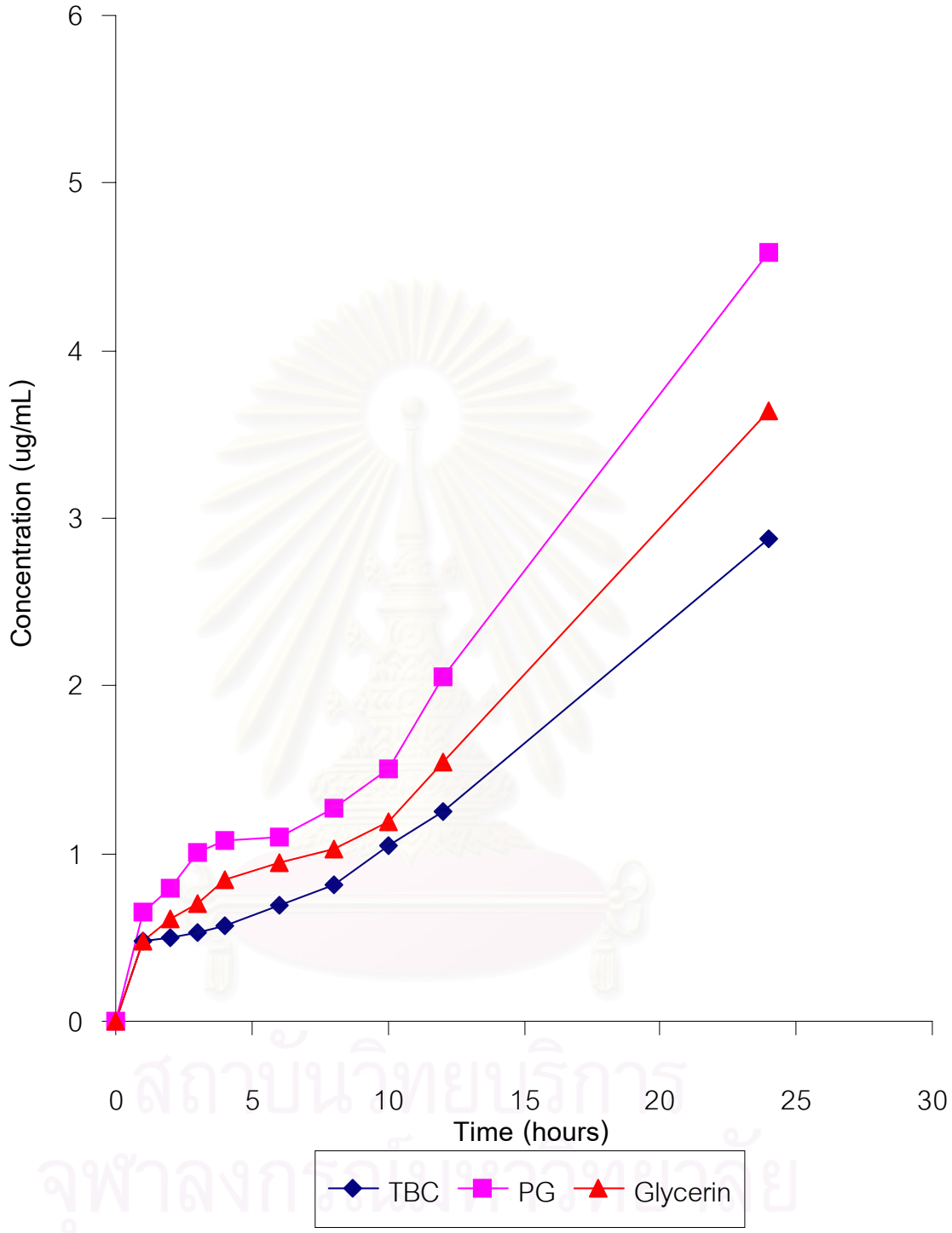


Figure 26. Plasma ketoprofen concentration-time curves of rabbit No.7 after administration of three formulations of ketoprofen transdermal patches.

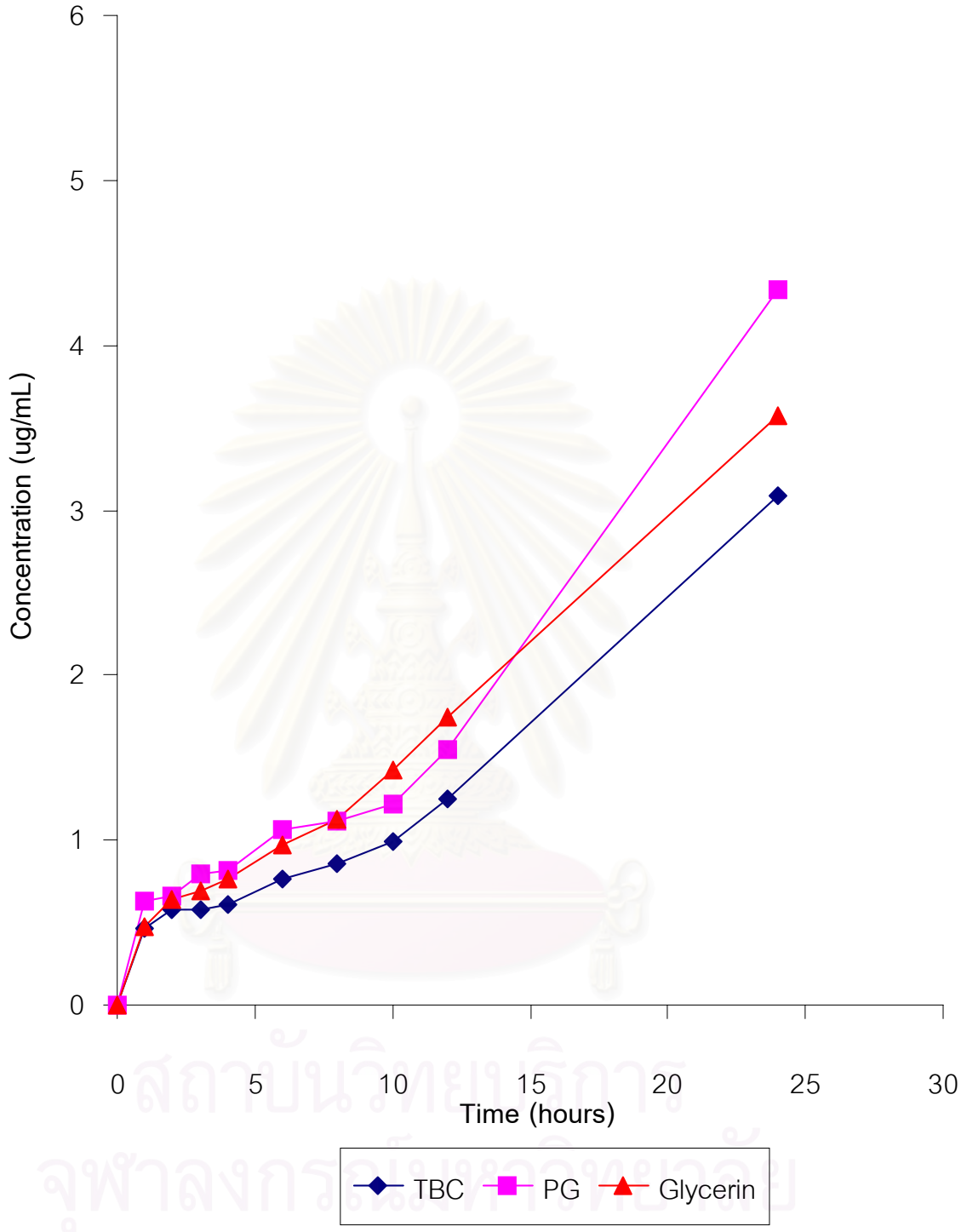


Figure 27. Plasma ketoprofen concentration-time curves of rabbit No.8 after administration of three formulations of ketoprofen transdermal patches.

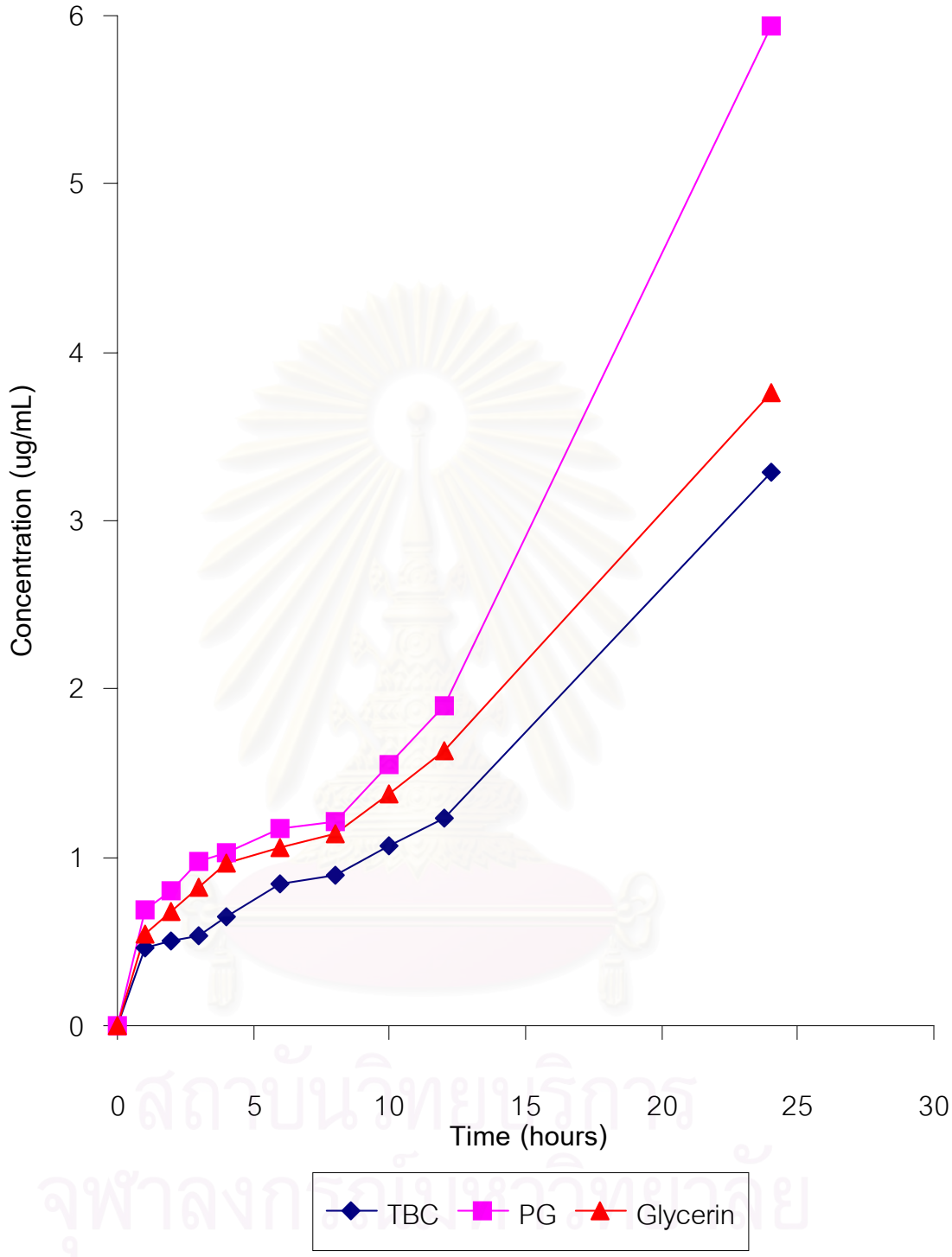


Figure 28. Plasma ketoprofen concentration-time curves of rabbit No.9 after administration of three formulations of ketoprofen transdermal patches.

