การพัฒนาสูตรตำรับระบบนำส่งยาทางผิวหนังของดิลไทอะเซมไฮโดรคลอไรด์ โดยใช้ซูโดเลเทกซ์เป็นสารพื้น

นางสาว ชมจรรย์ อำนวยกิจ

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FORMULATION DEVELOPMENT OF DILTIAZEM HYDROCHLORIDE TRANSDERMAL DRUG DELIVERY SYSTEM UTILIZING PSEUDOLATEX BASE

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ในการเตรียมตำรับและประเมินยาดิลไทอะเซมไฮโดรคลอไรด์ เพื่อที่จะพัฒนาและเตรียมดิลไทอะเซมไฮโดรคลอไรด์เป็นระบบการปลดปล่อยยาซึ่งใช้ทางผิวหนัง ซูโดเลเทกซ์ที่เตรียมขึ้นจากพอลีเมอร์ Eudragit RL100® และ Eudragit RS100® ถูกนำมาใช้เป็นสารพื้นในรูปของแผ่นแปะผิวหนัง โดยมีการประเมินผลการปลดปล่อยตัวยาในหลอดทดลองผ่านเมมเบรนที่มีรูพรุน และประเมินผลการซึมผ่านผิวหนังในหลอดทดลองโดยใช้หนังงูลอกคราบ พบว่า อัตราการปลดปล่อยของตำรับดิลไทอะเซมไฮโดรคลอไรด์ซูโดเลเทกซ์ให้ผลแตกต่างกันไปตามการเปลี่ยนแปลงอัตราส่วนผสมของพอลีเมอร์, ความเข้มข้นของสารก่อช่องทาง (PVP K30), ความเข้มข้นของสารลดแรงตึงผิว, ความเข้มข้นของสารให้ความยืดหยุ่น และความเข้มข้นของตัวยา แต่อย่างไรก็ตามรูปแบบการปลดปล่อยตัวยาในหลอดทดลองของตำรับซูโดเลเทกซ์ทั้งหมดเป็นไปตามจลนพลศาสตร์ฮิกูชิ ในขณะที่กลไกการปลดปล่อยเป็นไปตาม non fickian diffusion

ตำรับซูโดเลเทกซ์ที่เหมาะสมที่นำมาใช้เป็นแหล่งกักเก็บยาในแผ่นแปะประกอบด้วยดิลไทอะเซมไฮโดรคลอไรด์ 10%, Eudragit RL100® 12%, PVP K30 4%, Tween 80 10%, and dibutyl phthalate 4% โดยน้ำหนักของตำรับ ซึ่งให้ผลอัตราการซึมผ่านผิวหนังเป็น 6.5 ไมโครกรัมต่อตารางเซนติเมตรต่อรากที่สองของชั่วโมง ตามจลนพลศาสตร์การปลดปล่อยแบบ ฮิกูชิ การศึกษาขั้นตอนก่อนกำหนดสูตรตำรับของดิลไทอะเซมไฮโดรคลอไรด์ซูโดเลเทกซ์ ชี้ให้เห็นว่า ระบบนี้มีศักยภาพที่จะเตรียมในรูปแบบนำส่งยาทางผิวหนัง และมีความจำเป็นต้องมีการศึกษาและประเมินผลในสัตว์ทดลองต่อไป

ภาควิชา.....**เภสัชอุตสาหกรรม**..... **ลายมือชื่อนิสิต**.....
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DRUG DELIVERY SYSTEM/ PSEUDOLATEX BASE
CHOMCHAN AMNUAIKIT : FORMULATION DEVELOPMENT OF
DILTIAZEM HYDROCHLORIDE TRANSDERMAL DRUG DELIVERY SYSTEM
UTILIZING PSEUDOLATEX BASE. THESIS ADVISOR: ASSOC. PROF. KAISRI
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The formulation and an evaluation of diltiazem hydrochloride (DTZ HCl) with a view to developing and preparing an DTZ HCl releasing system for transdermal applications. Eudragit RL100® and Eudragit RS100® were used to prepare pseudolatex as a drug reservoir patch. These preparations were evaluated for in vitro release across porous membrane, and permeation of the drug across shed snake skin. The release rate of DTZ HCl pseudolatex can be varied by changing the polymer ratio, PVP K30, surfactant, platsticizer, and drug concentrations, respectively. However, In vitro drug release model of all pseudolatex formula appeared to be Higuchi's model while as in vitro drug release mechanism revealed that non fickian diffusion would be operated.

The suitable pseudolatex formula, which used to prepare as drug reservoir in patch, composed of DTZ HCl 10%, Eudragit RL100® 12%, PVP K30 4%, Tween 80 10%, and dibutyl phthalate 4% w/w of formulation. Then DTZ HCl patch exhibiting skin permeation rate as 6.5 mcg/cm^{2}hr$^{-1/2}$ followed by Higuchi's model. Preliminary studies on DTZ HCl pseudolatex are indicative of its potentiality for transdermal preparation and establish the further need for in vivo evaluation.

Department..Manufacturing Pharmacy.. Student's signature……...
Field of study..Industrial Pharmacy…… Advisor's signature……
Academic year...2000…… Co-advisor's signature……..

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LISTS OF ABBREVIATIONS

%	percentage
%RH	percent of relative humidity
>	more than
<	less than
°C	degree celcius
cm^2	square centrimeter
conc	concentration
cp	centipoise
DTZ	diltiazem
e.g.	for example, exempli gratia
et.al.	Et alli, and others
g	gram(s)
HCl	hydrochloride acid or hydrochloride salt
hr	hour(s)
kg	kilogram(s)
M	molar
μg, mcg	microgram(s)
mg	milligram(s)
ml	milliliter
mm	millimeter
mp	melting point
mPa.s	millipascal per second(s)
MW	molecular weight
nm	nanometer
No.	number
PCS	photon correlation spectrophotometer

LISTS OF ABBREVIATIONS (cont.)

pH	the negative logarithm of the hydrogen ion concentration
pK_a	the negative logarithm of the dissolution constant
r^2	correlation of determination
SD	standard deviation
SEM	scanning electron microscope
TDDS	transdermal drug delivery system
UV	ultraviolet
w/w	weight by weight
w/v	weight by volume
v/v	volume by volume

CHAPTER I

INTRODUCTION

Although oral administration is one of the best way which is acceptable to patients, this route is useful, and suitable for some drugs that do not have problem about hepatic first pass metabolism. However, diltiazem hydrochloride (DTZ HCl) has this problem then it may be not suitable to design in controlled or sustained oral dosage form. Moreover, it has a short elimination half life that, disadvantages to take drug more often for controlling constant drug concentration in blood level.

In order to get rid of detriment, new technology and many possible drug designs including drug delivery systems are used to improve desirable constant rate of drug deliver into blood stream. One of many choices that use to solve a problem is transdermal drug delivery systems (TDDSs) because TDDS is self contained, discrete dosage form, which deliver the drug through the skin at a controlled rate to the systemic circulation. Furthermore, TDDS is a highly complex controlled delivery system, developed with the cooperation of many researchers in various fields (P. Morganti et al., 1999). (See Fig.1)

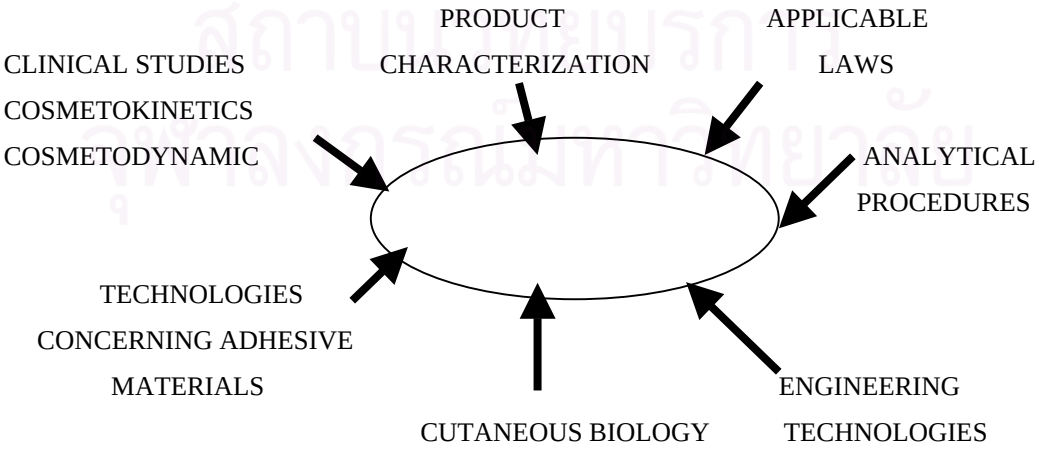


Figure 1 Cooperative of experts to develop TDDS.

Some drugs don't achieve their full potential because they are delivered via a suboptimal route for example, oral administration may be limited by GI irritation or side effects due to the peaks, and thoughs associated with its pharmacokinetic profiles. TDDS can give a drug with a great deal of potential, a better chance at clinical success.

With the limit of absorption through the skin, then several technologies have been successful in developing a device, which can control the releasing rate and the skin permeation of drugs. To release drug from transdermal patch, these will deal with designing drug formulation, and base which is made from polymeric matrix, gel, pseudolatex or another materials. For this study, pseudolatex base is interesting in the part of controlling drug, distribution and skin permeation.

Pseudolatexes may be colloidal dispersion containing spherical solid or semisolid particles less than 1 micron in diameter. The components of pseudolatex base are polymer and plasticizer. There are oil droplets that hold drug inside. Pseudolatex can deliver either polar or non polar drug by surfactant that helps drug distribute through skin regularly.

There are many types and variety of polymers that are used for controlling drug, some made from nature, and the others made from synthesis. The selection of using polymer in this study depends on principle of matrix system development for TDDS, applying to use in pseudolatex base. Acrylic polymers are widespread used in pharmaceutical field especially TDDS. Several branded systems are based on acrylics such as Minitrans® , Nitro-Dur® (nitroglycerin) and etc. Most of them are sold under the brand name of Eudragit®, then in this experiment aimed to use of these polymers, Eudragit®RL100 and Eudragit®RS100, in a single, and

combination with varying ratio for suitable controlling and releasing of DTZ.

Objectives of the study

1. Formulate the preparation of TDDS of diltiazem hydrochloride utilizing pseudolatex base.
2. Investigate physical characteristic of formulation when the components are changed such as the ratio of polymer RL100 and RS100, chanelling agent, effect of surfactant, effect of plasticizer, and effect of drug concentration.
3. Study the release characteristic of pseudolatex and TDDS.

Literature Review

Transdermal Drug Delivery Systems

TDDS is the results of sophisticated procedures, where technology prevailed over a well-known pharmacological component, resulting in the development of the system in a short time. Such development progressed through three stages, or generations, aimed at improving delivery and absorption, reducing patch size and making it easier to use. (Fig. 2)

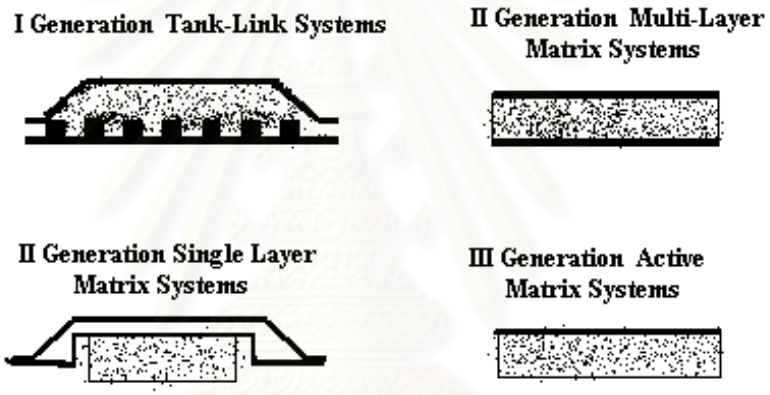


Figure 2 Various concepts of transdermal drug delivery systems in order to control release of active drug.

Furthermore, the therapeutic benefits of TDDS are an important issue in the development of any drug products. Therefore drug developers are turning to TDDS as a way to combine the advantage of IV infusion with the convenience of oral administration

The advantages are

- Adaptability to drugs with a short half-life.
- Avoidance of variation in gastrointestinal absorption.
- By pass of the hepatic first pass metabolism.
- Ease of self-administration.

- Good patient compliance.
- Production of sustained and constant plasma concentrations of drugs
- Reduction in repeated dosing intervals.
- Reduction of potential adverse side effects.
- Removal of TDDS provokes an immediate decrease of drug plasma levels
- Substitute for oral or parenteral administration in certain clinical situation (pediatrics, geriatrics, nausea, etc.)
- Suitable for drugs which produce a therapeutic response at very low plasma concentrations.

Table 1 TDDS offers the best of IV and oral administration

Topic	IV	Oral	TDD
Reduced first pass effects	Yes	No	Yes
Constant drug levels	Yes	No*	Yes
Self administration	No	Yes	Yes
Unrestricted patient activity	No	Yes	Yes
Non-invasive	No	Yes	Yes

*Sometimes can be achieved with controlled release.

3M Pharmaceuticals,St.Paul, Minn. entered the transdermal drug delivery market in the late 1970s, drawing on a variety of its corporate technologies including those in pressure sensitive adhesive, specialty films and membrane (See in Fig. 3).

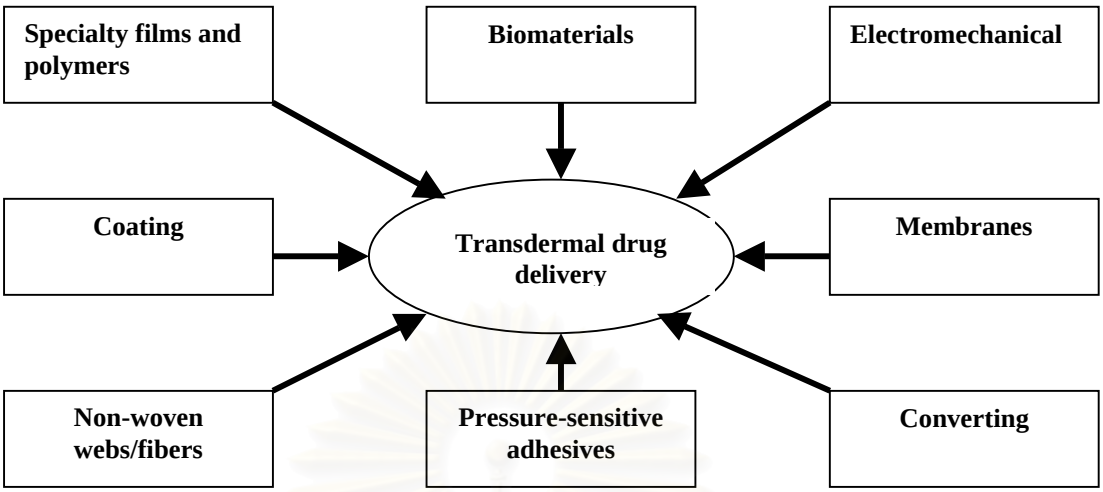


Figure 3 Pharmaceutical's core technologies from 3M Co., Ltd. in Transdermal drug delivery systems (Steven M. Wick, R. Ph., 1995).

All of technologies can classified into the four common configurations that are used in TDDS as shown in Fig. 4.

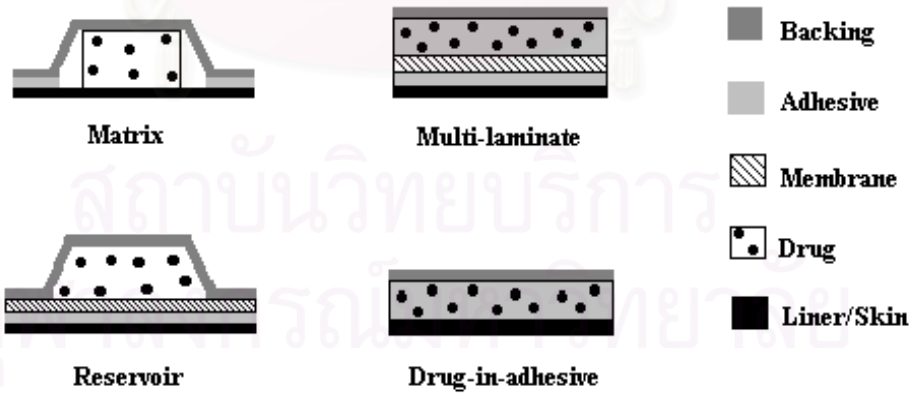


Figure 4 Basic Approaches for patch constructions.