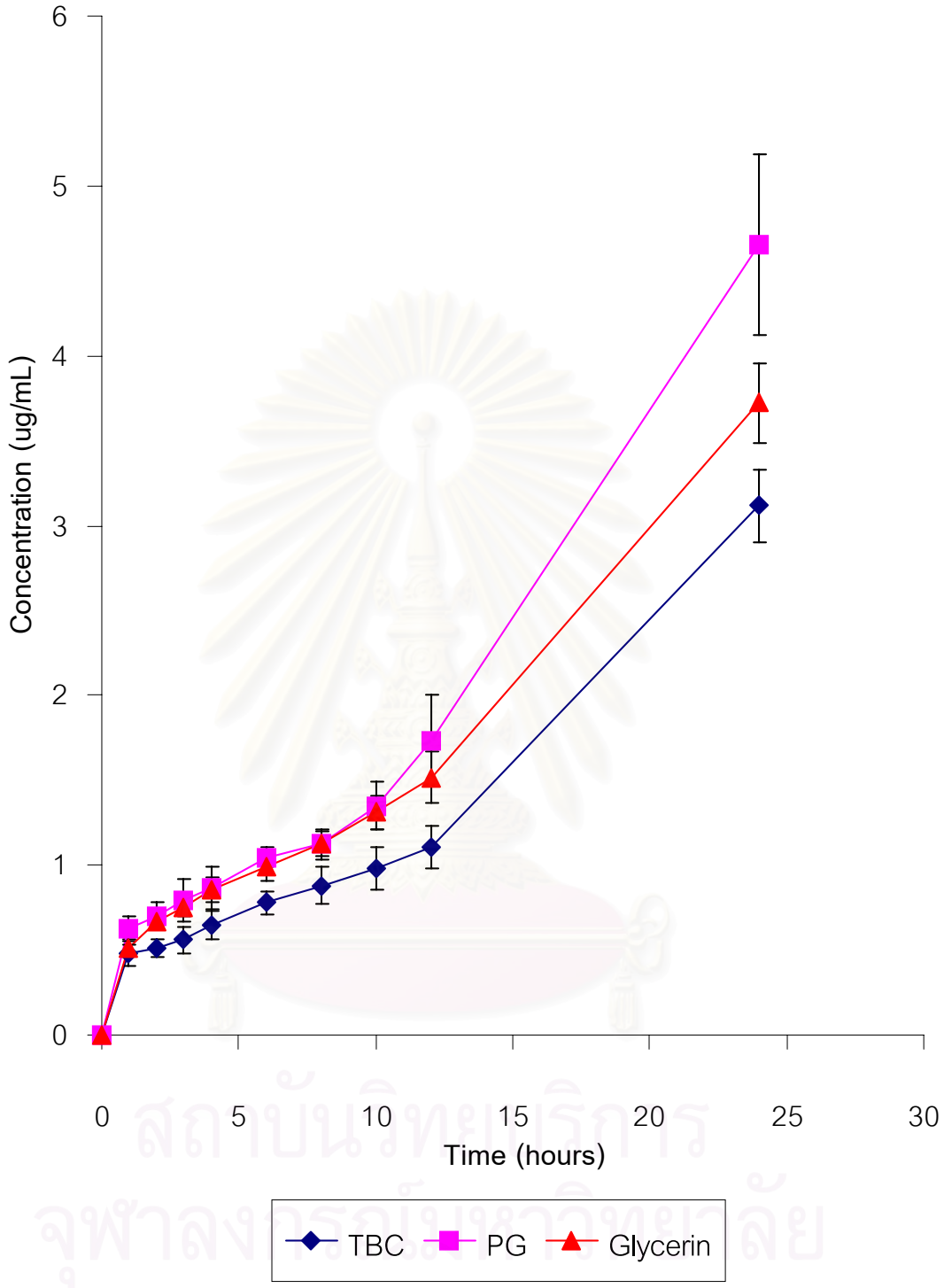


Figure 29. Mean plasma ketoprofen concentration-time curves of nine rabbits after administration of three formulations of ketoprofen transdermal patches.

From Figure 29, it can be seen that propylene glycol gave the best results in rabbit plasma. The mechanism of the enhancing effect of propylene glycol is reported to be solvation of the stratum corneum by unfolding the keratinized protein matrix, and it is also known that this vehicle penetrates the skin (Niazy et al. 1990). The change in driving force and/or the change in the barrier property of the skin with time may have resulted in various permeation profiles (Cho and Choi, 1998). Rate of diffusion of drug through the film depend on film thickness and plasticizers used (Rao and Diwan, 1997). On the other hand, tributyl citrate gave the lowest amount of ketoprofen release from transdermal patch in rabbit plasma maybe due to its hydrophobic action like ketoprofen cause solubility of tributyl citrate in ketoprofen is high, leading to low percutaneous absorption and low drug concentration in rabbit plasma. The one it would possibly be due to the limit absorption of drug through rabbit skin, the conditon of skin or any other variations. All of these are waiting to be proven in the future.

From the data, the mean concentration of the drug in nine rabbits from the formulations using tributyl citrate, propylene glycol, and glycerin as plasticizer were 3.12μg/mL, 4.65 μg/mL, and 3.72 μg/mL, respectively.

5.3 Pharmacokinetics studies.

The pharmacokinetics of ketoprofen following administration of ketoprofen transdermal patches in nine rabbits were evaluated. As stated earlier, the C_{max} and t_{max} values were not able to observe due to incompletion of early absorption phase. Thus, the pharmacokinetic parameters presented are those calculated from the available experimental data. They are; the area under the plasma concentration-time curve (AUC_{0-24}), the area under the moment curve ($AUMC_{0-24}$) and the mean residence time (MRT), respectively. Detail of each individual was summarized as follows:

Area under the plasma concentration-time curve (AUC_{0-24}).

This parameter represented the amount of ketoprofen absorption after administration into systemic circulation from time 0 upto 24 hours. The AUC_{0-24} (Mean± S.D.) from transdermal patches with tributyl citrate, propylene glycol, and glycerin as plasticizer were 34.27±2.23, 50.53±4.82 and 43.01±2.04 μg.hr/mL (Table 11), respectively. Both propylene glycol and glycerin provided the greater amount of drug absorption than that tributyl citrate did. This is expected since they are polar whereas tributyl citrate is nonpolar. The AUC_{0-24} value from the formula with propylene glycol appeared to be the highest figure followed by those with glycerin and tributyl citrate, respectively. These results related to *in vitro* release of the drug. Statistical comparisons were made to determine if the AUC_{0-24} values obtained were different. Results in Table 12 and 13 indicate that these values were different among each other ($P<0.05$).

Table 11. Area under the plasma concentration-time curve from time 0 upto 24 hours (AUC_{0-24}) of nine rabbits after administration of three formulations of ketoprofen transdermal patches.

Rabbit No.	**AUC_{0-24} (μg.hr/mL)**		
	Tributyl citrate	Propylene glycol	Glycerin
1	31.76	45.03	42.53
2	31.30	53.68	41.25
3	33.83	50.33	40.79
4	32.58	47.75	45.17
5	36.38	48.17	46.3
6	37.65	48.39	40.71
7	33.49	53.68	41.99
8	35.01	46.98	43.68
9	36.44	60.79	44.69
Mean	34.27	50.53	43.01
S.D.	2.23	4.82	2.04

Table 12. Analysis of variance for three way crossover design of AUC_{0-24} of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	26	1451.40	55.82	-		
Sequence	2	38.30	19.15	1.13	5.14	NS
Subject (Seq)	6	101.32	16.89	2.52	2.85	NS
Period	2	25.89	12.94	1.93	3.74	NS
Formulation	2	1192.30	596.15	89.13	3.74	S
Error	14	93.64	6.63	-		

Table 13. Least significant difference test of AUC_{0-24} of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

$i-i^{/}$	$Xi-Xi^{/}$	n_i	$LSD_{0.05}$	Significance
B-A	16.26	9	2.51	B differ A
B-C	7.52	9	2.51	B differ C
C-A	8.74	9	2.51	C differ A

A = Formulation with tributyl citrate as plasticizer.

B = Formulation with propylene glycol as plasticizer.

C = Formulation with glycerin as plasticizer.

Area under the moment curve ($AUMC_{0-24}$).

Area under the moment curve was calculated from the plot of concentration*time versus time profile using linear trapezoidal rule. Table 14 present the average AUMC (Mean±S.D.) of the formulation with tributyl citrate, propylene glycol, and glycerin as plasticizer with the values of 592.95±39.37, 883.86±92.16 and 725.17± 34.92 μg.hr/mL, respectively. This parameter is related to the area under the plasma drug concentration versus time curve. It is the first moment and has been used for calculating the mean residence time of the drug in noncompartmental method. Statistical tests of them appear to be the same manner of those with the AUC_{0-24} values ($P<0.05$) as seen in Tables 15 and 16.

Table 14 Area under the moment curve from time 0 upto 24 hours ($AUMC_{0-24}$) of nine rabbits after administration of three formulations of ketoprofen transdermal patches.

Rabbit No.	**$AUMC_{0-24}$ (μg.hr/mL)**		
	Tributyl citrate	Propylene glycol	Glycerin
1	575.44	794.33	707.95
2	549.54	933.25	707.95
3	575.44	870.96	676.08
4	549.54	812.83	758.58
5	645.65	851.14	794.33
6	645.65	870.96	707.95
7	562.34	912.01	707.95
8	602.00	812.83	724.44
9	630.96	1096.48	741.31
Mean	592.95	883.86	725.17
S.D.	39.37	92.16	34.92

Table 15. Analysis of variance for three way crossover design of $AUMC_{0-24}$ of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	26	472034.00	18155.15	-		
Sequence	2	9022.90	4511.45	0.72	5.14	NS
Subject (Seq)	6	37466.00	6244.33	2.39	2.85	NS
Period	2	7016.20	3508.10	1.34	3.74	NS
Formulation	2	381920.00	190960.00	73.03	3.74	S
Error	14	36609.00	2614.93	-		

Table 16. Least significant difference test of $AUMC_{0-24}$ of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

$i\text{-}i^{/}$	$Xi\text{-}Xi^{/}$	n_i	$LSD_{0.05}$	Significance
B-A	290.91	9	49.66	B differ A
B-C	157.94	9	49.66	B differ C
C-A	132.22	9	49.66	C differ A

A = Formulation with tributyl citrate as plasticizer.