- Reservoir, in which the drug is placed in liquid and delivered to the skin across a rate moderating membrane. The early years in the transdermal

drug delivery market were dominated by interest in reservoir-type configurations. One of the first transdermal systems, introduced by Ciba-Geigy, East Lansing, Mich, in 1980. The examples of this type are Duragesic®(fentanyl), Transderm-Nitro (nitroglycerin) and Transderm Scop®(scopolamine).

- Matrix, in which the drug is placed within a non-adhesive polymeric material, typically a hydrogel or soft polymer. Several TDDSs have been successfully developed from this type are examplified by Habitrol®(nicotene) and Nitrodisc®(nitroglycerin).
- Drug in adhesive, in which the drug is placed within an adhesive polymer. This configuration has been utilized in the development of Catapress TTS®(clonidine), Climara®(estradiol) and Nitro Dur®(nitroglycerin).
- Multi-laminate, which is similar to the drug in-adhesive design but which incorporates an additional layer of pressure sensitive adhesive to cover the entire patch and affix it to the skin.

The basic components of TDDS are also shown in Figure 4.

1. Drug formulation or drug reservoir: This may be a single or multi-layered part where the required amount of drug is stored in a stable form.
2. An adhesive: to maintain contact with the patient's skin, and should not irritate the skin.
3. A release liner that protects the patch during storage and is removed prior to skin application.
4. A backing that protects the patch from external factors during the application period.

In-vitro study of TDDS

When drug is absorbed through the skin, it can be measured directly by analyzing drug concentration profile in the blood or in the urine. However, during the development of TDDS, a quantitative assessment of the mechanisms, and rates of transdermal permeation of drug can be achieved by analyzing the drug permeation profile through an excised skin mounted on the diffusion cell (Keshary and Chien, 1984). The aim of the in-vitro experiment in TDDS is to understand and/or predict the delivery, and penetration of drug molecules from the delivery device into the body via the skin of a living animal (Gummer, 1989). The in-vitro experiment is useful in an evaluation of a dosage form because it is cost efficient and is able to test a large number of formulations in a relatively short time. In addition, the in-vitro studies can be used to screen for suitable formulations as well as test the effects of various ingredients on skin permeation.

The general major assumptions that may be made when conducting in-vitro experiments are as follows:

- The stratum corneum is the rate-limiting barrier to permeation,
- The skin's barrier properties are not compromised by the removal from living organism, and
- The possibility of metabolism with the skin is ignored (Zatz, 1990).

1. Diffusion cell

The Franz diffusion cell is one of the most widely used systems for in-vitro skin permeation studies. First disclosed in 1978 and subsequently marketed, this cell has a small donor compartment and a dumbbell-shaped receptor chamber (David R. Friend, 1992) as seen in Fig. 5. The bottom

portion of the dumbbell-shaped chamber connected with a narrower cylindrical tube which widens in the upper part of chamber bear the area of contact with the membrane. The central part of the receptor chamber is enclosed in a water-jacket for temperature control. Portions of the receptor chamber and the entire donor compartment are open to ambient conditions. The receptor chamber is agitated with a Teflon-coated magnetic stir bar. A number of modifications have been introduced into the original design.

Moreover, Chien and Valia (1984) had deigned the horizontal diffusion cell by aiming to minimized the potential inefficiencies observed in the Franz diffusion cell (Fig. 6). It is composed of a skin permeation cell and a magnetic driving unit, and two half-cells in mirror image of each others. Each of the half-cells contains a solution chamber within a stirring platform, which will rotate the magnetic stirrer at a synchronous speed. However, in this experiment modified Franz diffusion cell is used to evaluate drug permeation from pseudolatex and TDDS

2. Skin Model

TDDS is dealing with the skin that is one of the most extensive and readily accessible organs of the human body. The skin of an average adult cover over 3,000 square inches of surface area, and receives abort on-third of all blood circulating through the body. It is elastic, rugged, and, under normal physiological condition, self-regenerating. It separates the underlying blood circulation network from the outside environment and serves as barrier against physical and chemical attacks acts as a thermostat in maintaining body temperature, plays a major role in the regulation of blood pressure, shields the body from invasion by microorganisms, and protects against the penetration of ultraviolet rays

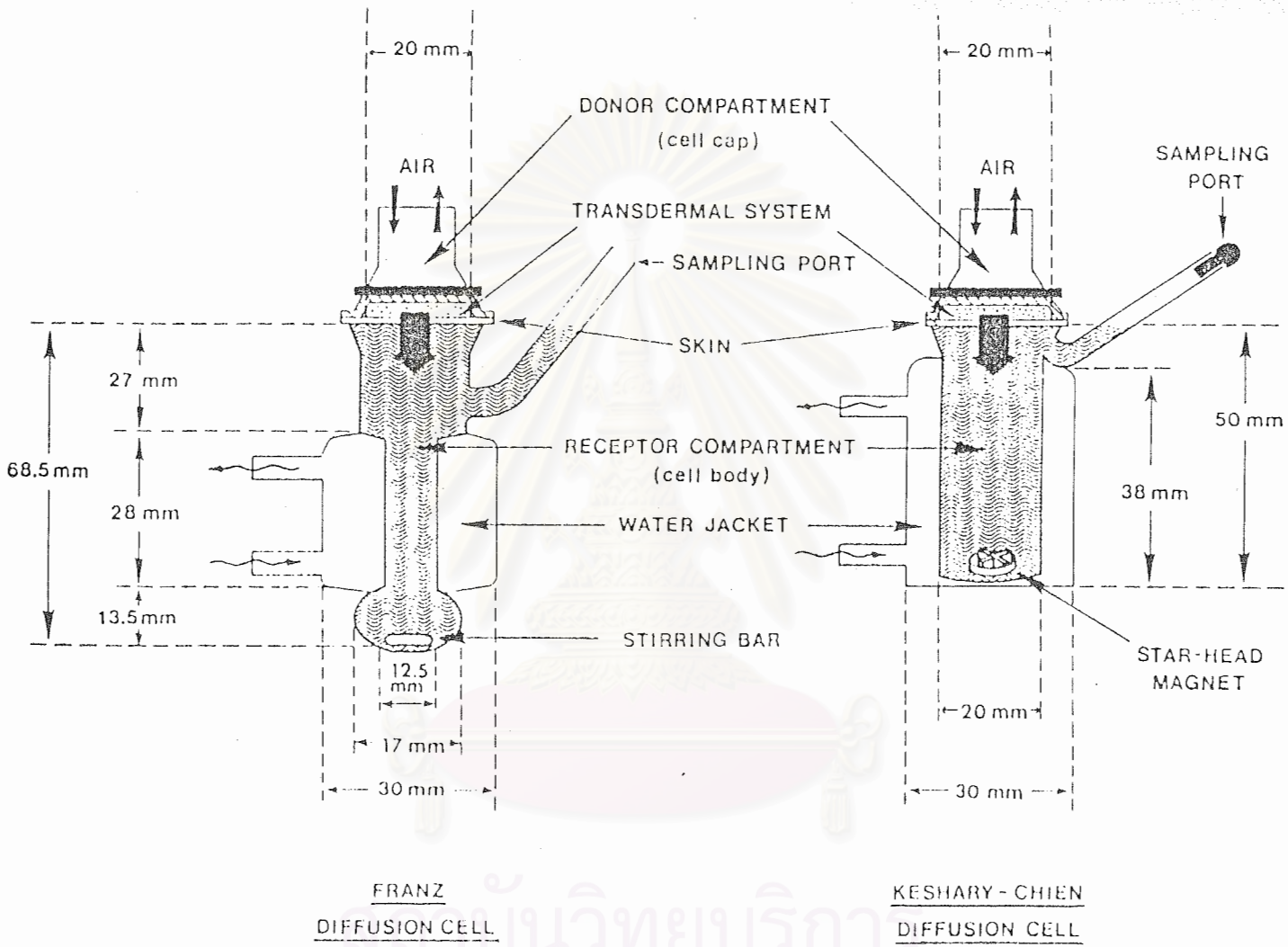


Figure 5 Diagrammatic illustration and comparison of Franz[7] and Keshary-Chien[7] diffusion cells.

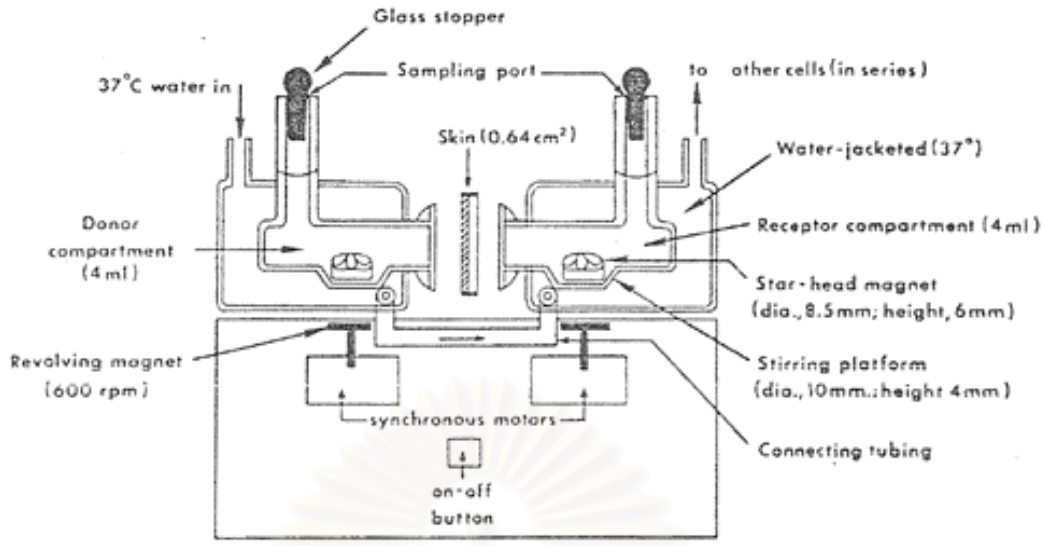


Figure 6 Schematic illustration of Valia-Chien's horizontal diffusion cell.

When microscopically considered, the skin appears to consist of various histologic layers. As a rule, three major layers are distinguished: the epidermis, dermis and subcutaneous fat (Fig. 7).

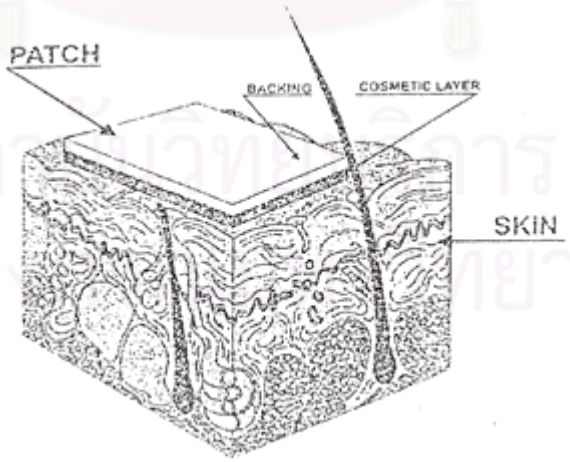


Figure 7 Three major layers of the skin.

The epidermis is divided into five layers. The outermost layer, the stratum corneum (SC), is a barrier to substances passing through the skin because it is highly compact, as well as rich in keratinized cell and special purpose lipidic lamellas.

In general, excised human skin is the most accurate, preferred membrane for the in-vitro skin permeation study. However, human skin is in short supply and has a variety of conditions that could induce a high permeability variation between individuals. If one assumes that the in-vitro experiment should reflect exactly the in-vivo situation, then only human skin can be used (Zatz, 1990).

In-vitro permeation studies can be conducted using animal skin, such as hairless mouse, guinea pig, fuzzy rat, rabbit , snake, etc. Although there are a number of similarities between the two types of skins, but no animal skin could completely mimics the penetration characteristics of a human skin (Gummer, 1987). Moreover, the use of artificial membrane in transdermal research is limited because they lack keratinized proteins and lipids which are primary component in the stratum corneum of mammalian skins (Itoh et al., 1990).

Stratum corneum is a major contribution to human skin which provides nearly impermeable barrier to the transport of most drugs (Bhatt et al., 1991). The unique barrier properties of the stratum corneum are due chiefly to its lipodal material (Bronaugh and Stewart, 1986).

There are many studies reported about the similarities between human stratum corneum and snake skin and the use of snake skin in percutaneous absorption study (Bhattachar et al.,1992 ; Itoh et al., 1990).

Both shed snake skin and human stratum corneum are composed of keratinized proteins and lipids, and have similar water permeation. It has found that snake skin has some features that make it useful as a model membrane . Since shed snake skin has no living tissue, it can be stored at a refrigerated temperature for a relatively long period.

The model skin examined in this study is shed snake skin, which is a non living pure stratum corneum with no hair follicles. Human stratum corneum consists of 10-20 layers an alpha-keratin-rich intracellular layer, and lipid-rich intercellular layer, but shed snake skin consists of three distinctive layers. These are the beta-keratin-rich outermost beta layer, alpha-keratin-and lipid-rich intermediate mesos layer, and alpha-keratin-rich innermost alpha layer. Further, the mesos layer show three to five layers of multilayer structure with cornified cells surrounded by intercellular lipids, which is similar to human stratum corneum.

Table 2 Comparison of thickness, lipid content and water evaporation rate between human stratum corneum, and shed snake skin (Itoh et al.,1990).

	Human stratum corneum	Shed snake skin (*Elaphe obsoleta)*
Thickness	13-15 μm	10-20 μm
Lipid content	2.0-6.5 %	6.0 %
Water evaporation rate	0.1-.8 mg /cm^2hr	0.15-0.22 mg /cm^2hr

Table 2 shows the similarities between the shed snake skin of *Elaphe obsoleta* (black rat snake) and the human stratum corneum in terms of thickness and lipid content. Also, shed snake skin and human stratum corneum have similar lipid compositions, that is, neutral lipids are a major

lipid component in both skins and fatty acids , with carbon-chain lengths of C_{15} and C_{18} predominant.

Harada et al. (1992) has been examined in-vitro permeability studies of salicylic acid using cadaver, rodent and shed snake skin to select model membrane for mimic human skin. Shed snake and hairless rat skin were found to show similar permeability to human breast and thigh skin, where wistar rat, and mouse skin showed similar permeability to human cheek, neck, and inguinal skin.

Pseudolatex

Pseudolatex emulsions are water-based systems that will examine colloidal aqueous dispersions of FDA-approved polymers. They are useful in mediating drug release from a reservoir. Pseudolatexes contain spherical solid or semisolid typically less than 1 μm. They are fluid even at polymer concentrations of 30 %. Pseudolatex can be prepared by dissolving the polymer in a suitable solvent system, and introducing the organic phase into water phase in order to form an emulsion by employing surfactant as stabilizers. After homogenization, the solvent is removed by vacuum distillation. Particle size is the key to pseudolatex stability or resistance to settling and sedimentation. According to Stoke's law for spherical particles, rate of sedimentation can given by equation (1).

$$R_s = (D^2/18\eta)(d_p - d_m)g \qquad (1)$$

where R_s is rate of sedimentation, D is particle diameter, η is the viscosity of the medium, d_p and d_m are the densities of the particles and the medium, respectively, and g is the gravitational constant.

The tendency for colloidal particles to settle upon standing is offset by their Brownian motion, and convection current arising from small temperature gradients in the sample. Brownian motion, which results from the unbalanced collisions of solvent molecules with the colloidal particles, increase in intensity with decreasing particle size. One criteria for settling is that a sedimentation rate of 1 mm /24 hrs will be offset by thermal convection currents, and Brownian motion within the sample. Substituting this sedimentation rate into the Stoke's equation enables to verify the largest particle size that, in any particular instance, will not settle out upon standing.

Drug delivery systems which control drug released by matrix, and pseudolatex base are similar in dispersibility of drug within polymer. The rate of drug release from matrices, and pseudolatexes may be altered by variations of the polymer matrix material and the drug concentration in the base. The physicochemical properties of the drug molecule and differences in the condition of the skin, region, age and sex will also play an important role in the permeation of the drug through the skin (K. H. Valia and Y. W. Chien, 1984).

P. Rama Rao and Prakash V. Diwan designed to develop a suitable matrix model TDDS of DTZ HCl and indomethacin, employing ethylcellulose (EC) and polyvinyl pyrrolidone (PVP) as film formers. Dibutyl phthalate was incorporated into the formulation as plasticizer. Most the release rate of drug from matrix model TDDS followed Higuchi equation in which the amount of drug release is linear to the square root of time. The release rate constant has been found to be dependent on initial drug loading as well as the film composition (Lisbeth Illium et al., 1987 ; Rande, V. V. et al.,1996). Moreover, delivery rates from hydrophilic

polymer matrix such as PVP, polyethylene oxide (PEO) base are higher compared to the hydrophobic TDDS matrices (M. M. Feldstein et al., 1996).

There are the studies of comparative release rate from two base systems; pseudolatex and matrix diffusion controlled systems for salbutamol. This study has been found that pseudolatices demonstrated better skin permeation than matrices and followed zero order of pharmacokinetic (Jain, Sanjay K. et al., 1994). In addition, bromhexine from pseudolatex base system, the drug release and across the skin permeability, recorded to be better and uniform (Deepak Thassu and S. P. Vyas, 1991). From these studies, the use of pseudolatex base will be a good chance to increase potential TDDS in the future.

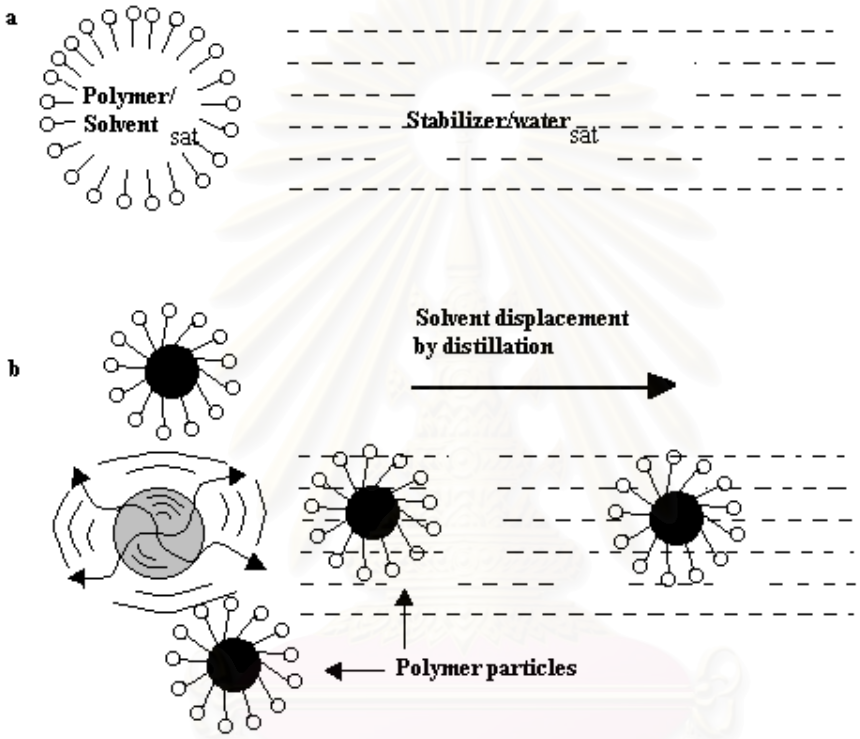
In many studies which utilize the dispersion of a drug in a pseudolatex made up of polymer, surfactant, channeling agent, plasticizer and oil phase. There are various drugs which can be used in this base, including large molecules for example diclofenac sodium and bromhexine hydrochloride, and small molecules for example ephedrine and salbutamol.

Suresh P. Vyas et al (1991) have developed prolonged and controlled release of diclofenac by using polymeric pseudolatex dispersion. To achieve the desired release rate, different combination of hydrophilic and hydrophobic polymer were used for the preparation. The designed system exhibited linear relationship between drug release as a function of square root of time. The TDDS could maintain a constant, and effective plasma level for 24 hours. Moreover, some reports have been studied comparison of drug plasma profile between pseudolatex based system for TDDS and conventional oral dose of isosorbide dinitrate and ephedrine.

The result showed that designed pseudolatex TDDS of isosorbide dinitrate could be used successfully with improved performance (S. P. Vyas et al.,1994) including a constant and comparatively higher ephedrine blood level could be achieved (Sanjay K. Jain et al.,1990). Then the most promising in-vivo availability of the drug was recorded with selected pseudolatices.

Pseudolatexes are typically prepared by the emulsification-evaporation technique patented by Vanderhoff et al. (1979): a polymer solution in a water-immiscible organic solvent is emulsified in an aqueous phase containing emulsifiers. This crude emulsion is then submitted to a high energy source, e.g. ultrasound radiation, or is passed through homogenizers, high pressure dispersers, etc. The polymer emulsion resulting from such treatment is very stable, and contains very small droplets (below 0.5 μm. diameter). This emulsification procedure is followed by the removal of the solvent by vacuum steam distillation, producing a fine aqueous dispersion of polymeric particles averaging less than 0.5 μm. Recently, a new method, the emulsification-diffusion, has been proposed to prepare nanoparticles from preformed polymers (Quintanar-Guerrero et al.,1999). Pseudolatex using an emulsification-diffusion technique, involving partially water miscible solvents. The preparation method consisted of emulsifying an organic solution of polymer (saturated with water) in an aqueous solution of a stabilizing agent using conventional stirrers, followed by direct solvent distillation. The technique relies on the rapid displacement of the solvent from the internal into the external phase which thereby provokes polymer aggregation. Nanoparticle formation is believed to occur because rapid solvent diffusion produces regions of local supersaturation near the interface, and nanoparticles are formed due to the ensuing interfacial phase

transformations, and polymer aggregation that occur in these interfacial domains (see Fig. 8). Using this method, it was possible to prepare pseudolatexes of biodegradable and non-biodegradable polymers. Therefore, an optimization step in this study would be required for each polymer/solvent/stabilizer system in order to find the component ratio



necessary to produce only nanoparticles.

Figure 8 Schematic description of the proposed mechanism of formation of the nanoparticles by the emulsification-diffusion method based on solvent displacement by distillation (a: emulsification step, b: evaporation step).

Acrylic polymers (M. Dittgen et al.,1997)

An acrylic homopolymer consisting of polymethyl methacrylate, gained recognition in the 1937 World Trade Show as an outstanding

commercial product. At present, the pharmaceutical uses of acrylic polymers are widespread, mainly due to the variety of acrylic copolymers. These can be designed from different combinations of monomers, and comonomers to achieve distinguishing properties. The performance properties of these polymers can be uniquely designed by a creative combination of variety of monomers and polymerization techniques

Table 3 Chemical structure of (meth)acrylic monomers, general formula: $CH_2=C(R_1)-CO-(R_2)$

Monomers	R_1	R_2	Chemical name
AA	H	OH	acrylic acid
AAm	H	NH_2	acrylamide
BCA	C∫N	$O-C_4H_9$	butyl cyanoacrylate
BMA	CH_3	$O-C_4H_9$	butyl methacrylate
DEAEMA	CH_3	$O-CH_2-CH_2-N(C_2H_2)_2$	N,N-diethyl aminoethyl methacrylate
DHPMA	CH_3	$O-CH_2-CHOH-CH_2-OH$	dihydroxypropyl-methacrylate
DMAEMA	CH_3	$O-CH_2-CH_2-N(CH_3)_2$	N,N-dimethyl aminoethyl methacrylate
EA	H	$O-C_2H_5$	ethylacrylate
ECA	C∫N	$O-C_2H_5$	ethyl cyanoacrylate
EGDMA	CH_3	$O-CH_2-CH_2-O-CO-C(CH_3)= CH_2$	ethylene glycol dimethacrylate
HECA	C∫N	$O-CH_2-CH_2-OH$	hydroxyethyl cyanoacrylate
HEEMA	CH_3	$O-CH_2-CH_2-O-CH_2-CH_2-OH$	hydroxyethoxyethyl methacrylate
HEMA	CH_3	$O-CH_2-CH_2-OH$	hydroxyethyl methacrylate
HPMAm	CH_3	$NH-CH_2CH-OH-CH_3$	N-(2-hydroxypropyl) methacrylamide
IBCA	C∫N	$O-CH_2-CH_2-(CH_2)_2$	isobutyl cyanoacrylate
IPAAm	H	$NH-CH_2-(CH_2)_2$	N-isopropyl acrylamide

Table 3 (cont.)

Monomers	R_1	R_2	Chemical name
MA	CH_3	OH	methacrylic acid
MeA	H	O- CH_3	methylacrylate
MMA	CH_3	O- CH_3	methyl methacrylate
TAMCl	CH_3	O-CH_2-CH_2-N(CH_3)$_3$Cl	trimethyl ammonioethyl methacrylate chloride

Table 4 Composition, monographs, abbreviated and brand names of (meth)acrylic polymers for pharmaceutical application.

Abbreviated name:poly	Weigh ratio	Monographs	Brand names
AA		carbomer NF/USP	Carbopol
BMA/MMA/DEAEMA	1/1/2	aminoalkyl methacrylate copolymer EJPE/DAB	Eudragit E100
EA/MMA	2/1	polyacrylate dispersion 30% EP	Eudragit NE30D
EA/MMA/TAMCl	1/2/0.2	ammonioethyl methacrylate copolymers, type A,NF/USP	Eudragit RL100
EA/MMA/TAMCl	1/2/0.1	ammonioethyl methacrylate copolymers, type B,NF/USP	Eudragit RS100
MA/EA	1/1	methacrylic acid copolymers, type C,NF/USP	Eudragit L100-55
MA/MMA	1/1	methacrylic acid copolymers, type A,NF/USP	Eudragit L100
MA/MMA	1/2	methacrylic acid copolymers, type B,NF/USP	Eudragit S100
MeA/MMA/MA	1/1/0.2	new	Eudragit pre.4110D

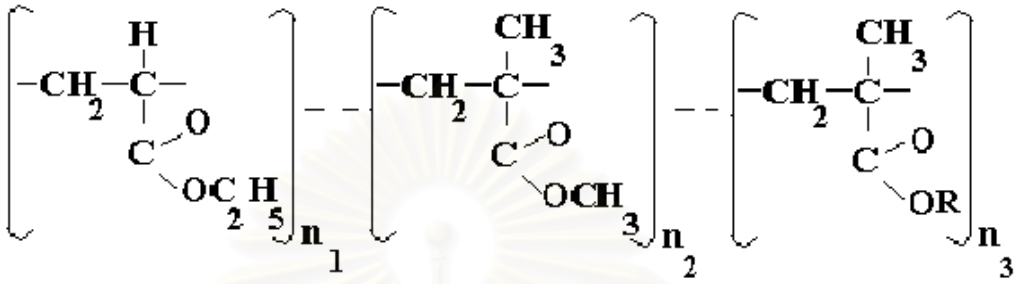
The physical and chemical stability of polymethacrylates are due to the presence of a polymeric backbone made up of carbon atoms. In the case of polymethacrylates, the carbon backbone is further stabilized by the methyl side group. The monomeric units of methacrylic derivatives contribute to their rigid, hard, and brittle nature. Acrylic derivatives contribute more to their softness, and flexibility. There are also characteristic differences among these polymers due to the chemical properties of acrylic, and methacrylic functional groups. The absence of the hydrophobic methyl group in acrylic derivatives results in their greater reactivity and hydrophilicity as compare to the methacrylic derivatives.
Acrylic acid is a stronger acid (pK_a 4.25). Linear polyacrylic acid is water soluble even at low pH values. Methacrylic acid, due to the presence of the methyl group, is a weaker acid (pK_a 4.66), and is insoluble in water, except as a water-soluble carboxylate polyanion at neutral or alkaline pH. The ester groups in polymethacrylic esters are stable against hydrolytic attack by dilute acids or bases.

Ammonio methacrylate Copolymer Type A (Eudragit RL100®) and Type B (Eudragit RS100®) compose of trimethyl ammonioethyl methacrylate chloride, ethylacrylate and methylmethacrylate. These polymers are quarternary ammonium groups, due to their hydrophilic nature, increase the permeability of polymethacrylate films. Type A has a ratio between ammonium group and neutral methacrylates of 1:20, and type B 1:40 which make differently permeability. Type A can give more permeable than type B (See Table 5).

Eudragit RS/RL pseudolatexes enjoy several advantages such as stability to heat and mechanical shear , and dilutability with organic solvents. The film forming properties are outstanding, as a result of which

controlled release properties can be obtained by using Eudragit RS/RL pseudolatexes (Rong-kum Chang and Charles Hsiao, 1989).

Table 5 Methacrylate ester copolymers



Scientific name	$n_1 : n_2 : n_3$	MW	Behavior in digestive juices	Eudragit type
Poly(ethylacrylate methylmethacrylate)	2 : 1	800,000	Insoluble films of medium permeability	NE 30 D (30% aqueous dispersion)
Poly(ethylacrylate methylmethacrylate) trimethyl ammonioethylmethacrylate chloride R: CH_2-CH_2-$N^+(CH_3)_3Cl^-$	1 : 2 : 0.2	150,000	Insoluble films of high permeability	RL 30 D (30% aqueous dispersion) RL100 (Granules)
Poly(ethylacrylate methylmethacrylate) trimethyl ammonioethylmethacrylate chloride R: CH_2-CH_2-$N^+(CH_3)_3Cl^-$	1 : 2 : 0.1	150,000	Insoluble films of low permeability	RS 30 D (30% aqueous dispersion) RS100 (Granules)

Materials Information

1. **Diltiazem Hydrochloride (DTZ HCl)** is a calcium ion influx inhibitor (slow calcium channel blocker)

1.1 Physicochemical properties (David J. Mazzo et al., 1994)

Chemical name : (2S-cis)-3-(acetyloxy-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxy-phenyl)-1,5-benzothia-zepin-4(5H)-one monohydrochloride.

Empirical name : $C_{22}H_{26}N_2O_4S \bullet HCl$

Molecular weight : 450.98 g/mole

Chemical structure is shown in Figure 9

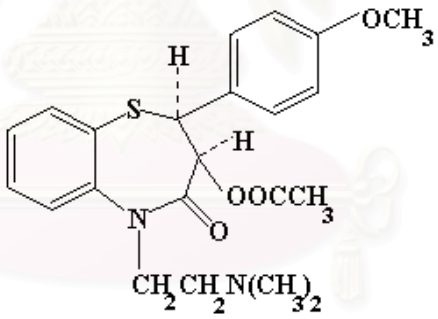


Figure 9 Chemical structure of diltiazem hydrochloride.

Appearance : DTZ HCl is a white to off-white crystalline power. It is odorless and has a bitter taste.

Solubilities : The solubility of DTZ HCl in a variety of solvents is presented in Table 6.

Table 6 Solubility of DTZ HCl in various solvent systems at 25 °C

Solvent	Solubility
Chloroform	Freely soluble
Formic acid	Freely soluble
Methanol	Freely soluble
Water	Freely soluble
Dehydrated alcohol	Sparingly soluble
Benzene	Practically insoluble
Ether	Insoluble

1.2 Pharmacology (Micaela M.-T. Buckley et al., 1990)

DTZ HCl is a calcium antagonist effective in the treatment of stable, variant and unstable angina pectoris and mild to moderate systemic hypertension, with a generally favourable adverse effect profile. It is also effective in terminating supraventricular tachycardia and in controlling the ventricular response to atrial fibrillation/flutter.

1.3 Pharmacokinetic (Micaela M.-T. Buckley et al., 1990)

Approximately 90% of an orally administered dose of DTZ HCl is absorbed. After administration of single oral doses, including a sustained release tablet, the mean absolute bioavailability is about 30 to 40% and is dose related. The area under the plasma concentration-time curve (AUC) increases after multiple dosing, indicating that first-pass metabolism decrease with multiple dosing.

DTZ HCl undergoes substantial first pass metabolism to form several metabolites. The most important of these are N-

monodemethyldiltiazem, with an estimated 20% of the potency of diltiazem and deacetyl diltiazem, which is about half as potent as the parent drug. Steady state diltiazem concentrations in plasma are achieved within 3 to 5 days. The pharmacokinetics of DTZ HCl are unaffected by renal disease.