B = Formulation with propylene glycol as plasticizer.

C = Formulation with glycerin as plasticizer.

Mean residence time (MRT).

The mean residence time is the time of drug residing in the body. It is approximately calculated from the ratio of $AUMC_{0-24}$ to AUC_{0-24} and represented the time for 63.2 percent of the dose administered to be eliminated. The MRTs computed were 17.27±0.36, 17.47±0.37 and 16.95±0.24 hours for the formulations with tributyl citrate, propylene glycol and glycerin as plasticizer, respectively. These values are comparable. However, statistical tests in Tables 18 and 19, only the formulations with tributyl citrate and propylene glycol are not different (P>0.05).

Table 17. Mean residence time (MRT) of nine rabbits after administration of three formulations of ketoprofen transdermal patches.

Rabbit No.	**MRT (hr)**		
	Tributyl citrate	Propylene glycol	Glycerin
1	18.00	17.67	16.75
2	17.39	17.41	17.02
3	16.92	17.46	16.75
4	16.91	16.90	16.94
5	17.61	17.66	17.29
6	17.28	17.84	17.36
7	16.97	16.92	17.00
8	17.11	17.43	16.67
9	17.28	17.94	16.74
Mean	17.27	17.47	16.95
S.D.	0.36	0.37	0.25

Table 18. Analysis of variance for three way crossover design of MRT of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	26	3.85	0.15	-		
Sequence	2	0.18	0.09	0.83	5.14	NS
Subject (Seq)	6	1.36	0.23	2.86	2.85	S
Period	2	0.04	0.02	1.67	3.74	NS
Formulation	2	1.26	0.63	106.19	3.74	S
Error	14	1.01	0.07	-		

Table 19. Least significant difference test of MRT of nine rabbits after administration of three formulations of ketoprofen transdermal patches (α=0.05).

$i\text{-}i'$	$Xi\text{-}Xi'$	n_i	$LSD_{0.05}$	Significance
B-A	0.2	9	0.26	B = A
B-C	0.52	9	0.26	B differ C
C-A	0.32	9	0.26	C differ A

A = Formulation with tributyl citrate as plasticizer.

B = Formulation with propylene glycol as plasticizer.

C = Formulation with glycerin as plasticizer.

Comparison of pharmacokinetic parameters

The pharmacokinetic parameters obtained from this study revealed intersubject variations among all rabbits. Factor affected these variations may be due to sex, age, and weight of rabbits as well as the composition of inactive ingredients in each formulation.

Finally, according to *in vitro* and *in vivo* data, it can be concluded that concentration of ketoprofen loaded, concentration of plasticizer, and types of plasticizers affected ketoprofen released from ketoprofen transdermal patches and also behavior of the drug in the body.

CHAPTER V

CONCLUSIONS

Development and evaluation of ketoprofen transdermal patch was accomplished. The best three formulations were established based on excellent releasing characteristics of the drug. All of them contained Eudragit® E 100 as pressure-sensitive adhesive. Tributyl citrate, propylene glycol, and glycerin are plasticizer. Each plasticizer was assigned in individual formulation.

In vitro evaluations revealed that ketoprofen was slowly released from the patches. Concentration of drug loaded, type and concentration of plasticizers affected the release of the drug from all formulations.

In vivo studies of these three formulations were performed using a white New Zealand rabbits in a crossover manner. Ketoprofen was shown to be absorbed from the patches into systemic circulation. The corresponding pharmacokinetic parameters obtained from the individual formulation were significantly different with each other, implying that drug loaded, type and concentration of plasticizer in each formulation could also affect the behavior of the drug in the body.

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Appendix I

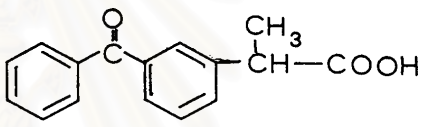
Characteristic of Constituents

1. **Ketoprofen** (Reynold, 1993 and Boylan, 1986)

1.1. Physicochemical properties

Chemical name : 2 - (3 - Benzoylphenyl) propionic acid, 3 – benzoyl - α - methylbenzene acetic acid.

Structure :



Molecular formula : $C_{16}H_{14}O_3$

Molecular weight : 254.29

Appearance : White or almost white, odorless, crystalline powder.

Melting range : 93-96 ^{o}C

Solubility : Ketoprofen is slightly soluble in water, freely soluble in ethanol, chloroform, ether and acetone, soluble in benzene.

pH : The pH of a 3.95 x 10^{-4} M solution in water is 6.5.

pKa : 4.45 in water.

Stability : Ketoprofen must be protected from light and moisture. It is stable at room temperature. It has been dissolved in ethyl acetate and stored for several weeks at 4^{o}C with no detectable decomposition. If ketoprofen is heated in an acid solution pH 1 at 98^{o}C for 30 minutes. No decompostion is detected.

1.2. Pharmacological properties (Reynold, 1993).

Ketoprofen has pharmacological actions similar to those of other NSAIDs. The drug exhibits anti-inflammatory, analgesic and antipyretic activity. Ketoprofen is used to treat musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis, peri-artricular disorders such as bursitis and tendinitis, mild to moderate pain such as dysmenorrhea or postoperative pain, and other painful and inflammatory conditions such as acute gout or soft-tissue disorders.

1.3. Dosage and administration.

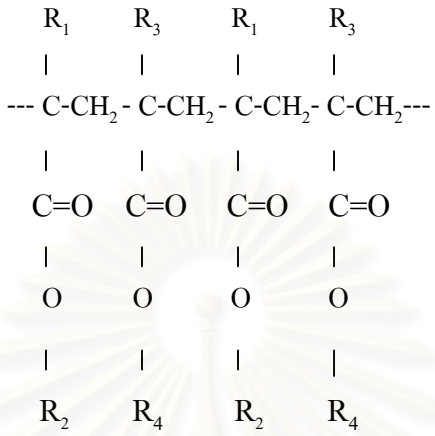
The usual daily dose orally is 100 to 200 mg in 2 to 4 divided doses with food, controlled formulations taken once daily may also be used. Ketoprofen may also be administered rectally as suppositories in a usual dose of 100 mg at night. The total daily dose by mouth and by rectum should not exceed 200 mg. The therapeutic range of ketoprofen is reported to be about 0.4-6.0 μg/mL.

1.4. Pharmacokinetic properties.

Ketoprofen is well absorbed after parenteral, oral, or rectal administration. Ketoprofen is readily absorbed from the gastro-intestinal tract; peak plasma concentrations occur about 0.5 to 2 hours after dosing. It is 99% bound to plasma proteins. The plasma elimination half-life is about 1-3 hours. Ketoprofen is metabolized mainly by conjugation with glucuronic acid, and is excreted mainly in the urine.

2. Eudragit® E 100 (Boylan, 1986)

Structure :



Where $R_1, R_3 = CH_3$

$R_2 = CH_2CH_2N(CH_3)_2$

$R_4 = CH_3, C_4H_9$

Chemical name: Poly(butyl methacrylate, (2-dimethyl aminoethyl methacrylate, methyl methacrylate) 1:2:1

Properties: Eudragit E® 100 is cationic polymer based on dimethylaminoethyl methacrylate and other neutral methacrylic acid esters available as 12.5% ready-to-use solution in isopropanol/acetone (60:40); light yellow in color with the characteristic odor of the solvent; solvent-free granules contain 98% dried weight content. It is soluble in gastric fluid below pH 5. It is colorless, transparent, shiny coating, dust-free and resistant to handling.

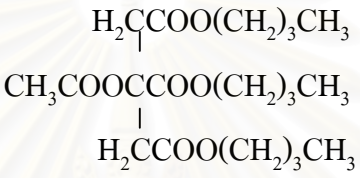
Solubility: Soluble in polar organic solvents, such as alcohols (ethanol, isopropanol), acetone, esters, chloroform, etc. Insoluble in

water, petroleum, ether, etc., and saliva. Swells and dissolves in acidic media.

Application: Eudragit E® has been used as granulation binder in concentration between 5 and 20 %, used as a film coating agent, soluble in gastric fluid.

3. Tributyl citrate (Doolittle, 1989)

Structure:



Molecular formula: $C_{20}H_{34}O_8$

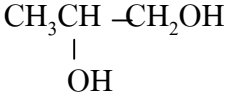
Molecular weight : 402

Properties : Soluble in organic solvents and hydrocarbons, insoluble in water. Compatible with most commercially available resins except cellulose acetate.

Tributyl citrate is a primary plasticizer for most resins, including vinyl chloride types. It has a low volatility and a number of other features of interest as an all-purpose plasticizer.

4. Propylene glycol (Boylan, 1986)

Structure :



Molecular formula: $C_3H_8O_2$

Molecular weight : 76.09

Properties: A clear, colorless, viscous and practically odorless liquid having a sweet, slightly acrid taste resembling glycerol. Used as humectant, solvent, plasticizer.

Solubility: Miscible with water, acetone, alcohol, glycerin and chloroform; soluble in a ratio of 1:6 ether; immiscible with light mineral oil; immiscible with fixed oils, but will dissolve in some essential oils.

Stability: Under ordinary conditions, propylene glycol is stable in well-closed containers, but at high temperatures in the open it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid and acetic acid. It is chemically stable when mixed with glycerin, water or alcohol. Propylene glycol withstands autoclave sterilization in sealed containers. Store in a well-closed container. Protect from light. Absorbs moisture when exposed to moist air.

5. **Glycerin** (Boylan, 1986)

Structure : $CH_2OH\text{-}CHOH\text{-}CH_2OH$

Molecular formula: $C_3H_8O_3$

Molecular weight : 92.09

Properties: A clear colorless, odorless, syrupy and hygroscopic liquid. Used as humectant, solvent, plasticizer and tonicity agent.

Solubility: Miscible with water, alcohol and methanol. One part of glycerin dissolves in 11 parts of ethyl acetate and in about 500 parts of ethyl ether. Insoluble in benzene, chloroform, ether, mineral oil, fixed and volatile oils, halogenated hydrocarbons and aromatic hydrocarbons.

Stability: Pure glycerin decomposes on heating, with the evolution of toxic acrolein. Mixtures of glycerin with water, ethyl alcohol and propylene glycol are chemically stable. Preserve in a tight container to avoid moisture absorption.

Application: Uses as emollient, humectant, plasticizer in tablet film coating, preservative in liquid pharmaceuticals, solvent for parenteral formulations and sweetener in high alcoholic elixirs.

Appendix II

Reagent Preparation

Phosphate buffer pH 7.4

Dissolved 27.22 g of potassium dihydrogen phosphate in water and adjust to 1 liter with water, take 50 mL of this solution to mix with 39.1 mL of 0.2 N Sodium hydroxide solution and adjust the resulting solution with water to a pH 7.2±0.02.

Sodium acetate buffer pH 4.2

Dissolved 1.6256 g of sodium acetate trihydrate in water, mix with 2.4 mL of glacial acetic acid, adjust with water to 500 mL and to a pH of 4.2±0.02.

Appendix III

Validation of Analytical Method for *In Vitro* Studies

1. At λ = 255 nm

1.1 Accuracy

Table 20. Accuracy of analytical method for determination of ketoprofen in methanol at λ = 255 nm.

Concentration (μg/mL)	Absorbance (n=3)	Inversely estimated concentration (μg/mL)	%Recovery
1.4	0.093	1.46	104.56
3.6	0.229	3.51	97.56
6	0.406	6.18	102.96
7.2	0.47	7.14	99.19
10.8	0.7	10.61	98.20
12	0.802	12.14	101.18

1.2 Precision

1.2.1 Within Run Precision

Table 21. Within run precision of analytical method for determination of ketoprofen in methanol at λ = 255 nm.

Concentration	Absorbance			Mean	S.D.	%C.V.
(μg/mL)	1	2	3			
1.4	0.092	0.095	0.092	0.093	0.002	1.86
3.6	0.229	0.235	0.229	0.231	0.003	1.50
6	0.392	0.392	0.393	0.392	0.001	0.15
7.2	0.467	0.47	0.468	0.468	0.002	0.33
10.8	0.716	0.718	0.714	0.716	0.002	0.28
12	0.803	0.8	0.801	0.801	0.002	0.19

1.2.2 Between Run Precision

Table 22. Between run precision of analytical method for determination of ketoprofen in methanol at λ = 255 nm.

Concentration (μg/mL)	Absorbance			Mean	S.D.	%C.V.
	1	2	3			
1.4	0.092	0.096	0.098	0.095	0.003	3.20
3.6	0.229	0.234	0.245	0.236	0.008	3.47
6	0.392	0.402	0.403	0.399	0.006	1.52
7.2	0.467	0.471	0.464	0.467	0.004	0.75
10.8	0.716	0.715	0.72	0.717	0.003	0.37
12	0.803	0.8	0.81	0.804	0.005	0.64

2. At λ = 260 nm

2.1 Accuracy

Table 23. Accuracy of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm.

Concentration (μg/mL)	Absorbance (n=3)	Inversely estimated concentration (μg/mL)	%Recovery
1.4	0.096	1.40	99.72
3.6	0.235	3.49	96.93
6	0.419	6.26	104.34
7.2	0.479	7.16	99.50
10.8	0.715	10.72	99.24
12	0.802	12.03	100.24

2.2 Precision

2.2.1 Within Run Precision

Table 24. Within run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm.

Concentration	Absorbance			Mean	S.D.	%C.V.
(μg/mL)	1	2	3			
1.4	0.095	0.094	0.096	0.095	0.001	1.05
3.6	0.238	0.236	0.234	0.236	0.002	0.85
6	0.401	0.403	0.399	0.401	0.002	0.50
7.2	0.476	0.488	0.482	0.482	0.006	1.24
10.8	0.683	0.694	0.695	0.691	0.007	0.96
12	0.806	0.811	0.815	0.811	0.005	0.56

2.2.2 Between Run Precision.

Table 25. Between run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm.

Concentration	Absorbance			Mean	S.D.	%C.V.
(μg/mL)	1	2	3			
1.4	0.095	0.096	0.098	0.096	0.002	1.59
3.6	0.238	0.245	0.245	0.243	0.004	1.66
6	0.401	0.402	0.412	0.405	0.006	1.50
7.2	0.476	0.481	0.482	0.480	0.003	0.67
10.8	0.683	0.696	0.694	0.691	0.007	1.01
12	0.806	0.817	0.8	0.808	0.009	1.07

3. Calibration curves.

Table 26. Typical calibration curve data for determination of ketoprofen in methanol estimated using linear regression.

Concentration (μg/mL)	Absorbance	Inversely estimated concentration (μg/mL)	%Recovery
1.4	0.095	1.46	104.52
3.6	0.238	3.61	100.20
4.8	0.312	4.72	98.26
6	0.401	6.05	100.85
7.2	0.476	7.17	99.66
9.6	0.634	9.54	99.42
10.8	0.715	10.76	99.62
12	0.806	12.12	101.02
		mean	100.44
		S.D.	1.74
		%C.V.	1.73

1. $R^2 = 0.9996$, Y = 0.0673X – 0.0109 (Y=Absorbance, X=Conc.)
2. Inversely estimated concentration = (Absorbance + 0.0109)/ 0.0673
3. %Recovery = (Inversely estimated conc. / Conc.) x 100
4. %C.V. = (S.D./Mean) x 100

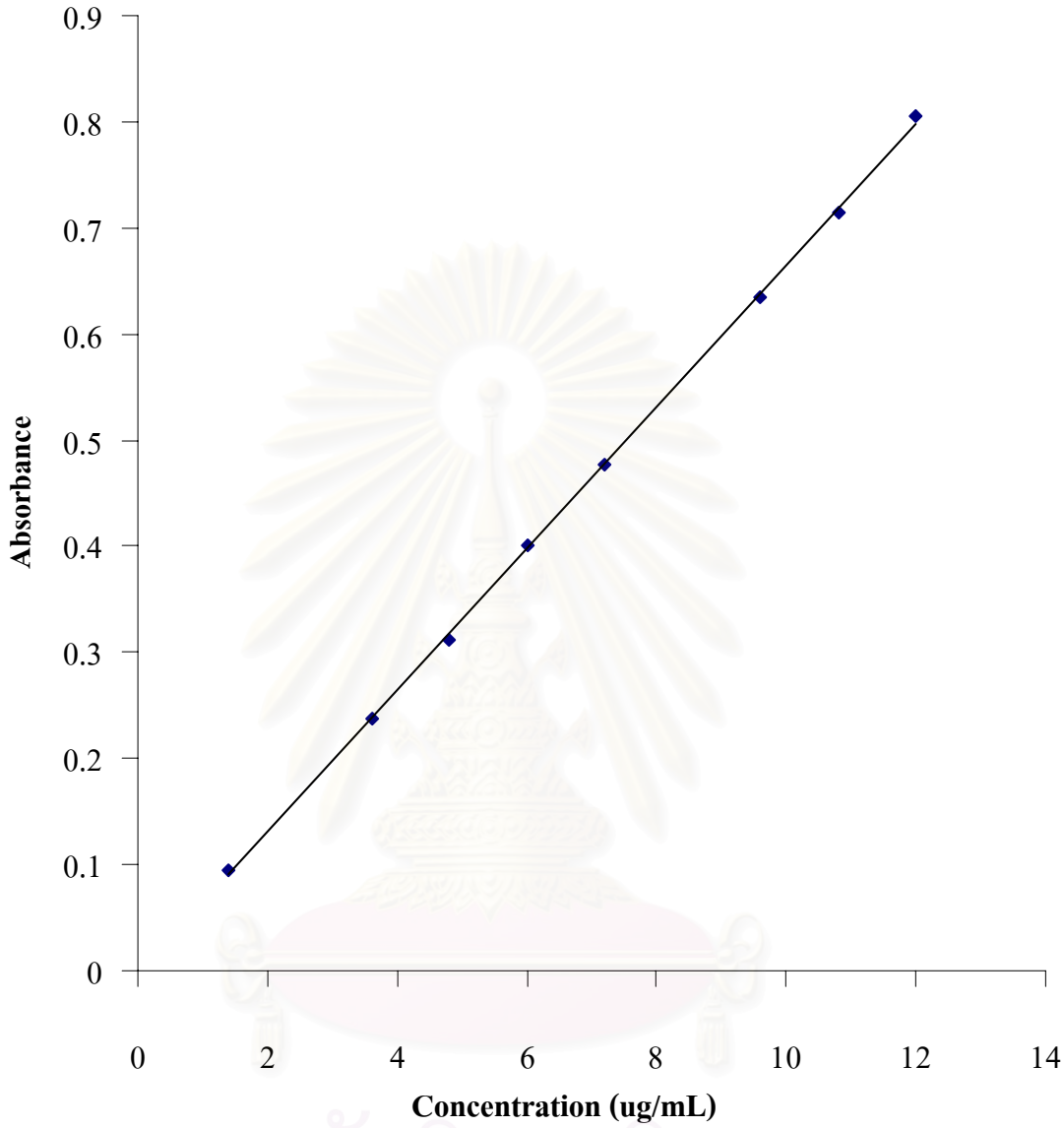


Figure 30. Typical calibration curve for determination of ketoprofen in methanol at λ = 255 nm.

Table 27. Typical calibration curve data for determination of ketoprofen in phosphate buffer pH 7.4 estimated using linear regression.

Concentration (μg/mL)	Absorbance	Inversely estimated concentration (μg/mL)	%Recovery
1.4	0.092	1.53	109.21
3.6	0.229	3.56	99.02
4.8	0.308	4.74	98.72
6	0.392	5.99	99.78
7.2	0.467	7.10	98.62
9.6	0.637	9.63	100.28
10.8	0.716	10.80	100.01
12	0.803	12.09	100.78
		mean	100.80
		S.D.	3.26
		%C.V.	3.23

5. $R^2 = 0.9996$, $Y = 0.0667X - 0.0026$ (Y=Absorbance, X=Conc.)
6. Inversely estimated concentration = (Absorbance + 0.0026)/ 0.0667
7. %Recovery = (Inversely estimated conc. / Conc.) x 100
8. %C.V. = (S.D./Mean) x 100