Table 7 Pharmacokinetics of DTZ HCl (Triggle, D.J.)

	Diltiazem
Absorption, oral (%)	>90
Bioavailability (%)	~40
Onset of action : oral (min)	<30
Peak effect	3-5 hrs.
Protein binding (%)	90
Plasma half life	5 hrs.
Metabolism	60% of first dose ; 10% steady state
Excretion	
-Renal (%)	30
-Fecal (%)	70

1.4 Dosage and Preparation

Oral dosages employed in the treatment of systemic hypertension and angina pectoris. In systemic hypertension, oral dosages between 90 to 180 mg/day are employed in Japan and Southeast Asia.

Treatment of stable or variant angina pectoris should be initiated at 120 mg/day divide with stepwise titration up to a maximum of 360

mg/day. The dosage of 90 mg/day is normally employed in angina pectoris in Southeast Asia

DTZ HCl (Cardizem, Dilacor) is available as tablets, sustained release capsules, and injectable forms. Therapy is individualized and generally begins with 30 mg four times a day up to a maximum of 360 mg daily. Intravenous therapy usually begins with a dose of 0.2 mg/kg over 2 min, followed by an additional dose of 0.3 mg/kg. Infusions are usually given in dose of 10 mg/hr and can be maintained for up to24 hr.

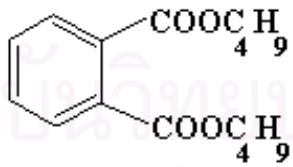
2. Dibuthyl Phthalate (Ainley Wade and Paul J. Weller, 1994)

Synonyms : 1,2 benzenedicarboxylic acid dibutyl ester ; n-butyl phthalate ; DBP ; dibutyl benzene-1,2-dicarboxylate ; di-n-butylphthalate ; Kodaflex DBP ; phthalic acid dibutyl ester.

Empirical name : $C_{16}H_{22}O$

Molecular weight : 278.35

Structural formula



Functional category : solvent ; plasticizer

Appearance : a clear, colorless or faintly colored oily liquid

Applications : dibutyl phthalate is used as a plasticizer in film coating ; has limited compatibility with cellulose acetate. It is also used as an insect repellant, primarily for the impregnation of clothing.

Typical properties :

-density 1.05 g/cm^3

-boiling point 340 °C

-flash point 171 °C

-freezing point –35 °C

-refractive index n_D^{20} = 1.491-1.493

-solubility very soluble in acetone, benzene, ethanol (95%), and ether ; soluble 1 in 2500 of water

- viscosity 15mPas (15cP) at 25 °C

3. Mineral Oil

Nonproprietary names

BP : Liquid paraffin

Ph Eur : Paraffinum liquidum

USP : Mineral oil

Synonyms : 905 (mineral hydrocarbons) ; Avatech ; Citation ; heavy liquid petrolatum ; heavy mineral oil ; liquid petrolatum ; paraffin oil ; white mineral oil

Empirical name : Mineral oil is a mixture of refined liquid saturated hydrocarbons obtained from petroleum.

Functional category : Emollient ; solvent ; tablet and capsule lubricant ; therapeutic agent ; oleaginous vehicle.

Applications : Mineral oil used primarily as an excipient in topical pharmaceutical formulations where its emollient properties are exploited as an ingredient in ointment bases. It is additionally used in oil-in water emulsions, as a solvent, and as a lubricant in capsule and tablet formulations , and to a limited extent, as a mold release agent for

cocoa buffer suppositories. Moreover, mineral oil is also used in cosmetic and food products.

Description : Mineral oil is a transparent, colorless, viscous liquid, free from fluorescence in day light. It is practically tasteless and odorless when cold, and has faint odor when heated. Mineral oil should be stored in an airtight container, protected from light, in a cold, dry, place.

Typical properties

-boiling point > 360 °C

-flash point 210-224 °C

-pour point -12.2 to –9.4 °C

-refractive index n_D^{20} = 1.4756-1.4800

-surface tension 35 mN/m (dynes/cm) at 25 °C

-solubility practically insoluble in ethanol (95%), glycerin, and water ; soluble in acetone, benzene, chloroform, carbon disulfide, ether, and petroleum ether. Miscible with volatile oils and fixed oils, with the exception of castor oil.

-viscosity 110-230 mPas at 20 °C

-incompatabilities with strong oxidizing agents.

4. Polyvinyl pyrrolidone K30

Nonproprietary name

BP : Povidone

PhEur : Polyvidonum

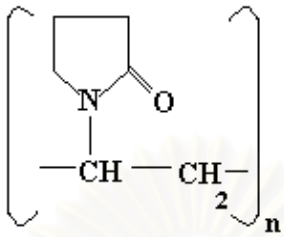
USP : Povidone

Synonyms : E1201 ; Kollidon ; Plasdone ; poly [1-(2-oxo-1-pyrolidinyl) ethylene] ; polyvidone ; PVP ; 1-vinyl-2-pyrrolidinone polymer

Empirical name : $(C_6H_9NO)_n$

Molecular weight : approximate 50,000

Structural formular



Functional category : suspending agent ; tablet binder

Applications : It is primarily used in solid dosage forms. In tableting, povidone solution are used as binders in wet granulation processes. Povidone solution may also be used as coating agents. Povidone is additionally used as a suspending, stabilizing or viscosity increasing agent in a number of topical and oral suspensions and solutions. The solubility of a number of poorly soluble active drugs may be increased by mixing with povidone.

Description : Povidone is a fine, white to creaming-white colored, odorless, hygroscopic powder. Povidones with K-value equal to or lower than 30 are manufactured by spray drying and exist as spheres. It should be stored in an airtight container in a cool, dry, place

Typical properties

-density (bulk) 0.31 g/cm^3 , tabbed 0.40 g/cm^3 for plasdone K-30

-hygroscopicity very hygroscopic, significant amounts of moisture being absorbed at low relative humidities.

-melting point softens at 150 °C

-solubility freely soluble in acids, chloroform, ethanol, ketones, methanol and water ; practically insoluble in ether, hydrocarbon and mineral oil. In water the concentration of a solution is limited only

by the viscosity of the resulting solution which is a function of the K-value

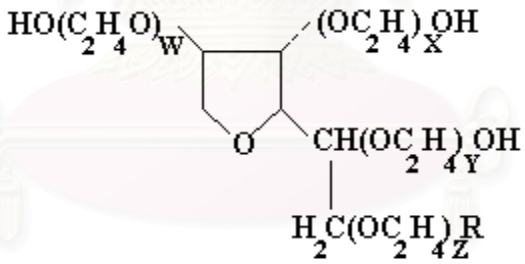
-viscosity K30 in ethanol 3.4 mPas

-incompatibilities : the efficacy of some preservatives, e.g. thimerosal, may be adversely affected by the formation of complexes with povidone.

5. Tween 80

Synonyms : sorbitan mono-9-octadecenoate poly (oxy-1,2-ethanediyl) derive ; polyoxyethylene(20)sorbitan mono-oleate ; sorethytan (20)mono-oleate ; polyethylene oxide sorbitan mono-oleate ; sorlate ; Polysorbate 80 ; Monitan ; Olothorb

Structural formular



Sum of W, X, Y, Z is 20 : R is $(C_{17}H_{33})COO$

Functional category : Emulsifying agent ; nonionic surfactant ; solubilizing agent ; wetting agent

Description : Amber-colored, viscous liquid. vicosity 270-430 centristokes, very soluble in water ; soluble in alcohol, cotton seed oil, corn oil, ethyl acetate, methanol, toluene. Insoluble in mineral oil, pH of 5% solution between 5 and 7

Applications : shown in Table 8.

Table 8 Application of Tween 80 in pharmaceutical formulations

Use	Concentration (%)
Emulsifying agent	
Used alone in water-in-oil emulsions	1-15
Used in combination with hydrophilic emulsifiers in oil-in water emulsions	1-10
Used to increase the water-holding properties of ointments	1-10
Solubilizing agent	
For poorly soluble, active constituents in lipophilic bases	1-10
Wetting agent	
For insoluble, active constituents in lipophilic bases	0.1-3

CHAPTER II

MATERIALS AND METHODS

Materials

A. Chemical for Preformulation Studies

1. Diltiazem HCl : Lot No. 0690798 Distributed by Siam Chemical Product Co., Ltd. Thailand.
2. Eudragit RL-100® : Lot No. 0860406945 Supported by JJ-Degussa-huls Co., Ltd. Thailand.
3. Eudragit RS-100® : Lot No. 0841208165 Supported by JJ-Degussa-huls Co., Ltd. Thailand.
4. Dibutyl phthalate : Lot No. 61219343 Distributed by Triton (Thailand) Ltd.
5. Polyvinyl pyrrolidone K30 : BASF Co., Ltd. Germany.
6. Tween 80 : Lot No. 807870 Distributed by B.L. Hua Co., Ltd. Thailand.
7. Liquid paraffin : Lot No. LDB89/843 Distributed by Srichan saha O. sod Ltd. Thailand.
8. Chloroform : Lot No. 4440 Mallinckrodt Baker, Inc. USA.
9. Methanol : Lot No. 9070-68 Mallinckrodt Baker, Inc. USA.
10. Dichloromethane : Lot No. V183KPHD Mallinckrodt Baker, Inc. USA.
11. Potassium dihydrogen phosphate : E Merck, Darmstadt Germany.
12. Sodium hydroxide : E Merck, Darmstadt Germany.

B. Membrane

1. Co Tran™ 9711 porous membrane ethylene vinyl acetate 9 % : Lot 101
2. Scotchpak™ Film No. 1009 heat sealable polyester film backing membrane Lot # 751 : Lot No. 2710916
3. Co Tran™ Adhesive (PGTA) 9871 : Lot No. PH14120015

All of these membranes manufactured by Drug Delivery Systems 3M. Pharmaceuticals, Co., USA.

C. Shed Snake Skin

Shed snake skin of *Elaphe obsoleta* (Black rat snake) donated by Pata department store's zoo was used throughout the experiment.

Apparatus

1. Analytical balance : Mettler Toledo model PB 3002, Switzerland.
2. Analytical balance : A 200S, Satorious, Germany.
3. Homogenizer : Ystral GmbH D-7801 Dottingen type : ×1020 #2, Germany.
4. Light microscope : Olympus model BH-2, Japan.
5. Magnetic stirrer : Sybron/Thermolyne nuova 7 stir plate, USA.
6. Rotary evaporator, Buchi model RE 311, Buchi Laboratoriums Techink, Germany and Aspirator, Eyela model A-35 Tokyo Rkakikai Co., Ltd., Japan.
7. Cone & Plate viscometer : Brookfield digital cp# 41, USA.
8. pH : Orion pH meter model 420 A., USA.
9. Diffusion cell : Modified from Keshary-Chien diffusion cell.
10. Spectrophotometer : UV 160 A Shitmadzu, Japan

11. Dissolution Apparatus : Hanson Research Model SR-2, USA.
12. Scanning Electron Microscope with cryo method : JSM-5410 LV JEOL, Japan.
13. Photon correlation spectrophotometer : LoC version PCS :v1.23 Malvern Instruments, England.

Methods

1. Technique and Condition of Preparing Pseudolatex

In this experiment, solvent removal method was used to prepare pseudolatex. The preparation was composed of two phases, oil and water phase.

Oil phase

A suitable grade of Eudragit approximately 14% w/w was dissolved in 205 ml of chloroform, using magnetic stirrer until it was truly soluble. After that 6% w/w of PVP K30 were added and stirred until its dissolve. Then about 2% w/w of liquid paraffin and 4%w/w of dibutyl phthalate were filled respectively.

Water phase

Tween 80 was dissolved in water, and stirred until completely soluble.

Two phases were mixed together by pouring water phase into oil phase and mixing with homogenizer for 30 minutes (Fig. 10). In this step, emulsion-like system occurred then poured emulsion-like system into round bottom flask to remove solvent by rotary evaporator.

The rotary evaporator is an apparatus, which used throughout the study with controlling temperature (40±2°C) and vacuum condition (Fig.11).

Remark: The polymer and their weight fraction ratio, drug and plasticizer concentration used in the pseudolatex system were changed to many ratios and concentrations with mention in preformulation of pseudolatex in order to prescreening to obtain a suitable composition for TDDS.

Figure 10 A homogenizer for mixing two phases of pseudolatex.

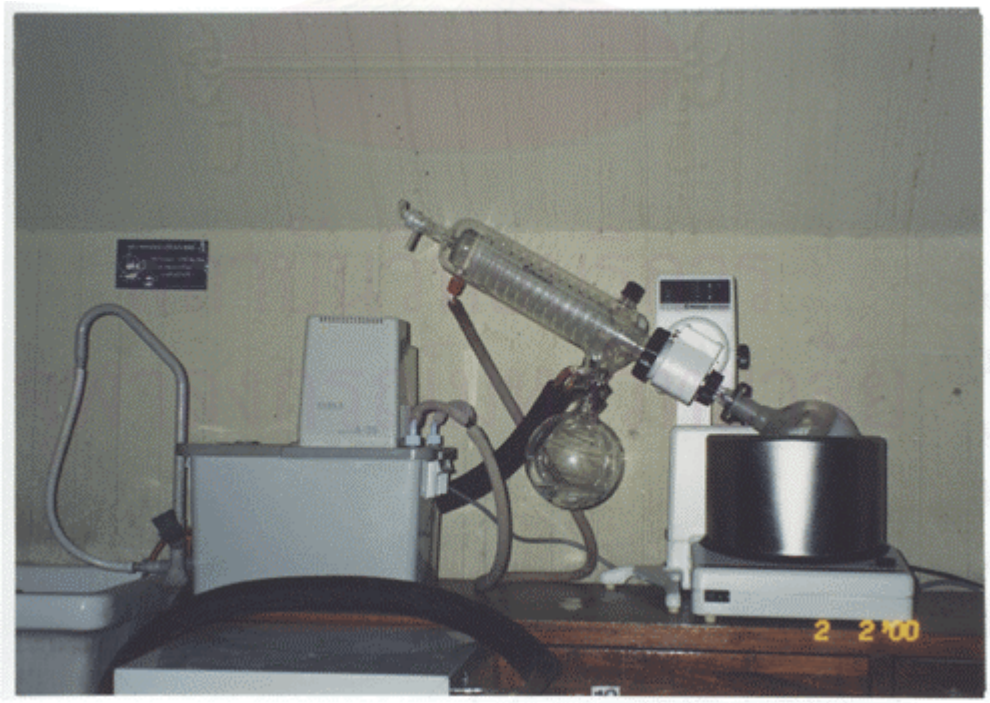


Figure 11 A rotary evaporator, using to prepare pseudolatex.

2. Preformulation of Pseudolatex as a Drug Reservoir for DTZ HCl TDDS

These parameters will be employed for further formulation development.

2.1 Determination of Water Removal Time from Pseudolatex without Drug.

Core formula of pseudolatex base as shown in Table 9 in order to determine suitable water removal time.

Table 9 Core formula of pseudolatex base without drug containing.

Ingredient (s)	% w/w of formulation
Eudragit RL/RS-100®	14.36
PVP K30	6.15
Liquid paraffin	2.05
Dibutyl phthalate	4.10
Tween 80	10.26
Water	63.08

The water removal time was started to measure when organic solvent (chloroform) has been completely removed. To distinguish between chloroform and water in round bottom flask which containing solvent removal from emulsion-like system was clearly determined because two solvents were immiscible. Products were kept with water removal time at 1, 2, 4 and 6 hours. All of products were evaluated as described in section 3 for physicochemical characteristics in order to chose suitable water removal time of products.

2.2 Determination of Water Removal Time from Pseudolatex with Drug (DTZ HCl 2% of formulation)

Core formula of pseudolatex, containing 2% of DTZ HCl as shown in Table 10 in order to determine suitable water removal time.

Table 10 Core formula of pseudolatex base with drug containing.

Ingredient (s)	% w/w of formulation
DTZ HCl	2
Eudragit RL/RS-100®	14
PVP K30	6
Liquid paraffin	2
Dibutyl phthalate	4
Tween 80	10
Water	62

Products were kept with water removal time at 4, 6 and 7 hours. All of products were evaluated as same as topic 2.1, as mentioned before.

2.3 Effect of Polymer Ratios between RL100 and RS100

When a suitable removal time of core formula was defined already, effect of polymer ratios between RL100 and RS100 on physicochemical characteristics, and the release profile were determined. There were two parts of formulations, first part composed no drug in pseudolatex formulas and another was formulas, which containing 2% w/w of DTZ HCl in the same composition. The formulas with various components are shown in Table 11.

At first, the ratio of polymer RL100 and RS100 used in the descending value as follows at 100:0, 80:20, 50:50, 20:80 and 0:100, respectively. From the formulation as indicated above, polymer had 14 % w/w in preparation which divided in the ratio of Eudragit RL100® and RS-100® for example; in the ratio 80:20 showed that preparation composed of 11.2 % Eudragit RL100® and 2.8 % Eudragit RS-100®.

Table 11 Formulas of pseudolatex and its composition with various polymer ratios between Eudragit RL/RS.

Formula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100®/RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#1	-	14.36	-	6.15	2.05	10.26	4.10	63.08
F#2	-	11.49	2.87	6.15	2.05	10.26	4.10	63.08
F#3	-	7.18	7.18	6.15	2.05	10.26	4.10	63.08
F#4	-	2.87	11.49	6.15	2.05	10.26	4.10	63.08
F#5	-	-	14.36	6.15	2.05	10.26	4.10	63.08
F#6	2	14	-	6	2	10	4	62
F#7	2	11.20	2.80	6	2	10	4	62
F#8	2	7	7	6	2	10	4	62
F#9	2	2.80	11.20	6	2	10	4	62
F#10	2	-	14	6	2	10	4	62

2.4 Effect of Channeling Agent

After the concentration of polymer in the formulation was determined, and the suitable ratio of polymer RL100 and RS100 was chosen. Then, the concentration of polymer at quantities of 10, 12, 14 and 16 % w/w in the formulation were evaluated along with the effect of channeling agent.

Polyvinyl pyrrolidone (PVP K30) was used in this study as channeling agent in the preparation. The concentrations employed in the formulations are at 4, 6, 8 and 10 %, respectively. It was noticed that all formulations as indicated above containing the same drug content of 2% w/w. Formulas are given in Table 12.

Table 12 Formulas of pseudolatex and its composition used to determine the effects of polymer concentration, and channeling agent.

For mula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100® /RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#1	-	14.36	-	6.15	2.05	10.26	4.10	63.08
F#11	-	10.36	-	6.15	2.05	10.26	4.10	67.08
F#12	-	12.36	-	6.15	2.05	10.26	4.10	65.08
F#13	-	16.36	-	6.15	2.05	10.26	4.10	61.08
F#6	2	14	-	6	2	10	4	62
F#14	2	10	-	6	2	10	4	66
F#15	2	12	-	6	2	10	4	64

Table 12 Continued

Formula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100® /RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#16	2	16	-	6	2	10	4	60
F#17	-	10.36	-	4.15	2.05	10.26	4.10	69.08
F#18	-	12.36	-	4.15	2.05	10.26	4.10	67.08
F#19	-	14.36	-	4.15	2.05	10.26	4.10	65.08
F#20	-	16.36	-	4.15	2.05	10.26	4.10	63.08
F#21	2	10	-	4	2	10	4	68
F#22	2	12	-	4	2	10	4	66
F#23	2	14	-	4	2	10	4	64
F#24	2	16	-	4	2	10	4	62
F#25	-	12.36	-	8.15	2.05	10.26	4.10	63.08
F#26	-	12.36	-	10.15	2.05	10.26	4.10	61.08

2.5 Effect of Surfactant

With the step by step of consideration, an appropriate quantity of PVP K30 was chosen, and further studied for the effect of surfactant will be observed. From preliminary studied, Tween 80 seemed to be available to this system. The following experiments, we will study effect of Tween 80 at various concentrations containing in the formulas as indicated in Table 13. Tween 80 was used in the preparation with the concentration at 6, 10 and 14 % in the

formulation, respectively which the same drug content at 2 % w/w in each formulation.

Table 13 Formulas of pseudolatex and its composition used in order to observe the effect of surfactant.

Formula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100® /RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#18	-	12.36	-	4.15	2.05	10.26	4.10	67.08
F#27	-	12.36	-	4.15	2.05	6.26	4.10	71.08
F#28	-	12.36	-	4.15	2.05	14.26	4.10	63.08
F#22	2	12	-	4	2	10	4	66
F#29	2	12	-	4	2	6	4	70
F#30	2	12	-	4	2	14	4	62

2.6 Effect of Plasticizer

At the same consideration in each effect, an appropriate quantity of Tween 80 was chosen to study effect of plasticizer as shown in Table 14. Dibutyl phthalate was used in the preparation as plasticizer with the concentration at 2, 4, 6 and 8 % in the formulation, respectively which the same drug content at 2% w/w in each formulation.

Table 14 Formulas of pseudolatex and its composition used in order to observe the effect of plasticizer.

Formula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100® /RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#18	-	12.36	-	4.15	2.05	10.26	4.10	67.08
F#31	-	12.36	-	4.15	2.05	10.26	2.10	69.08
F#32	-	12.36	-	4.15	2.05	10.26	6.10	65.08
F#33	-	12.36	-	4.15	2.05	10.26	8.10	63.08
F#22	2	12	-	4	2	10	4	66
F#34	2	12	-	4	2	10	6	64
F#35	2	12	-	4	2	10	8	62

2.7 Effect of Drug Concentration

Effect of various components in topic 2.3-2.6 which had the same drug content (2%w/w) in every formulation was chosen the best composition of formulation in order to continue studying the effect of drug concentration as shown in Table 15. Then, DTZ HCl at 2, 4, 6 and 10 % w/w of formulation, respectively were used in order to evaluate physicochemical properties and skin permeation profiles.

Table 15 Formulas of pseudolatex and its composition used in order to observe the effect of drug concentration.

Formula No.	Components of formulation (% w/w)							
	DTZ HCl	Eudragit RL100® /RS100®		PVP K30	Liquid Paraffin	Tween 80	Dibutyl Phthalate	Water
		RL	RS					
F#22	2	12	-	4	2	10	4	66
F#36	4	12	-	4	2	10	4	64
F#37	6	12	-	4	2	10	4	62
F#38	10	12	-	4	2	10	4	58
F#39	12	12	-	4	2	10	4	56

Table 16 Summary of various effects used in this study and its correspondence formulas.

Effect	Comparative of each effect among formulas.	
	No Drug	Drug
• Effect of polymer ratio RL: RS	F#1-F#5	F#6-F#10
• Effect of % of polymer and channeling agent	F#1, F#11-F#13, F#17-F#20 and F#25-F#26	F#6,F#14-F#16, F#21-F#24
• Effect of surfactant	F#27, F#18, F#28	F#22, F#29, F#30
• Effect of plasticizer	F#31, F#18, F#32, F#33	F#22, F#34, F#35
• Effect of drug conc	-	F#22, F#36-F#39

3. Evaluation of Physicochemical Characteristics of Pseudolatex Formulations

3.1 Identification of Particle Size of Pseudolatex

Particle sizes of pseudolatex in various preformulations were evaluated by scanning electron microscope (SEM). Due to pseudolatex base was a special specimen, low temperature scanning electron microscopy (cryo) method was used. Specimen stages of this method were maintained at temperature as low as -170°C, it was possible to observe specimens in the scanning electron microscope in a frozen-hydrated state. Frozen hydrate specimens displayed little evidence of preparation damage. All of the cryo process was done under liquid nitrogen condition in order to keep a low temperature. For this reason above, special equipment was necessary, which it consisted of a transfer device, an airlock loading system on the SEM chamber and a cold stage. Moreover, a cold stage for JSM-2 SEM in which a knife was mounted in an antechamber so that specimens may be fractured while the specimen was scanned by SEM, preparing specimens on stub were done by dropping pseudolatex base in the hole of stub and dipped into liquid nitrogen until freezing. After that transferred stub with specimen immediately in the microscope chamber.

3.2 Physical Stabilities of Pseudolatex Formulations

3.2.1 Evaluation of Sedimentation

Freshly prepared pseudolatex formulas were filled in 15 ml of vial, and then settled for one month to observe sedimentation of pseudolatex among various formulations.

3.2.2 Measurement of viscosity

Pseudolatex formulations were measured using Brookfield digital cone & plate viscometer.

3.2.3 Measurement of pH Value

Pseudolatex formulations were measured pH by using Orion pH meter model 420 A. Orion pH meter had special probe, which can direct measure pseudolatex base without any dilution.

3.3 Evaluation of Size Distribution

Particle size distributions of various formulations were determined by photon correlation spectrophotoscopy (PCS). The 1-2 drops of samples were diluted with filtered water, and filled in rectangular vessel volume of 10 ml before they were measured. This method was reported as mean particle size by volume, number, and intensity.

4. Analytical Development, In Vitro Release and Permeation of DTZ HCl pseudolatex formulations

4.1 Calibration curve of DTZ HCl

Calibration curve of DTZ HCl in various media were done to determine an amount of drug dissolved during determination of drug content and dissolution testing. These curves are presented in the Appendix A

For determining drug content, about 22 mg of DTZ HCl was weighed accurately and transferred to 100 ml volumetric flask, then adjusted to volume with mixed solvent between methylene chloride and methanol at the ratio 1:1 volume by volume as stock solution. Stock solution (concentration about 220 μg/ml) was diluted to 2.2, 4.4, 8.8, 11, 13.2 and 15.4 μg/ml with mixed solvent, respectively. The absorbance of dilute solutions was determined at a wavelength of 241 nm by using ultraviolet spectrophotometer. In the case of dissolution medium, preparing of calibration curve was to weigh about 50 mg DTZ HCl and transferred to 500-ml volumetric flask. After that this flask was adjusted to volume with phosphate buffer pH 7.4 as a stock solution (concentration about 100 μg/ml). Stock solution was diluted to 4, 6, 10, 12, 14, 16 and 18 μg/ml with dissolution medium, respectively. Although preparation of calibration curves in mixed solvent and dissolution medium, were similar but the wavelength of absorbances were different. For phosphate medium absorbance at 236 nm was used.

4.2 DTZ HCl Content in Pseudolatex base

Determination of DTZ HCl content, weighed accurately about 0.5 g of pseudolatex equivalent to about 10 mg of DTZ HCl which drug contained 2% of formulation, transferred to a 25 ml volumetric flask. Added approximately 15 ml of mixed solvent (methylene chloride and methanol) and shook until completely soluble, then adjusted to volume with mixed solvent. Dilution of mixture was 1:50 to suitable concentration for UV determination. If drug contained of formulation were changed such as 4%, 6% and 10%, pseudolatex base would be weighed and diluted by calculation of suitable concentration for UV determination. Moreover, blanks of all formulations were made from pseudolatex base without drug formulation that had the same ratio of ingredients.

4.3 The Release of DTZ HCl from Pseudolatex

4.3.1 Preparation of Dissolution Medium

Dissolution medium was 0.067 M phosphate buffer (pH 7.4) for the semisolid formulations (Yamaguchi et al.,1996). Then a 10 liters of medium was prepared from 91.18 g of potassium dihydrogen orthophosphate and 20.5 g of sodium hydroxide, added water to adjust volume which this medium was pH 7.4 by itself.

4.3.2 Pretreatment of Membrane

Porous membrane ethylene vinyl acetate 9% was a membrane which used in dissolution test, to be supporting layer for release

study of DTZ HCl from pseudolatex base. Prior to the experiment, the membranes were cut into pieces ($\pi(3.5)^2$ cm^2 each) and then soaked in the dissolution medium for 24 hours before used.

4.3.3 Release Study of DTZ HCl Pseudolatex

JPXII Apparatus 2 was employed for the release study. In this study disk assembly method (DA): a DA diffusion cell (Bottari et al.) was modified to apply for experiment (Fig. 12). A cell had a semisolid formulation loading part with 1 cm depth and 6 cm in diameter, and cover part which membrane was between in low parts of this cell. Two parts were hold together with screws. Effective release rate was $\pi(3)^2$ cm^2. About 8-9 g of pseudolatex formulation was filled in a disk and put into vessel that contained 500 ml of dissolution medium. Each formulation was compared with its blank.

Paddle rotating speed was set at 100 rpm and dissolution medium was equilibrated to 37±0.5°C through a duration time of study. A ten ml of sample was withdrawn at predetermined time interval of 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10 and 12 hours, respectively. The same amount of fresh medium was added to keep the volume constant throughout the experiment. The samples were diluted in suitable concentration in order to determined by an ultraviolet detector at 236 nm. The release amount of DTZ HCl at any time interval was calculated from a calibration curve. A cumulative amount of drug release as a function of time was determined.

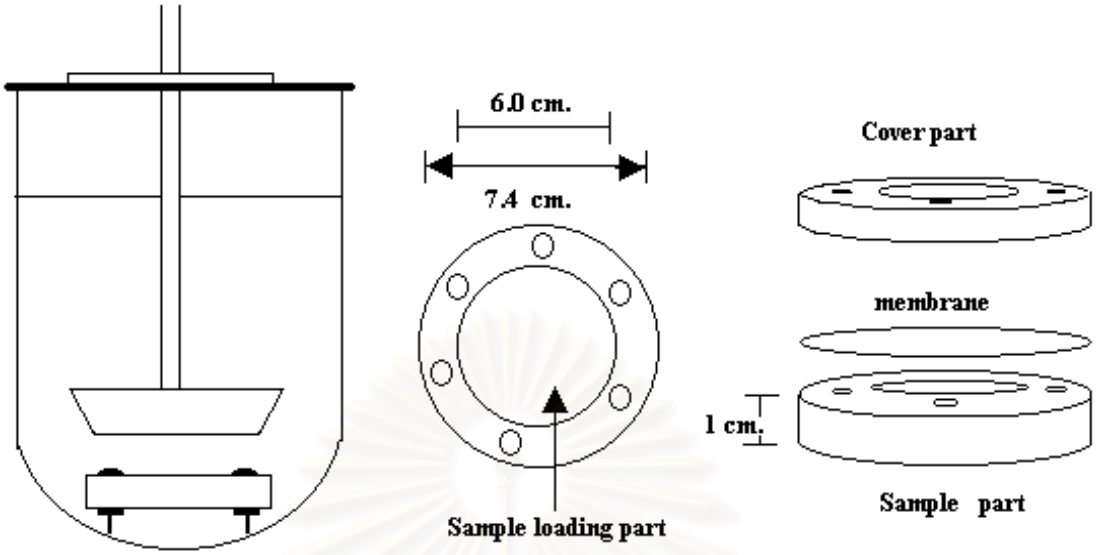


Figure 12 Schematic illustration of disk assembly method (JPXII).

4.4 The In-Vitro Skin Permeation of DTZ HCl for Pseudolatex

4.4.1 Pretreatment of Shed Snake Skin

Shed snake skin specimens from *Elaphe obsolata* were selected as the representative of the stratum corneum, the major barrier to percutaneous drug absorption. They were kept in the freezer. Prior to the experiment, they were thawed at room temperature and the dorsal part of the specimens were cut into pieces (6×6 cm^2 each) and then soaked in the dissolution medium for 24 hours before used.

4.4.2 Permeation Study of DTZ HCl Pseudolatex

Although the method and condition were in the same way of release study of pseudolatex, shed snake skin specimens were used instead of ethylene vinyl acetate 9% porous membrane in this experiment.

5. Selection of DTZ HCl Pseudolatex Formulations

Procedures in the topics 3 and 4 were used to evaluate DTZ HCl pseudolatex formulations in order to prepare DTZ HCl TDDS formulations.

6. Evaluation of DTZ HCl TDDS Formulations

6.1 Preparation of Transdermal Patch

The transdermal patch used in this study was prepared using impermeable backing membrane of heat sealable tan polyester film laminate. The adhesive layer of the system was hypoallergenic acrylate pressure sensitive transfer adhesive. Preparation of transdermal patch was cut backing membrane which had adhesive layer together with into 13×13 cm pieces and put on a glass plate as basement for casting pseudolatex matrix. A rectangular stainless steel (12.5×12.5 cm, 3 mm height) was then placed on the glass plate to limit boundary of pseudolatex matrix. Before pseudolatex preparation was poured, the glass plate with membrane and rectangular stainless steel had been weighed. When pseudolatex was spread across the plate surface within

the area bound by rectangular steel, excessive amount was got rid of plate and weighed again.