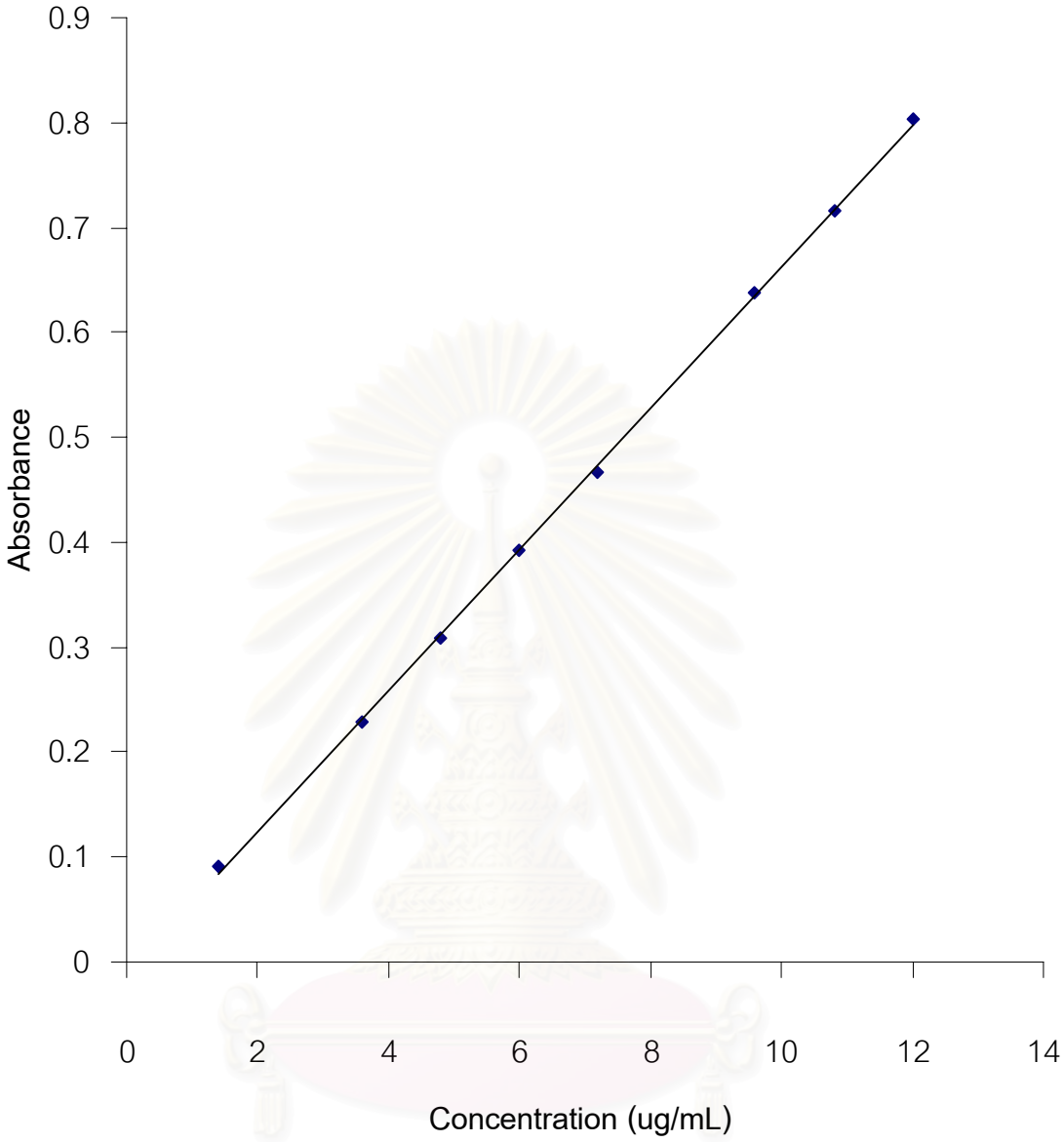


Figure 31. Typical calibration curve for determination of ketoprofen in phosphate buffer pH 7.4 at λ= 260 nm.

Appendix IV

Results of *in vitro* release studies

Table 28. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 20% tributyl citrate of Eudragit® E 100 (Rx1).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	25.81	30.02	27.49	26.65	25.81	28.34	27.35	1.64
0.17	48.72	47.06	47.89	48.72	47.03	47.89	47.88	0.75
0.42	108.87	111.41	109.71	108.87	109.70	110.56	109.85	0.99
0.67	156.69	162.63	159.23	161.76	158.38	158.40	159.51	2.25
1	214.06	226.77	214.09	215.78	223.34	224.21	219.71	5.70
1.5	302.95	321.63	311.40	307.21	304.69	315.68	310.59	7.12
2	363.65	376.53	365.40	369.61	369.61	368.86	368.94	4.45
2.5	437.32	441.00	439.93	438.26	437.42	440.04	439.00	1.54
3	497.06	502.44	497.16	498.01	497.16	499.79	498.60	2.15
4	545.31	605.54	553.84	575.78	594.32	580.10	575.82	23.06
5	630.08	713.41	662.27	681.80	672.62	674.33	672.42	27.08
6	698.43	794.03	715.62	742.01	750.48	747.15	741.29	32.85
8	821.12	936.64	829.97	839.63	830.45	831.31	848.19	43.73
10	960.83	1093.85	978.16	1020.76	1003.93	1008.18	1010.95	46.07
12	1130.47	1141.93	1126.81	1123.27	1127.43	1131.69	1130.27	6.44
24	1613.06	1780.58	1735.87	1652.19	1656.37	1660.66	1683.12	62.24

Table 29. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 40% tributyl citrate of Eudragit® E 100 (Rx2).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	40.14	40.99	40.14	41.83	42.67	40.99	41.13	0.99
0.17	76.63	74.10	76.63	75.79	74.96	76.63	75.79	1.06
0.42	172.35	179.93	178.25	169.82	168.98	173.20	173.76	4.45
0.67	238.24	244.18	232.37	237.39	237.38	230.66	236.70	4.78
1	317.14	318.89	321.36	312.91	312.06	317.95	316.72	3.58
1.5	420.09	415.94	417.58	427.64	426.79	420.90	421.49	4.78
2	503.35	504.25	499.99	501.67	503.34	502.49	502.51	1.52
2.5	578.64	582.91	581.16	575.26	577.78	578.61	579.06	2.67
3	656.85	654.40	652.64	655.14	656.00	657.67	655.45	1.80
4	758.26	759.17	753.18	751.48	755.71	758.23	756.00	3.12
5	855.14	856.06	845.82	839.89	844.15	846.69	847.96	6.37
6	963.85	1022.96	1043.87	1001.64	1022.79	1010.16	1010.88	27.11
8	1150.38	1150.78	1133.86	1167.30	1142.18	1137.92	1147.07	11.97
10	1312.62	1317.23	1300.22	1333.85	1317.02	1308.52	1314.91	11.23
12	1467.29	1471.93	1463.25	1467.55	1475.93	1458.95	1467.48	6.03
24	2204.67	2209.34	2200.62	2204.94	2196.50	2204.72	2203.47	4.39

Table 30. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 20% tributyl citrate of Eudragit® E 100 (Rx3).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	45.20	51.95	49.42	48.58	50.26	48.58	49.00	2.25
0.17	85.93	91.87	85.11	89.33	88.49	91.01	88.62	2.70
0.42	193.51	199.49	196.06	197.77	196.93	194.40	196.36	2.20
0.67	270.48	285.77	275.58	278.13	279.82	282.34	278.69	5.33
1	378.23	400.35	384.20	390.14	391.00	389.32	388.87	7.39
1.5	525.37	553.51	543.17	544.94	538.20	544.10	541.55	9.34
2	621.87	633.30	624.59	627.20	631.40	625.52	627.31	4.31
2.5	717.20	727.00	721.62	722.56	723.41	720.03	721.97	3.30
3	817.26	824.58	818.33	823.50	824.34	820.95	821.49	3.16
4	1038.79	1123.73	1064.32	1077.10	1089.76	1072.85	1077.76	28.22
5	1221.54	1328.04	1306.25	1289.59	1285.45	1302.18	1288.84	36.22
6	1358.90	1503.94	1406.13	1418.89	1469.54	1473.72	1438.52	53.39
8	1644.57	1689.20	1649.89	1658.50	1663.05	1646.17	1658.56	16.63
10	1885.40	1909.18	1932.91	1912.05	1912.41	1916.52	1911.41	15.30
12	2114.87	2100.82	2137.34	2141.66	2133.59	2116.63	2124.15	15.85
24	3053.96	3077.76	3084.97	3110.40	3072.77	3081.02	3080.15	18.36

Table 31. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 40% tributyl citrate of Eudragit® E 100 (Rx4).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	49.42	48.58	47.73	46.89	46.05	48.58	47.87	1.24
0.17	101.14	101.98	96.07	96.91	100.27	99.45	99.30	2.35
0.42	249.28	246.75	245.87	250.09	245.88	247.58	247.57	1.77
0.67	368.73	368.71	366.98	366.16	365.31	369.55	367.57	1.67
1	496.42	494.72	492.14	498.90	496.36	496.40	495.82	2.25
1.5	749.62	748.75	747.84	747.06	751.25	749.60	749.02	1.48
2	848.54	852.72	844.22	823.19	865.35	844.30	846.39	13.80
2.5	1004.99	1013.42	996.43	987.93	1034.55	1013.38	1008.45	16.17
3	1162.28	1158.11	1132.60	1128.27	1162.49	1170.72	1152.41	17.56
4	1493.30	1468.02	1509.84	1497.05	1493.51	1472.27	1489.00	15.86
5	1741.79	1737.46	1724.69	1720.27	1746.22	1750.16	1736.76	11.94
6	2021.14	2025.21	1995.51	2016.36	2000.28	2037.99	2016.08	15.89
8	2082.71	2086.81	2090.67	2094.78	2099.69	2087.01	2090.28	6.15
10	2144.57	2178.21	2182.10	2186.22	2191.16	2178.40	2176.78	16.53
12	2430.19	2485.10	2526.96	2396.18	2384.27	2379.88	2433.76	60.16
24	3596.93	3698.52	3580.39	3554.29	3546.54	3571.63	3591.38	55.51

Table 32. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 20% tributyl citrate of Eudragit® E 100 (Rx5).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	52.79	53.64	51.95	53.64	54.48	52.79	53.21	0.88
0.17	123.93	123.93	124.76	123.09	125.62	126.46	124.63	1.24
0.42	248.58	250.27	249.42	247.74	251.97	250.28	249.71	1.48
0.67	365.49	358.76	364.65	363.80	361.31	362.99	362.83	2.46
1	500.76	489.77	494.01	494.84	497.40	490.65	494.57	4.13
1.5	777.60	739.56	748.88	758.15	757.35	752.25	755.63	12.70
2	903.65	835.89	852.86	857.11	886.67	873.95	868.36	24.63
2.5	1051.98	992.27	1013.55	1017.83	1043.33	1026.32	1024.21	21.52
3	1209.53	1145.28	1154.02	1158.33	1205.05	1162.65	1172.48	27.61
4	1528.16	1400.31	1434.40	1455.59	1477.28	1472.59	1461.39	43.19
5	1747.32	1601.90	1640.39	1653.27	1662.42	1691.45	1666.13	49.40
6	2014.03	1825.65	1927.61	1978.51	2017.22	1957.86	1953.48	71.25
8	2408.68	2177.10	2220.58	2229.60	2260.09	2238.35	2255.73	79.77
10	2830.76	2522.01	2561.51	2557.93	2567.50	2541.42	2596.86	115.77
12	3271.97	2953.11	2963.30	2993.42	2977.74	2951.53	3018.51	125.18
24	4485.51	4485.38	4495.62	4471.09	4489.05	4479.56	4484.37	8.37

Table 33. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 40% tributyl citrate of Eudragit® E 100 (Rx6).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	67.13	66.29	64.60	65.44	66.29	67.13	66.15	0.99
0.17	168.70	129.90	139.17	140.02	136.65	133.28	141.29	13.95
0.42	339.15	311.94	317.04	322.11	321.25	327.99	323.25	9.45
0.67	465.83	456.19	463.85	457.14	457.96	463.89	460.81	4.17
1	608.40	609.66	608.93	608.09	605.54	611.50	608.69	1.96
1.5	888.36	895.54	886.37	894.79	893.92	894.86	892.31	3.91
2	999.00	1036.58	1002.90	1019.81	1011.34	1024.08	1015.62	14.03
2.5	1173.13	1232.01	1197.96	1240.19	1223.28	1202.31	1211.48	24.99
3	1297.61	1373.68	1342.64	1325.88	1330.04	1334.34	1334.03	24.72
4	1599.84	1701.63	1622.42	1639.37	1647.75	1668.92	1646.65	35.64
5	1810.94	1938.59	1856.11	1889.96	1839.31	1852.07	1864.50	44.42
6	2010.52	2181.04	2094.29	2048.05	2031.08	2060.72	2070.95	60.89
8	2379.82	2509.11	2417.05	2429.80	2391.73	2442.49	2428.33	45.93
10	2751.11	2935.92	2757.05	2803.54	2816.02	2824.67	2814.72	66.76
12	3212.94	3394.54	3198.10	3236.41	3211.02	3219.71	3245.45	74.10
24	5206.21	5296.03	5334.92	5268.03	5217.19	5310.27	5272.11	51.67

Table 34. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 20% propylene glycol of Eudragit® E 100 (Rx7).

Time	Amount of ketoprofen released ($\mu g/cm^2$)						Mean	S.D.
(hours)	1	2	3	4	5	6	($\mu g/cm^2$)	
0	0	0	0	0	0	0	0	0
0.08	49.42	40.14	40.99	45.20	43.52	42.67	43.66	3.35
0.17	73.31	74.94	71.57	74.13	72.43	76.64	73.84	1.82
0.42	149.61	158.00	146.18	150.44	156.32	153.81	152.39	4.45
0.67	200.20	201.89	195.06	196.81	197.67	196.83	198.07	2.51
1	260.33	259.50	245.89	258.61	256.10	247.67	254.69	6.31
1.5	338.51	335.99	328.20	335.94	332.57	340.95	335.36	4.49
2	394.34	406.14	395.79	397.66	410.29	391.74	399.33	7.27
2.5	453.00	469.93	462.05	456.34	459.76	468.94	461.67	6.76
3	515.35	527.31	526.98	528.82	531.43	531.38	526.88	5.96
4	613.45	631.38	620.08	616.03	627.09	627.88	622.65	7.19
5	708.71	719.99	695.14	709.62	716.52	707.19	709.53	8.62
6	792.67	810.76	781.55	791.05	786.18	800.42	793.77	10.47
8	999.69	1050.77	991.05	1016.62	1025.22	991.46	1012.47	23.28
10	1140.04	1166.10	1114.48	1140.20	1148.84	1123.33	1138.83	18.33
12	1222.11	1231.44	1221.71	1230.70	1273.12	1247.47	1237.76	19.68
24	1785.29	1760.94	1700.56	1726.46	1710.08	1743.33	1737.78	31.95

Table 35. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 40% propylene glycol of Eudragit® E 100 (Rx8).

Time	Amount of ketoprofen released ($\mu g/cm^2$)						Mean	S.D.
(hours)	1	2	3	4	5	6	($\mu g/cm^2$)	
0	0	0	0	0	0	0	0	0
0.08	37.61	37.61	39.30	38.46	39.30	40.14	38.74	1.02
0.17	104.44	106.13	96.86	99.39	102.77	103.62	102.20	3.44
0.42	203.69	186.84	162.35	190.18	186.83	170.81	183.45	14.72
0.67	241.08	231.72	207.25	232.55	216.53	235.85	227.50	12.87
1	303.13	292.88	262.51	295.40	286.04	291.97	288.65	13.96
1.5	388.29	378.82	343.40	381.35	371.10	362.72	370.95	16.11
2	469.69	461.01	419.71	434.89	449.03	457.48	448.64	18.44
2.5	547.32	539.44	489.55	536.78	499.56	535.04	524.61	23.87
3	610.18	609.85	554.89	607.18	587.46	603.74	595.55	21.63
4	731.57	732.08	673.73	731.08	726.44	725.94	720.14	22.89
5	844.33	844.85	791.56	824.45	835.80	827.71	828.12	19.76
6	889.73	872.54	897.57	880.70	914.05	889.04	890.60	14.32
8	1088.50	1104.95	1062.65	1113.16	1045.50	1070.95	1080.95	25.93
10	1170.28	1186.83	1203.33	1237.25	1194.51	1194.81	1197.84	22.29
12	1345.26	1336.59	1311.02	1328.26	1319.02	1353.05	1332.20	15.89
24	2145.23	2136.52	2102.37	2077.54	2085.12	2136.21	2113.83	29.24

Table 36. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 20% propylene glycol of Eudragit® E 100 (Rx9).

Time	Amount of ketoprofen released ($\mu g/cm^2$)						Mean	S.D.
(hours)	1	2	3	4	5	6	($\mu g/cm^2$)	
0	0	0	0	0	0	0	0	0
0.08	50.26	45.20	59.54	48.58	54.48	53.64	51.95	5.03
0.17	122.22	117.14	123.96	122.22	120.56	119.71	120.97	2.39
0.42	227.47	217.30	232.60	221.56	223.27	229.17	225.23	5.57
0.67	298.73	294.40	301.35	295.31	299.56	297.90	297.88	2.62
1	387.24	374.45	389.87	380.43	378.80	386.41	382.87	5.90
1.5	511.65	505.54	521.89	505.65	523.41	504.07	512.03	8.64
2	609.76	614.58	626.80	618.90	618.20	619.84	618.01	5.68
2.5	720.20	714.93	732.27	726.02	727.01	721.06	723.58	6.10
3	842.20	816.66	843.37	832.03	827.12	828.73	831.68	10.03
4	1001.45	996.86	1052.39	1026.65	1039.42	992.97	1018.29	24.75
5	1175.57	1158.30	1193.05	1179.82	1175.79	1171.25	1175.63	11.32
6	1316.89	1303.74	1363.98	1342.25	1308.68	1329.42	1327.49	22.73
8	1568.59	1584.89	1666.54	1594.09	1640.45	1644.44	1616.50	39.13
10	1762.61	1779.01	1852.67	1792.47	1809.57	1796.72	1798.84	30.87
12	1970.32	1982.59	2081.95	2034.08	2017.54	2004.61	2015.18	40.02
24	3220.64	3190.81	3176.87	3217.28	3200.65	3229.82	3206.01	20.08

Table 37. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 40% propylene glycol of Eudragit® E 100 (Rx10).

Time	Amount of ketoprofen released ($\mu g/cm^2$)						Mean	S.D.
(hours)	1	2	3	4	5	6	($\mu g/cm^2$)	
0	0	0	0	0	0	0	0	0
0.08	61.23	51.95	54.48	57.01	56.17	59.54	56.73	3.36
0.17	146.74	131.51	143.33	141.66	145.03	140.83	141.52	5.36
0.42	249.60	237.66	259.66	246.17	243.66	247.03	247.30	7.29
0.67	340.37	324.99	345.43	332.71	333.55	337.78	335.81	7.06
1	435.86	430.51	447.69	440.80	440.81	437.47	438.86	5.77
1.5	586.68	601.55	654.24	611.90	612.74	598.43	610.92	23.29
2	696.16	747.37	796.99	3765.43	3630.50	3693.69	2221.69	1616.50
2.5	812.14	883.03	939.66	4191.26	4051.97	4085.72	2493.96	1770.95
3	952.68	1007.95	1068.26	1024.99	957.23	1003.66	1002.46	43.34
4	1113.89	1215.84	1301.78	1207.68	1190.14	1253.70	1213.84	63.11
5	1275.96	1399.56	1469.11	1370.27	1365.29	1349.08	1371.54	63.22
6	1409.39	1580.05	1662.62	1639.14	1457.04	1550.36	1549.77	99.94
8	1720.61	1866.91	1996.31	1884.16	1852.85	1820.20	1856.84	89.68
10	1923.89	2045.69	2213.74	2058.82	2099.02	2049.32	2065.08	93.65
12	2119.82	2259.14	2512.43	2335.58	2266.39	2283.87	2296.20	127.93
24	3813.68	3827.25	3845.79	3824.00	3830.34	3835.25	3829.39	10.81

Table 38. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 20% propylene glycol of Eudragit® E 100 (Rx11).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	50.26	44.36	48.58	49.42	52.79	51.95	49.56	2.99
0.17	139.09	128.94	128.12	130.65	135.73	133.20	132.62	4.23
0.42	335.51	301.69	298.34	321.97	311.90	314.41	313.97	13.62
0.67	474.84	434.08	432.39	454.47	448.56	454.45	449.80	15.63
1	639.38	580.68	579.83	598.66	595.24	616.35	601.69	22.83
1.5	809.88	732.31	728.92	772.31	782.37	742.03	761.30	32.20
2	1028.02	963.52	963.49	1023.14	972.53	993.54	990.71	29.20
2.5	1168.59	1070.01	1103.71	1125.73	1129.66	1104.40	1117.02	32.99
3	1318.35	1215.00	1206.73	1287.90	1249.69	1232.72	1251.73	43.56
4	1553.23	1436.67	1449.43	1497.32	1454.68	1496.65	1481.33	43.32
5	1856.84	1676.40	1722.96	1787.98	1736.67	1770.44	1758.55	61.90
6	2056.67	1875.26	1922.07	1979.00	1952.72	1999.32	1964.17	62.96
8	2468.40	2294.43	2282.47	2305.97	2321.71	2351.71	2337.45	68.50
10	2785.36	2559.86	2585.78	2706.38	2696.91	2651.17	2664.24	83.35
12	3150.40	2843.56	2903.35	3024.61	2994.01	3095.60	3001.92	115.06
24	4601.05	4655.18	4660.49	4605.30	4612.49	4609.22	4623.96	26.57

Table 39. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 40% tributyl citrate of Eudragit® E 100 (Rx12).