About 14 g of pseudolatex was allowed to evaporate overnight in a humidity controlled room at 30 °C and 40% relative humidity (RH), with losing water content about 15%. Transdermal patch in this study was prepared as matrix system-like type. The system delivered across pseudolatex base to the skin. The system had a contact surface area of 11.34 cm^2 and have a total DTZ HCl content of 101.61 mg (8.96 mg/ cm^2). The drug reservoir pseudolatex casting were prepared modified from the film preparations method developed by Balasubramanian V. Iyer and Ravindra C.Vasavada (1997).

6.2 Design of Improved Diffusion Cell

An in-vitro release and permeation study were carried out using a diffusion cell (Fig. 13) modified from Franz diffusion cell (Chien and Valia, 1984), Keshary Chien diffusion cell and Patch cell (Mueller, Roberts and Scott, 1990). This diffusion cell consisted of two compartments, the donor compartment in the upper and the receptor compartment in the lower. The capacity of the receptor compartment was 60 ml and the cross sectional area of the donor compartment which was corresponded to the effective permeation area of 12.5 cm^2 . In the meantime, the water jacket compartment was extended to envelope a greater surface area of the receptor compartment than the Franz diffusion cell to provide a better temperature control and equilibrium release of drug.

Figure 13 Diffusion cell modified from Franz's cell diffusion.

6.1 In vitro Drug Release of DTZ HCl Patch

In vitro drug release study, a modified diffusion cell (as described in 6.2) was used. The transdermal patch was placed on porous membrane ethylene vinyl acetate 9% which clamped between the donor and the receptor compartment, with the drug releasing surface facing to the receptor compartment. The receptor compartment contained pH 7.4 phosphate buffer solution and it was maintained at a temperature of 37±1°C by a circulating water bath. A predetermined optimal stirring rate using a magnetic stirrer at 90 rpm was controlled. The donor

compartment was exposed to ambient temperature (30±1°C). A portion (10 ml each) of solutions was withdrawn from the receptor compartment at predetermined time interval of 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10 and 12 hours. The freshly prepared buffer solution was replaced with an equal volume. The DTZ HCl concentrations in these samples were determined by UV method.

6.4 In Vitro Skin Permeation of DTZ HCl Patch

In vitro skin permeation was used apparatus, method and condition as the same as release of DTZ HCl patch but the transdermal patch was placed on shed snake skin which had pretreatment already. The DTZ HCl concentration in the samples were determined by UV method and calculated from a calibration curve.

7. Analysis Data with Statistics

Statistics, which was used to compare and analyze the data more than 3 groups, was F-test by Analysis of Variance (ANOVA). For two groups of data, the independent t-test or paired t- test was used depending on nature form of data.

CHAPTER III

RESULTS

The results of the studies will be summarized in the following order:

1. **Analytical quantitation of DTZ HCl**
   1.1 Spectrophotometric analysis of DTZ HCl
   1.2 DTZ HCl content in various formulations of pseudolatex

2. **Preformulation of pseudolatex as a drug reservoir for DTZ HCl TDDS**
   2.1 Determination of water removal time from free drug of pseudolatex and DTZ HCl pseudolatex.
   2.2 Effect of various components on the physicochemical characteristics
   2.3 Effect of various components on the release profile
   2.4 Effect of drug concentrations on the skin permeation profile

3. **Evaluation of DTZ HCl TDDS formulation**
   3.1 In vitro drug release of DTZ HCl patch
   3.2 In vitro skin permeation of DTZ HCl patch

1. Analytical Quantitation of DTZ HCl

1.1 Spectrophotometric Analysis of DTZ HCl

The UV scanning for maximum absorption wavelength of DTZ HCl was detected at the wavelength of 236 nm for phosphate medium pH 7.4 as shown in Fig.14 while analysis of DTZ HCl content in pseudolatex base that used mixed solvent (methylene chloride and methanol at the ratio 1:1 v/v), the UV scanning for maximum absorption wavelength was determined at 241 nm as shown in Fig.15.

The calibration curve of DTZ HCl was plotted between the concentration of drug as a function of absorbance. Appendix A shows a concentration of DTZ HCl in phosphate buffer solution and mixed solvent versus their absorbances. A typical calibration plot showed a linear relationship between the absorbance and DTZ HCl concentration. The calibration curves of DTZ HCl after regression analysis are illustrated in Appendix A.

1.2 DTZ HCl Content in Various Formulations of Pseudolatex

Determination of DTZ HCl content was calculated from equation, including example of calculation method that showed in Appendix B.. The results of percent content in various formulations are shown in Table 17

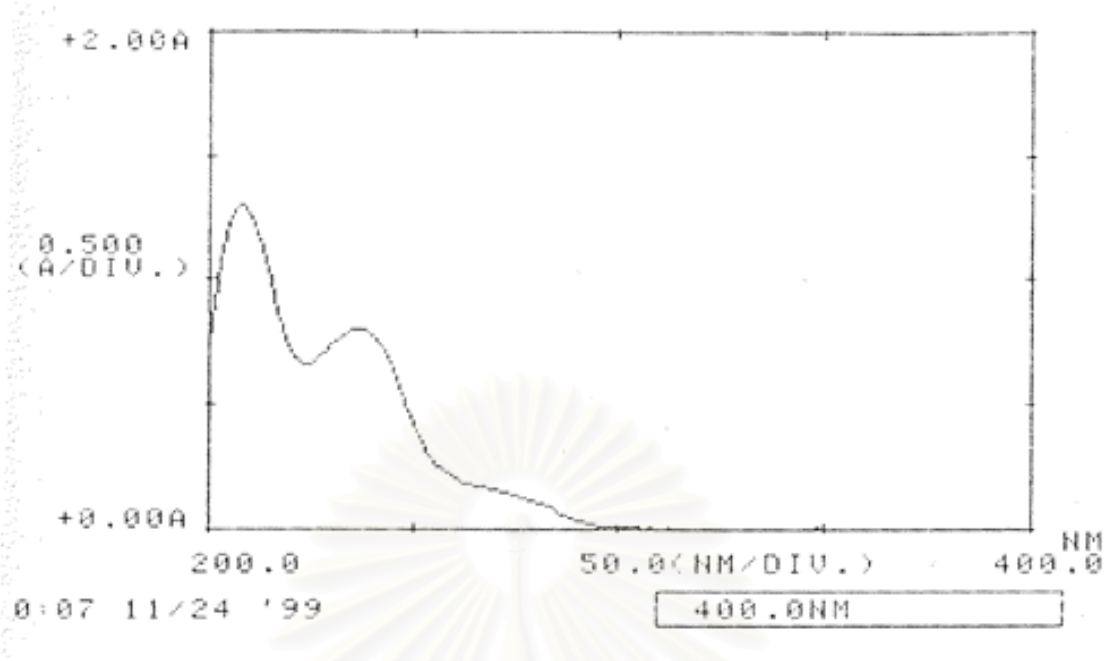


Figure 14 UV scanning curve of DTZ HCl showing maximum absorbance at 236 nm for phosphate medium pH 7.4.

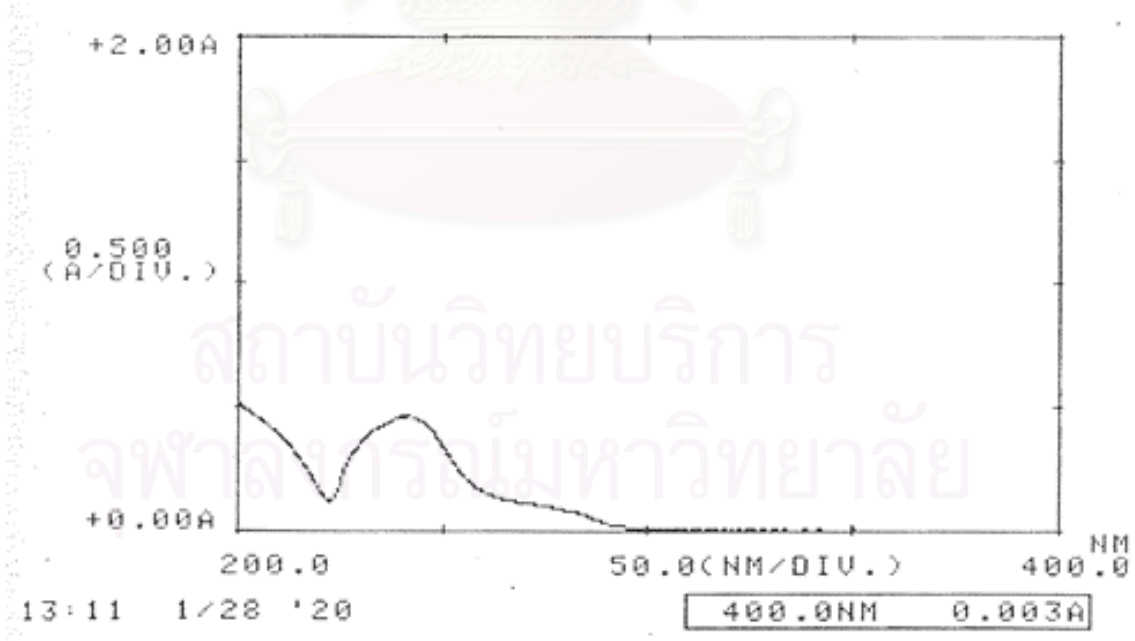


Figure 15 UV scanning curve of DTZ HCl showing maximum absorbance at 241 nm for mixed solvent (methylene chloride and methanol at the ratio 1:1 v/v).

Table 17 Percent of DTZ HCl content in various pseudolatex formulations*

Formula	Total weight of pseudolatex (g)	% content ± SD (n=3)
F#6(2%)	81.98	98.25±0.15
F#7(2%)	81.38	95.62±0.38
F#8(2%)	78.22	94.56±0.09
F#9(2%)	78.42	94.32±0.48
F#10(2%)	74.72	91.14±0.20
F#14(2%)	82.02	86.98±0.68
F#15(2%)	81.25	91.73±0.46
F#16(2%)	81.42	95.16±0.39
F#21(2%)	80.36	96.77±0.21
F#22(2%)	81.73	97.78±0.18
F#23(2%)	80.78	92.19±0.17
F#24(2%)	80.47	88.68±0.64
F#29(2%)	81.00	95.44±0.28
F#30(2%)	80.45	93.10±0.02
F#34(2%)	83.08	89.28±0.51
F#35(2%)	80.20	95.93±0.56
F#36(4%)	81.40	98.22±0.54
F#37(6%)	79.60	95.86±0.43
F#38(10%)	78.87	97.85±0.23

* Preparation's technique is the same in all formulations.

All of formulations had values of drug content in the range of 86.98-98.25% w/w. It should be indicated that in some formula DTZ HCl might lost during pseudolatex preparing at the most of approximately 11-13% w/w of initial drug amount (F#14, F#24, and

F#34). From these results, its might be assumed that pseudolatex formulations entrapped DTZ HCl very well.

2. Preformulation of Pseudolatex as a Drug Reservoir DTZ HCl TDDS

2.1 Determination of Water Removal Time With and Without DTZ HCl in Pseudolatex

In this part of study a suitable duration time for water removed from formulations was evaluated between formulas containing drug and formulas with no drug in pseudolatex preparations. In addition, physicochemical characteristics of drug, which were effected by water removal time were also determined. For pseudolatex with no drug in formulations, the water removal times at 1, 2, 4 and 6 hours were identified by sedimentation volume for one month (see Figs.16-20). A result showed that a suitable time to form pseudolatex was 6 hours, this may be indicated by no sedimentation of droplets occurred in these products. For pseudolatex formulations containing drug, the water removal time at 4, 6 and 7 hours were identified as same as mentioned above (see Fig. 21). Although a result showed no sedimentation volume occurred in the products at 6 and 7 hours, respectively. However a suitable time of 6 hours of preparation was selected because it took shorter time and gave the same physicochemical properties as compare with preparing at 7 hours. Then a water removal time of DTZ HCl pseudolatex in every formulation was 6 hours.

2.2 Effects of Various Components on the Physicochemical Characteristics

A shape and appearance of pseudolatex particle could identify rapidly by high power of magnified microscope. For pseudolatex system without DTZ HCl, at water removal time of less than 6 hours droplets of pseudolatex could not obtain. However, pseudolatex's droplets occurred at water removal time for 6 hours as may be seen in Fig.22. In addition, SEM technique was also used to justify formulations which were suitable for preparing TDDS. Furthermore, another physicochemical properties such as pH value, viscosity, and particle size distribution are summarized in Table 18.

The effect of polymer type, polymer concentration, channeling agent, surfactant and plasticizer on organoleptic properties of pseudolatex's particles as evaluated by SEM are illustrated in Figs. 23-28. In the case of effect of polymer, SEM pictures revealed that Eudragit RL100®: RS100® = 100: 0 was a best ratio, which gave a good appearance and shape of particles, for example particles had round shape with smooth surface than other ratios, and pseudolatex obtained from this polymer can include or probably encapsulate DTZ HCl in the droplets. Moreover, a high rate of release including easily preparing of this formulation were also considered. For effect of polymer concentration and channeling agent (PVP K30). Type of polymer was fixed, and polymer's concentrations were varied in accordance with channeling agent's concentrations as given in Table 12 (F#6, F#14-16, F#21-24). The results indicated that at all concentrations of Eudragit RL100 used, PVP K30 at 4% w/w gave more pseudolatex's particles than at 6% w/w. At 4% w/w of PVP K30 used, and at 12, 14 and 16%

w/w of Eudragit RL100 in this study, no sedimentation had been observed after one month of study. In the case of Eudragit RL 100 at 10% w/w, it was found that particles tended to fuse together as easily observed from photomicrograph (Fig 25-A). At 6% w/w of PVP K30 and at various polymer's concentrations as indicated above, photomicrograph (Fig 26-A) showed the same result as 4% w/w of PVP K30. Moreover an effect of surfactant as illustrated in Fig 27 revealed that concentration of surfactant at 10% w/w was suitable to form pseudolatex's particles with good size distribution and more quantity of particles than other concentrations (6 and 14% w/w of Tween 80). For effect of plasticizer, photomicrographs (Fig 28) showed a good appearance of particles included more particles at the concentration of dibutyl phthalate as 4% w/w while as another concentrations (6 and 8% w/w), particles distribution was poor and not regular. Then RL100 12%, PVP K30 4%, Tween 80 10% and Plasticizer (Dibutyl phthalate) 4%w/w of formulation probably seemed to have a good appearance and easy to prepare formulation.

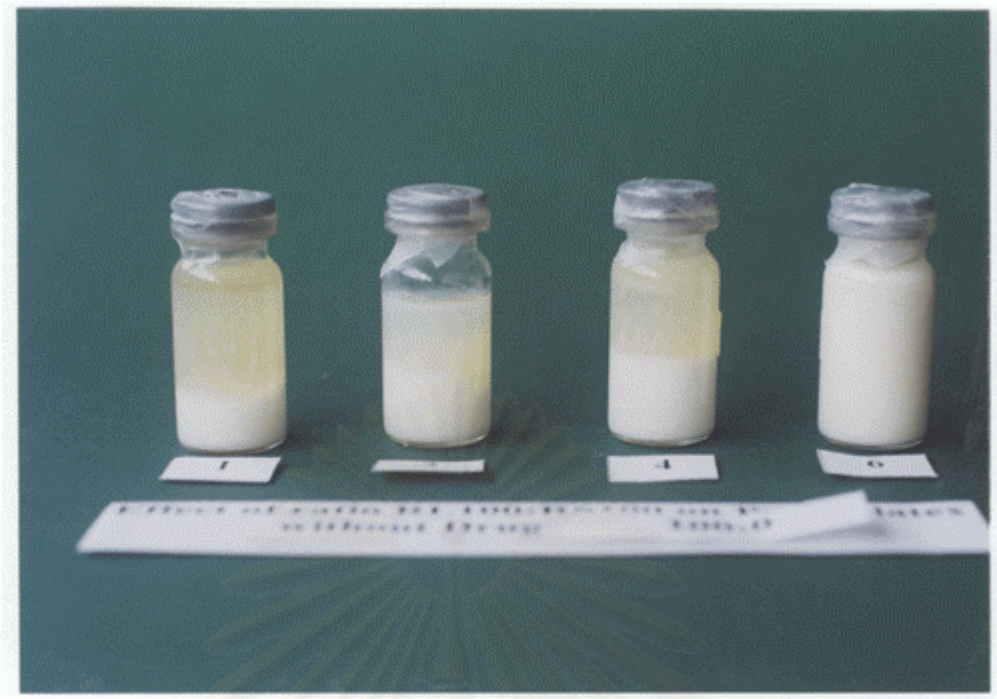


Figure 16 Effect of ratio RL100 : RS100 = 100 : 0 on water removal time of pseudolatex without drug at 1, 2, 4, and 6 hours, respectively (settle for one month).