Time	Amount of ketoprofen released ($\mu g/cm^2$)						Mean	S.D.
(hours)	1	2	3	4	5	6	($\mu g/cm^2$)	
0	0	0	0	0	0	0	0	0
0.08	93.27	95.80	93.27	94.12	94.12	92.43	93.83	1.15
0.17	141.86	147.78	145.23	142.71	145.24	144.39	144.53	2.10
0.42	331.55	327.38	330.73	328.19	328.20	329.87	329.32	1.65
0.67	487.71	479.30	477.61	484.33	483.50	485.18	482.94	3.79
1	684.37	668.33	664.09	680.97	673.39	672.55	673.95	7.60
1.5	861.87	847.42	839.79	844.11	846.61	844.08	847.31	7.61
2	1092.11	1075.05	1049.67	1083.53	1087.73	1070.85	1076.49	15.32
2.5	1262.54	1304.43	1207.22	1245.49	1262.36	1291.77	1262.30	34.52
3	1433.90	1505.53	1390.91	1420.96	1471.66	1421.11	1440.68	41.13
4	1694.71	1838.42	1643.06	1681.70	1652.57	1732.46	1707.15	71.78
5	1923.19	2147.82	1942.95	1952.28	1939.85	1910.55	1969.44	88.65
6	2186.63	2425.15	2130.60	2177.94	2119.05	2148.62	2198.00	114.32
8	2628.57	2978.03	2580.67	2598.75	2615.43	2565.05	2661.08	156.95
10	2916.89	3255.62	2851.86	2899.55	2924.76	2924.70	2962.23	146.33
12	3164.56	3640.07	3183.52	3168.21	3201.99	3189.28	3257.94	187.71
24	5176.08	5186.14	5186.72	5217.70	5213.72	5179.86	5193.37	17.80

Table 40. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 20% glycerin of Eudragit® E 100 (Rx13).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	30.02	25.81	30.87	26.65	31.71	32.55	29.60	2.76
0.17	48.74	47.88	57.18	52.10	58.87	46.23	51.83	5.19
0.42	143.47	99.58	119.90	124.91	115.70	117.32	120.15	14.26
0.67	194.02	143.14	155.99	153.43	155.13	160.98	160.45	17.46
1	260.02	191.16	245.40	220.06	223.46	235.24	229.22	23.72
1.5	314.58	239.44	302.41	302.23	268.54	277.01	284.04	27.86
2	352.57	311.59	362.26	334.25	342.54	325.76	338.16	18.34
2.5	394.14	368.96	417.37	376.56	398.39	383.20	389.77	17.35
3	439.30	410.62	470.25	430.90	420.81	432.52	434.07	20.37
4	515.06	501.40	565.58	517.58	528.51	526.80	525.82	21.77
5	584.47	568.23	649.62	597.14	565.96	561.71	587.85	33.05
6	637.40	647.21	711.33	661.94	656.74	689.57	667.36	27.83
8	763.12	761.19	855.17	767.57	802.81	791.98	790.31	35.97
10	835.55	841.21	940.76	856.89	843.42	852.76	861.77	39.47
12	951.71	979.34	1,097.13	1,010.28	1,001.79	1,027.21	1,011.24	49.57
24	1534.51	1402.07	1360.26	1428.96	1504.76	1386.95	1436.25	68.95

Table 41. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 15% ketoprofen and 40% glycerin of Eudragit® E 100 (Rx14).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	28.34	26.65	30.87	29.18	25.81	28.34	28.20	1.80
0.17	49.58	55.47	61.40	54.64	59.68	52.11	55.48	4.46
0.42	108.04	129.15	121.61	113.98	119.05	111.43	117.21	7.66
0.67	148.27	168.66	161.08	154.24	161.87	161.80	159.32	7.08
1	198.85	226.09	218.47	213.28	215.05	220.04	215.30	9.21
1.5	271.63	282.99	277.02	281.08	275.27	276.07	277.34	4.11
2	525.27	357.92	338.42	342.50	356.89	350.95	378.66	72.24
2.5	572.86	402.89	379.91	392.45	403.55	395.89	424.59	73.15
3	613.11	464.96	430.05	454.47	463.94	440.22	477.79	67.68
4	694.05	553.52	517.57	528.63	526.35	540.44	560.09	66.80
5	766.98	638.33	596.28	629.33	605.11	619.28	642.55	62.86
6	836.08	706.73	658.55	684.19	692.72	668.18	707.74	65.17
8	956.14	847.18	790.30	856.56	839.84	831.19	853.53	55.25
10	1071.27	969.32	900.33	977.07	960.26	964.21	973.74	55.15
12	1161.38	1063.11	1006.39	1032.96	1028.70	1045.32	1056.31	54.79
24	1644.11	1528.45	1450.35	1485.49	1472.77	1485.27	1511.07	69.97

Table 42. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 20% glycerin of Eudragit® E 100 (Rx15).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	56.17	55.32	62.07	57.01	57.85	59.54	57.99	2.47
0.17	112.14	107.07	102.89	103.71	105.40	108.78	106.67	3.44
0.42	211.43	193.69	200.45	203.80	195.38	204.68	201.57	6.54
0.67	297.77	275.72	280.83	285.04	286.69	289.30	285.89	7.53
1	378.69	355.67	365.87	372.63	372.61	366.80	368.71	7.90
1.5	485.34	461.35	471.61	475.88	470.80	479.29	474.05	8.20
2	604.39	576.05	594.79	598.25	588.07	591.56	592.18	9.70
2.5	692.03	662.69	678.17	684.17	675.63	679.13	678.64	9.73
3	780.14	757.39	762.83	764.65	770.40	768.01	767.24	7.75
4	936.19	909.94	931.43	920.61	922.18	914.72	922.51	9.89
5	1077.39	1063.66	1059.97	1064.26	1068.37	1072.67	1067.72	6.44
6	1218.17	1195.93	1204.87	1213.40	1200.67	1209.21	1207.04	8.22
8	1414.52	1421.68	1430.66	1413.94	1422.22	1426.59	1421.60	6.58
10	1645.65	1661.29	1670.32	1653.51	1657.61	1662.01	1658.40	8.37
12	1827.43	1822.08	1822.72	1826.90	1818.38	1822.80	1823.38	3.35
24	2617.36	2717.40	2646.36	2650.56	2667.29	2684.39	2663.89	34.48

Table 43. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 25% ketoprofen and 40% glycerin of Eudragit® E 100 (Rx16).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	52.79	51.95	53.64	53.64	51.11	52.79	52.65	0.99
0.17	106.22	100.31	108.75	102.85	103.68	106.22	104.67	2.99
0.42	231.62	227.36	234.17	230.76	229.06	232.46	230.90	2.43
0.67	340.84	339.10	348.47	343.36	341.65	340.01	342.24	3.38
1	435.49	440.48	443.16	438.02	441.36	439.71	439.70	2.68
1.5	561.01	572.78	576.31	566.08	571.13	568.63	569.33	5.37
2	703.24	717.60	714.41	710.03	712.58	713.43	711.88	4.90
2.5	815.89	826.95	817.84	818.49	821.06	821.91	820.36	3.90
3	914.29	952.41	910.35	935.47	906.00	922.88	923.57	17.57
4	1130.11	1134.70	1113.49	1130.31	1121.76	1138.74	1128.18	9.16
5	1309.14	1317.97	1271.34	1305.13	1313.40	1296.74	1302.29	16.81
6	1480.70	1476.93	1446.91	1472.45	1476.55	1489.31	1473.81	14.36
8	1733.29	1699.98	1686.67	1720.78	1699.60	1695.57	1705.98	17.45
10	2029.41	1995.91	1965.66	1983.09	1974.45	2012.56	1993.51	24.10
12	2128.93	2209.10	2275.66	2229.94	2174.87	2217.40	2205.99	49.95
24	3316.85	3110.72	2996.35	3131.68	3118.47	3102.21	3129.38	103.97

Table 44. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 20% glycerin of Eudragit® E 100 (Rx17).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	40.99	42.67	39.30	40.14	41.83	40.99	40.99	1.19
0.17	92.66	105.32	99.39	101.93	95.19	99.40	98.98	4.55
0.42	230.63	256.01	248.37	239.96	244.14	246.69	244.30	8.55
0.67	336.48	389.83	376.25	380.43	383.80	382.15	374.82	19.30
1	461.47	528.60	524.22	527.59	525.92	526.78	515.76	26.64
1.5	633.52	701.87	699.15	699.16	700.01	695.82	688.25	26.89
2	750.01	892.95	891.05	889.38	888.55	890.24	867.03	57.35
2.5	872.52	1047.45	1068.32	1051.46	1059.90	1064.13	1027.30	76.22
3	999.57	1204.98	1200.67	1196.36	1204.85	1192.23	1166.44	81.90
4	1198.99	1472.99	1456.00	1468.54	1464.42	1472.82	1422.29	109.58
5	1424.78	1742.44	1721.15	1725.32	1733.83	1729.63	1679.52	125.01
6	1596.97	1933.24	1920.25	1928.67	1924.57	1920.35	1870.68	134.18
8	1917.66	2319.00	2356.55	2335.50	2344.03	2352.43	2270.86	173.55
10	2244.30	2689.99	2668.71	2677.06	2668.77	2685.65	2605.75	177.28
12	2517.88	2919.60	2965.67	2952.98	2923.56	2961.62	2873.55	175.31
24	3855.51	4280.46	4217.16	4276.08	4221.20	4204.65	4175.85	160.13

Table 45. The cumulative amount of ketoprofen released from ketoprofen transdermal patches using 35% ketoprofen and 40% glycerin of Eudragit® E 100 (Rx18).

Time (hours)	Amount of ketoprofen released ($\mu g/cm^2$)						Mean ($\mu g/cm^2$)	S.D.
	1	2	3	4	5	6		
0	0	0	0	0	0	0	0	0
0.08	48.58	48.58	57.01	52.79	51.11	51.95	51.67	3.14
0.17	118.84	134.02	145.88	134.05	142.47	127.29	133.76	9.87
0.42	250.22	286.56	291.74	277.31	282.41	288.23	279.41	15.14
0.67	373.04	424.77	426.60	423.90	428.18	427.29	417.30	21.74
1	504.13	575.54	587.50	581.41	582.34	579.76	568.45	31.75
1.5	760.75	814.00	826.86	816.53	823.37	825.83	811.22	25.26
2	781.80	938.23	955.38	950.89	940.90	952.66	919.98	68.03
2.5	903.64	1116.59	1108.54	1112.46	1116.75	1120.98	1079.83	86.42
3	1001.34	1253.41	1287.48	1257.69	1291.52	1278.91	1228.39	112.31
4	1183.89	1521.67	1513.76	1509.11	1526.26	1505.15	1459.97	135.48
5	1418.02	1782.96	1762.35	1774.54	1779.13	1766.33	1713.89	145.15
6	1581.73	2011.91	1974.32	1995.01	2003.85	2016.28	1930.52	171.51
8	1914.98	2377.02	2364.51	2381.11	2368.91	2368.76	2295.88	186.70
10	2250.03	2744.09	2739.95	2748.20	2744.36	2731.56	2659.70	200.78
12	2540.50	3049.88	3024.63	3054.02	3045.94	3024.64	2956.60	204.24
24	4046.91	4445.18	4369.19	4377.66	4394.83	4419.80	4342.26	147.36

Appendix V

Validation of Analytical Method for *In Vivo* Studies

1. Accuracy

Table 46. Accuracy of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	PAR	Inversely estimated concentration (μg/mL)	%Recovery
0.56	0.093	0.598	106.848
1.2	0.184	1.224	102.017
2.8	0.410	2.779	99.234
3.6	0.537	3.652	101.444
6.4	0.942	6.437	100.585
8	1.169	7.999	99.983

2. Precision.

2.1. Within run precision.

Table 47. Within run precision of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	PAR			Mean	S.D.	%C.V.
	1	2	3			
0.56	0.093	0.093	0.096	0.094	0.002	1.905
1.2	0.184	0.183	0.177	0.181	0.004	1.974
2.8	0.410	0.431	0.428	0.423	0.011	2.632
3.6	0.537	0.541	0.555	0.544	0.009	1.706
6.4	0.942	0.932	0.946	0.940	0.007	0.793
8	1.169	1.182	1.196	1.182	0.013	1.130

2.2. Between run precision.

Table 48. Between run precision of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	PAR			Mean	S.D.	%C.V.
	1	2	3			
0.56	0.093	0.098	0.092	0.094	0.004	3.876
1.2	0.184	0.181	0.179	0.181	0.002	1.276
2.8	0.410	0.417	0.399	0.409	0.009	2.311
3.6	0.537	0.539	0.514	0.530	0.014	2.616
6.4	0.942	0.974	0.899	0.938	0.037	3.983
8	1.169	1.091	1.176	1.145	0.047	4.119

3. Calibration curve.

Table 49. Typical calibration curve data for determination of ketoprofen in rabbit plasma estimated using linear regression.

Concentration (μg/mL)	PAR	Inversely estimated concentration (μg/mL)	%Recovery
0.56	0.093	0.60	106.55
1.2	0.184	1.22	101.92
2	0.287	1.93	96.59
2.8	0.41	2.78	99.23
3.6	0.537	3.65	101.46
4.8	0.694	4.73	98.60
6.4	0.942	6.44	100.62
8	1.169	8.00	100.03
		Mean	100.62
		S.D.	2.93
		%C.V.	2.91

1. $R^2 = 0.9997$, $Y = 0.1453X + 0.0063$ (Y=Absorbance, X=Conc.)
2. Inversely estimated concentration = (Absorbance – 0.0063)/ 0.1453
3. %Recovery = (Inversely estimated conc. / Conc.) x 100
4. %C.V. = (S.D./Mean) x 100

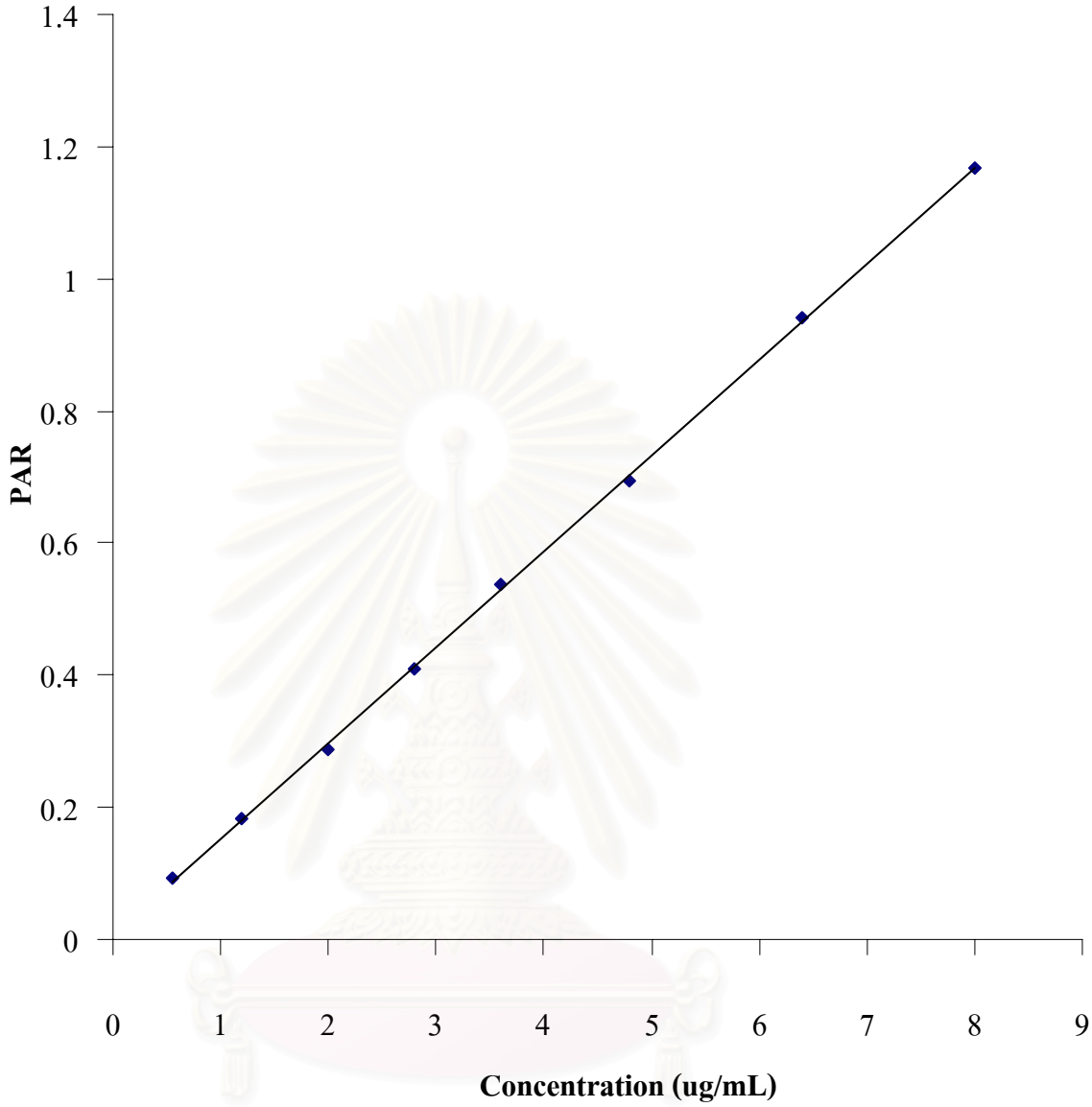


Figure 32. Typical calibration curve for determination of ketoprofen in rabbit plasma.

Appendix VI

Statistics

1. **Means (X)**

$$X = \sum X/n$$

2. **Standard deviation (S.D.)**

$$S.D. = \sqrt{\sum (X - X)^2 / n-1}$$

3. **Coefficient of variation (C.V.)**

$$C.V. = S.D. / Mean$$

4. **Area under the concentration time curve (AUC_{0-24})**

$$[AUC] = \frac{\sum (C_{n-1} + C_n)(t_n - t_{n-1})}{2}$$

5. **Area under the moment curve ($AUMC_{0-24}$)**

$$[AUMC] = \frac{\sum C_{n-1} t_{n-1} + C_n t_n (t_n - t_{n-1})}{2}$$

6. **Mean residence time (MRT).**

$$MRT = AUMC / AUC$$

7. Analysis of variance for three way crossover design.

The experimental design is:

Sequence	Subject/Sequence	Period		
		I	II	III
I	1,2,3	A	B	C
II	4,5,6	B	C	A
III	7,8,9	C	A	B

Where A = formulation with tributyl citrate

B = formulation with propylene glycol

C = formulation with glycerin

In statistical terms the calculations to set up an analysis of variance table are as follow:

Source of variation	d.f.	Sum of squares	Mean square
Total	g.n.t-1	SStotal	-
Sequence	g-1	SSsequence	MSsequence
Subjects (sequence)	g(n-1)	SSsubject	MSsubject
Period	p-1	SSperiod	MSperiod
Formulation	f-1	SSformulation	MSformulation
Error	(gn-2)(t-1)	SSerror	MSerror

Where

N = total number of subjects = gnt

f = number of formulation

g = number of sequence or group

p = number of time periods or week

n = number of subjects per sequence

C.T. = Correction term = $(\sum x)^2$ / g.n.t

Data presented next page are individual subject of the MRT of ketoprofen after administration of ketoprofen transdermal patches.

Sequence	Subject	Formulation A		Formulation B		Formulation C		Subject total
I	1,2,3	18	period I	17.67	period II	16.75	period III	52.42
		17.39	sum	17.41	sum	17.02	sum	51.82
		16.92	} 52.31	17.46	} 52.54	16.75	} 50.52	51.13
II	4,5,6	16.91	period III	16.9	period I	16.94	period II	50.75
		17.61	sum	17.66	sum	17.29	sum	52.56
		17.28	} 51.8	17.84	} 52.4	17.36	} 51.59	52.48
III	7,8,9	16.97	period II	16.92	period III	17	period I	50.89
		17.11	sum	17.43	sum	16.67	sum	51.21
		17.28	} 51.36	17.94	} 52.29	16.74	} 50.41	51.96
Formulation total		155.47		157.23		152.52		465.22

Period I = 52.31+52.40+50.41 = 155.12

Period II = 52.54+51.59+51.36 = 155.49

Period III = 50.52+51.80+52.29 = 154.61

1. Correction term = $(465.22)^2$ /27 = 8015.9

2. SStotal = $[(18)^2+(17.39)^2+\ldots+(16.74)^2]$ – C.T. = 3.8495

3. SSsequence = $[(52.42+51.82+51.13)^2+\ldots+(50.89+51.21+51.96)^2]$/9 – C.T.= 0.1809

4. SSsubject = $[(52.42)^2+(51.82)^2+\ldots.+(51.96)^2]/3 - C.T. = 1.3571$

5. SSperiod = $[(155.12)^2+(155.49)^2+(154.61)^2] - C.T. = 0.0434$

6. SSformulation = $[(155.47)^2+(157.23)^2+(152.52)^2]/9 - C.T. = 1.2587$

7. SSresidual = [3.8495-(0.1809+1.3571+0.0434+1.2587+1.0094)] = 1.0094

8. MS = SS/df

Analysis of variance table for three way crossover design.

Source of variation	d.f.	SS	MS	Fratio	Ftable	Sig.level
Total	26	3.85	0.15	-		
Sequence	2	0.18	0.09	0.40	5.14	NS
Subject (Seq)	6	1.36	0.23	3.14	2.85	S
Period	2	0.04	0.02	0.28	3.74	NS
Treatment	2	1.26	0.63	8.73	3.74	S
Error	14	1.01	0.07	-		

Where: F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are significant differences for the MRT values among three formulations.

8. **Least significant difference test.**

From the data of Table 11 obtained for example:

8.1. Rank mean from less to more:

	A	C	B
Mean (Xi)	34.27	43.01	50.53

8.2. Calculated Least Significant Difference (LSD)

$$LSD = t_{\alpha} \sqrt{(2 \times MSE/n_i)}$$

8.3. Compare LSD, if $|Xi-Xi^{/}|$ > LSD showed that $\mu i \neq \mu j$ at level 0.05

i-i/	Xi-Xi/	n	$LSD_{0.05}$	Significance
B-A	16.26	9	2.51	B differ A
B-C	7.52	9	2.51	B differ C
C-A	8.74	9	2.51	C differ A

VITA

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