Figure 17 Effect of ratio RL100 : RS100 = 80 : 20 on water removal time of pseudolatex without drug at 1, 2, 4, and 6 hours, respectively (settle for one month).

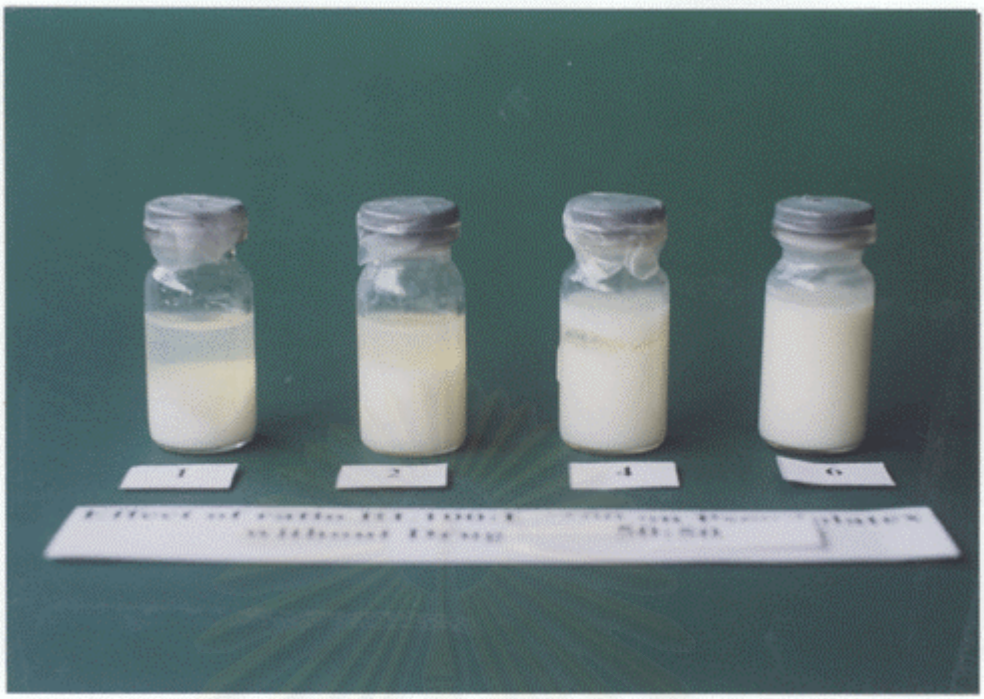


Figure 18 Effect of ratio RL100 : RS100 = 50 : 50 on water removal time of pseudolatex without drug at 1, 2, 4, and 6 hours, respectively (settle for one month).

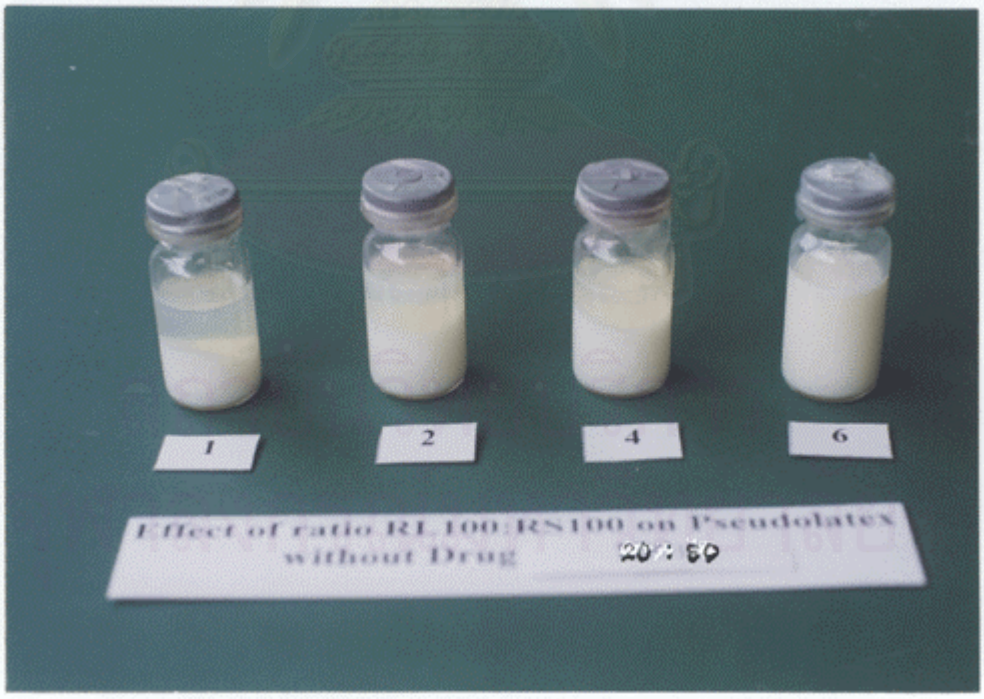


Figure 19 Effect of ratio RL100 : RS100 = 20 : 80 on water removal time of pseudolatex without drug at 1, 2, 4, and 6 hours, respectively (settle for one month).

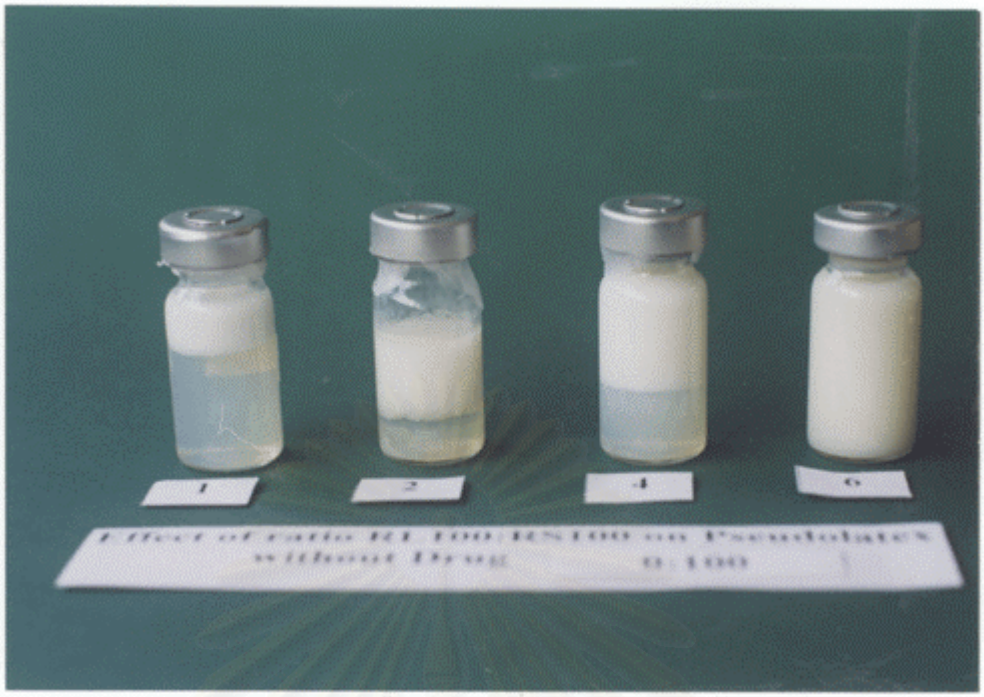


Figure 20 Effect of ratio RL100 : RS100 = 0 : 100 on water removal time of pseudolatex without drug at 1, 2, 4, and 6 hours, respectively (settle for one month).

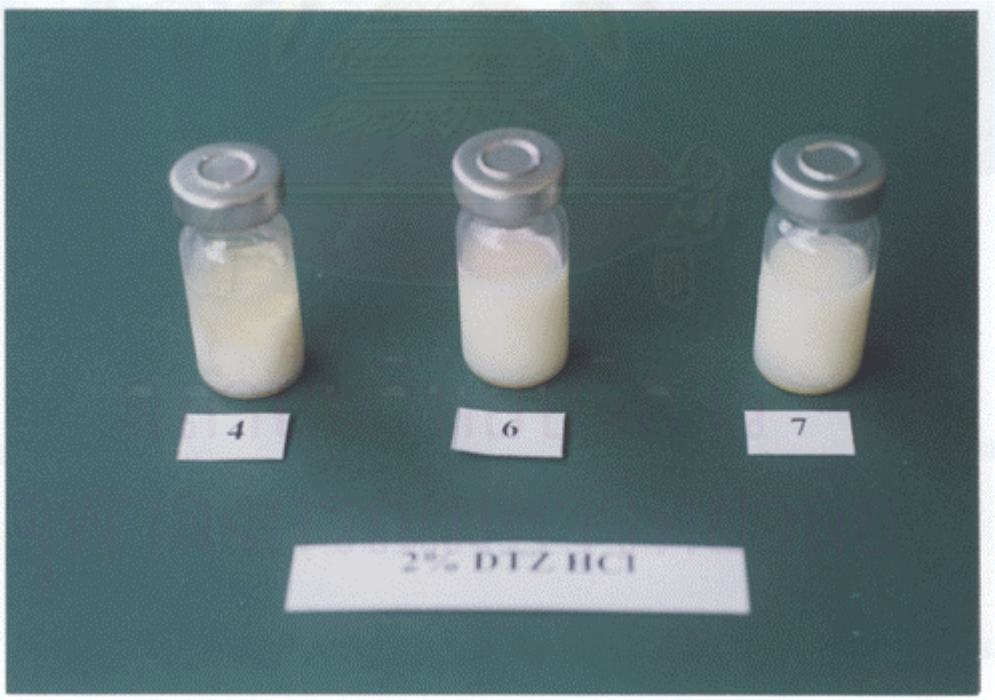


Figure 21 Effect of DTZ HCl with 2% w/w of formulation on water removal time of pseudolatex at 4, 6, and 7 hours, respectively (settle for one month).

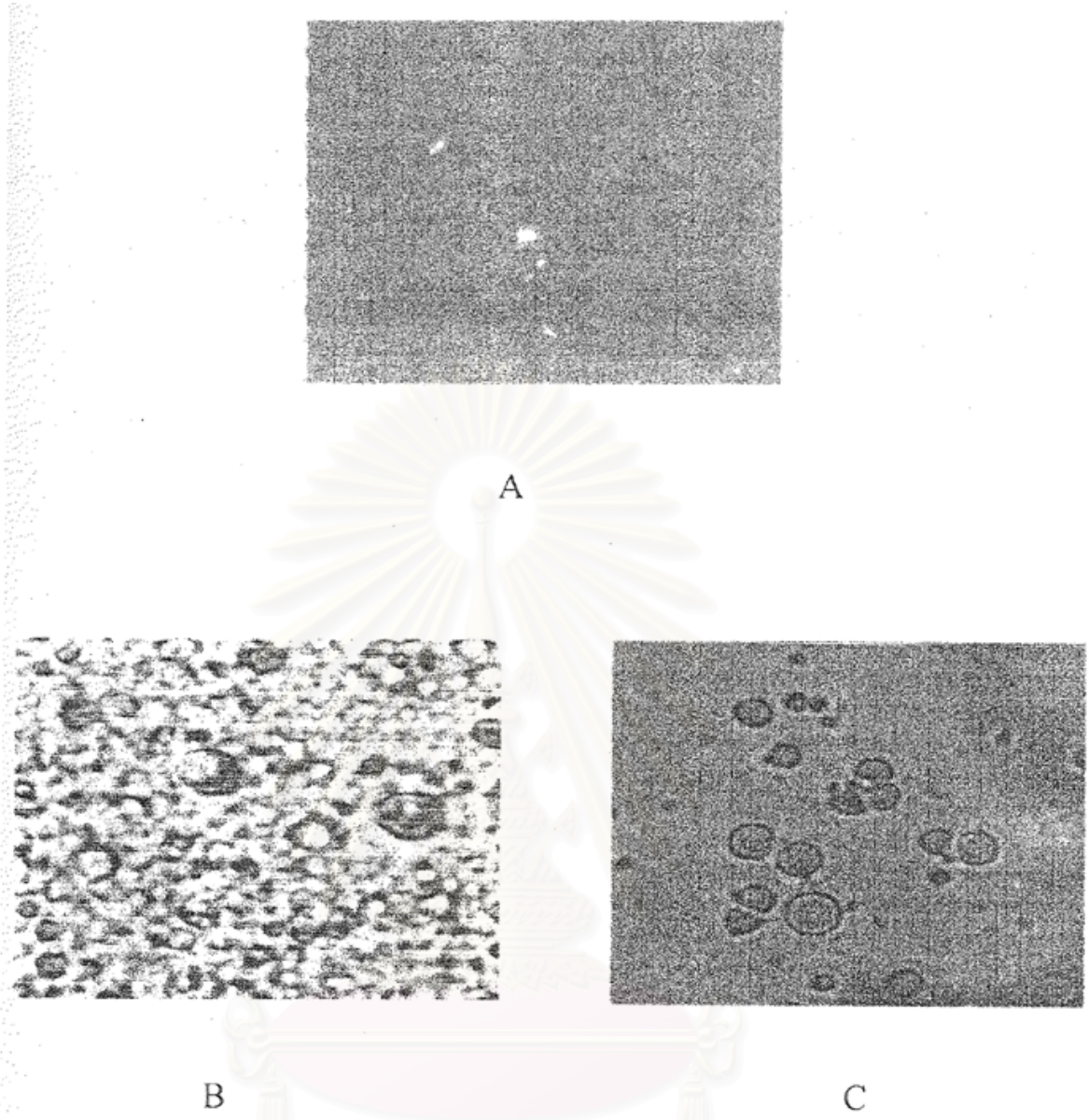


Figure 22 Photomicrographs of core formulation of pseudolatex without DTZ HCl (x1500 magnification), which A = particles of Pseudolatex system can not occur at less than 6 hrs of Preparation, B = particles of pseudolatex at 6 hrs of preparation, And C = the same as B but dilute with filtered water Approximately 2 times.

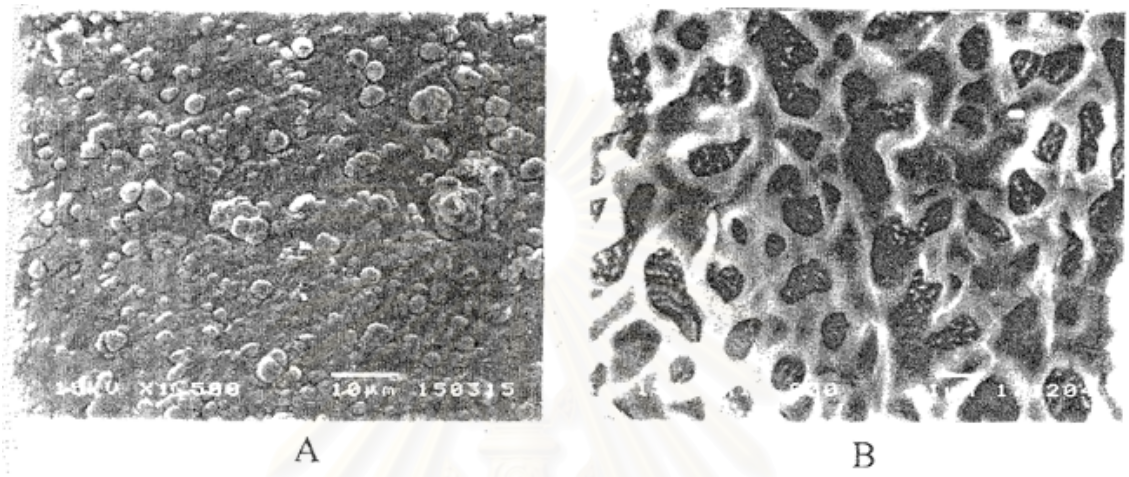


Figure 23 Photomicrographs from freeze fracture technique of core formulation of pseudolatex with DTZ HCl 2% w/w after one month of storage which A = overview of pseudolatex's particles (×1500), B = pseudolatex's particles not occurred (×10000).

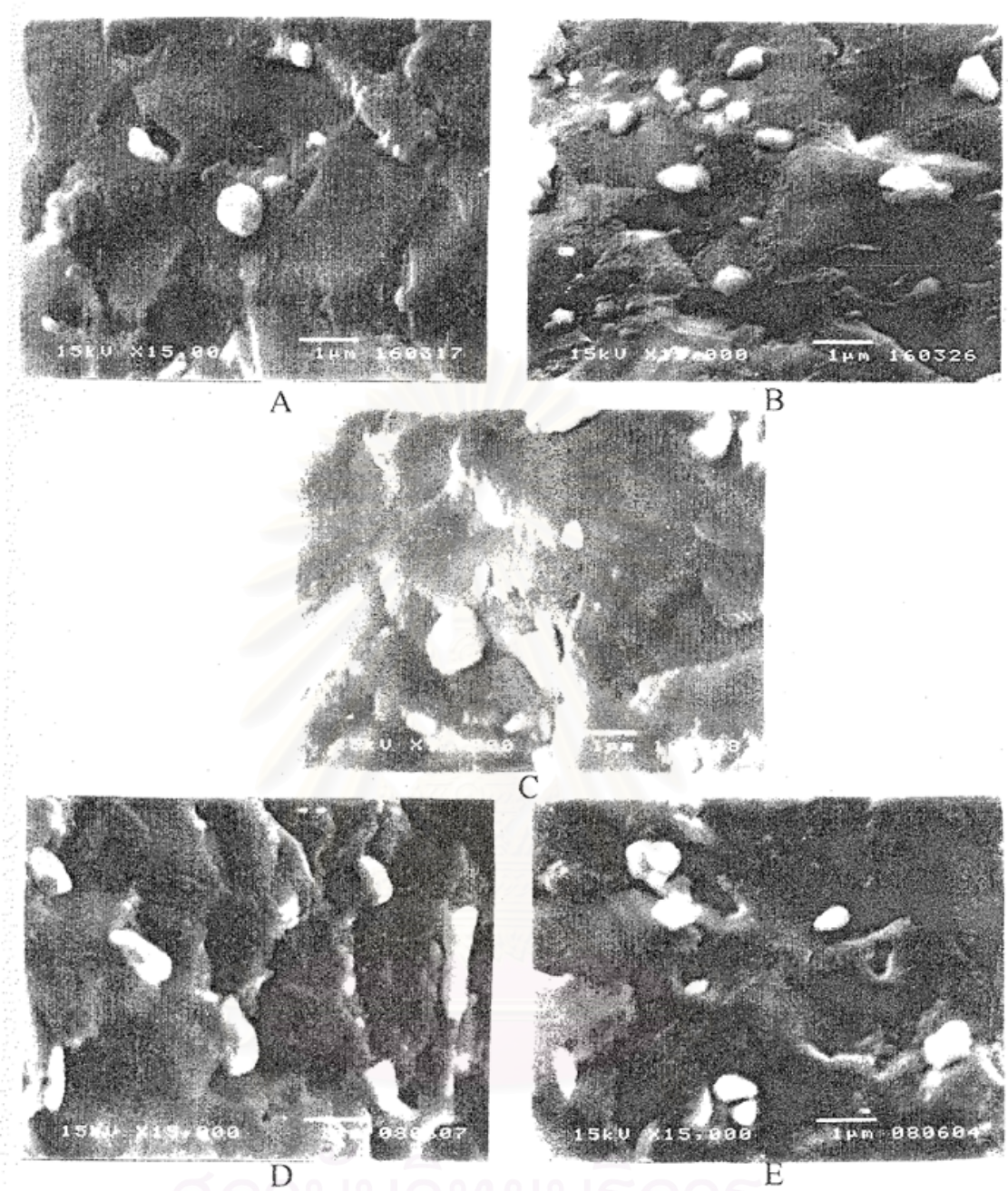


Figure 24 Photomicrographs from freeze fracture technique of pseudolatex formulations with DTZ HCl containing various ratios of polymers between RL100: RS100 in order to observe effect of polymer on particles appearance an size of pseudolatex (× 15000), after one month of storage, A-E are formulas containing 2% w/w of DTZ HCl with polymer ratios as 100:0, 80:20, 50:50, 20:80 and 0:100, respectively.

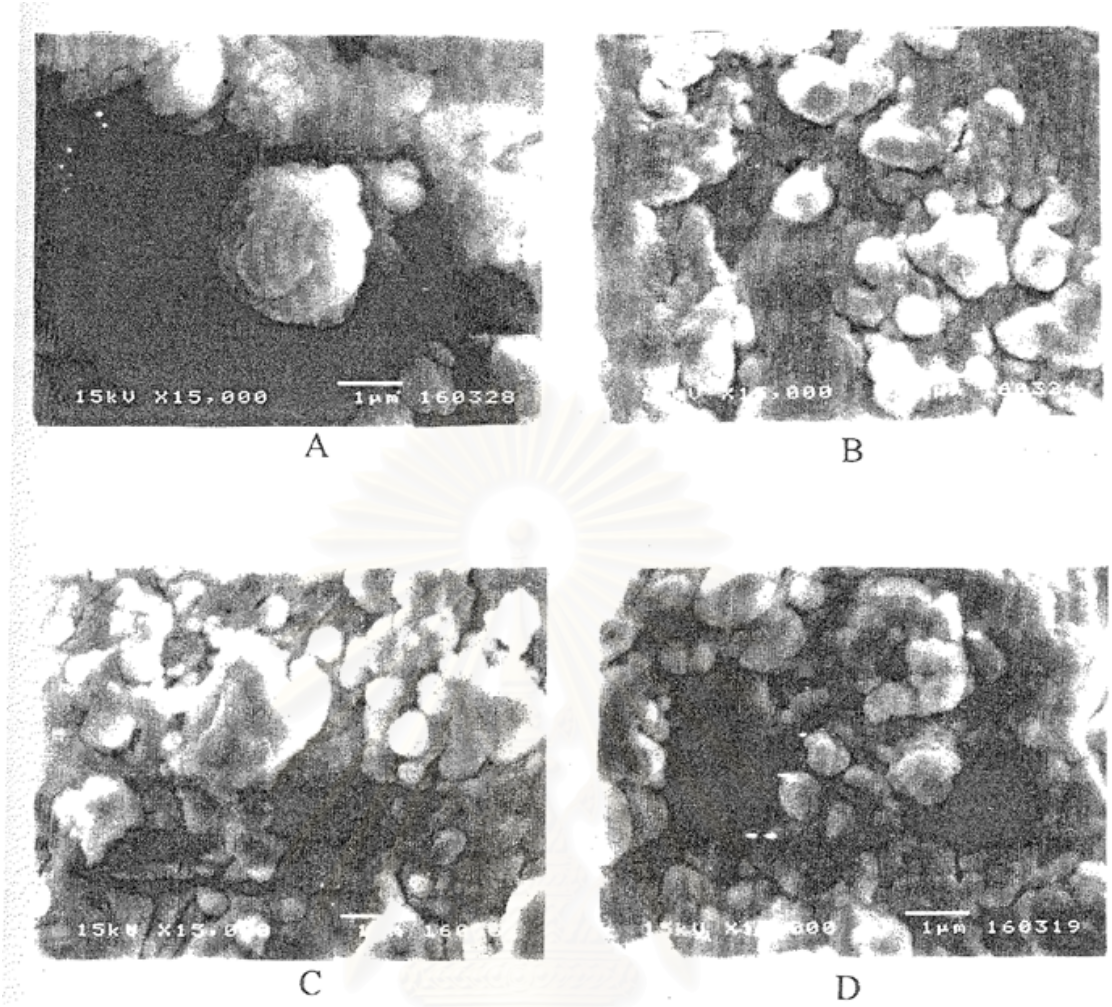


Figure 25 Photomicrographs from freeze fracture technique of pseudolatex formulations (×15000) after one month of storage, containing 2% w/w DTZ HCl, Tween 80 10% w/w, dibutyl phthalate 4% w/w, Eudragit RL100 at 10, 12, 14, and 16% w/w, respectively and 4% w/w of PVP K30 (A-D) in order to observe effect of polymer concentration and channeling agent on particle appearance.

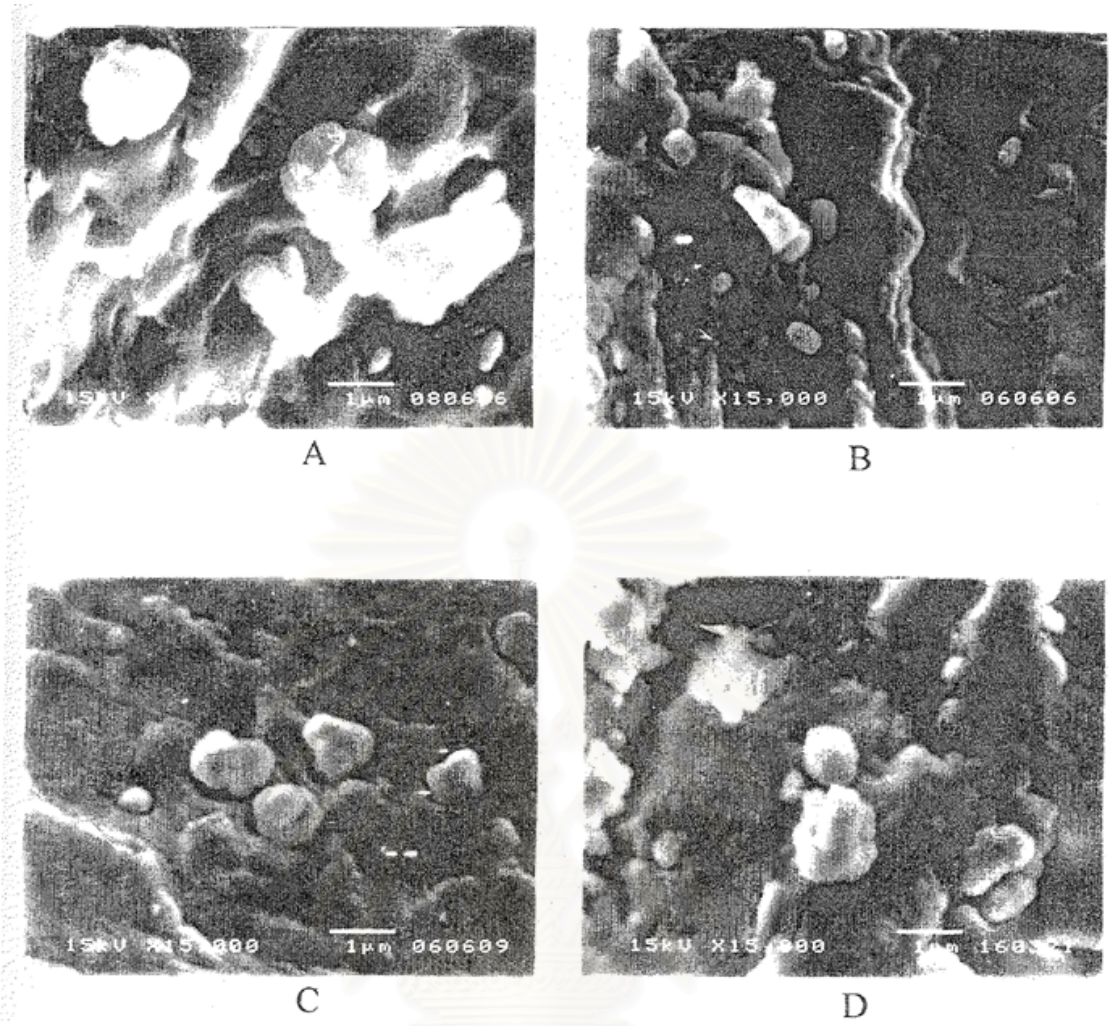


Figure 26 Photomicrographs from freeze fracture technique of pseudolatex formulations (×15000) after one month of storage, containing 2% w/w DTZ HCl, Tween 80 10% w/w, dibutyl phthalate 4% w/w, Eudragit RL100 at 10, 12, 14, and 16% w/w, respectively and 6% w/w of PVP K30 (A-D) in order to observe effect of polymer concentration and channeling agent on particle appearance.

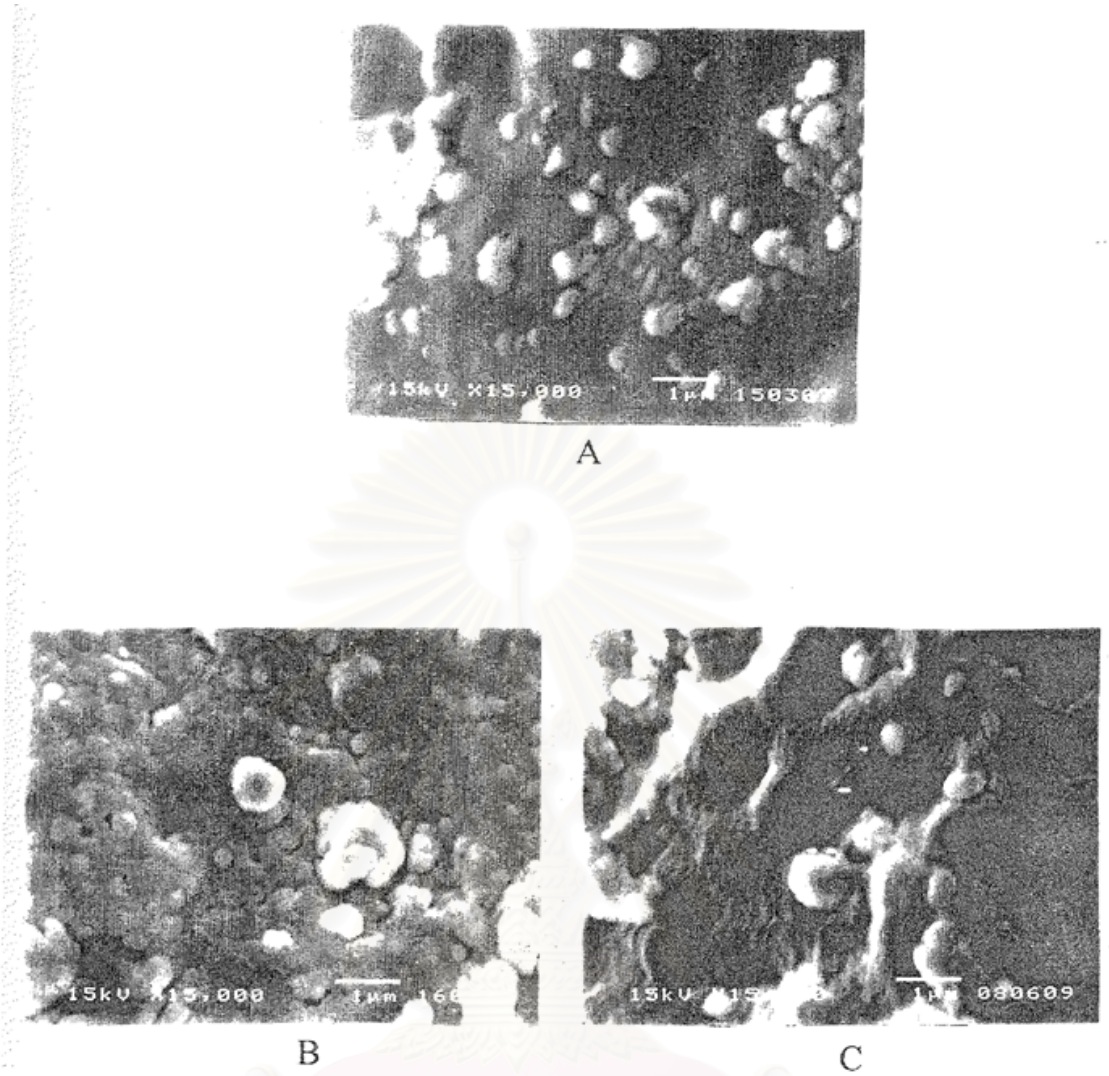


Figure 27 Photomicrographs from freeze fracture technique of pseudolatex formulations (×15000) after one month of storage, containing 2% w/w DTZ HCl, Eudragit RL100 12% w/w, PVP K30 4% w/w, dibutyl phthalate 4% w/w and Tween 80 at 6, 10, and 14% w/w, respectively (A-C) in order to observe effect of surfactant concentration on particle appearance.

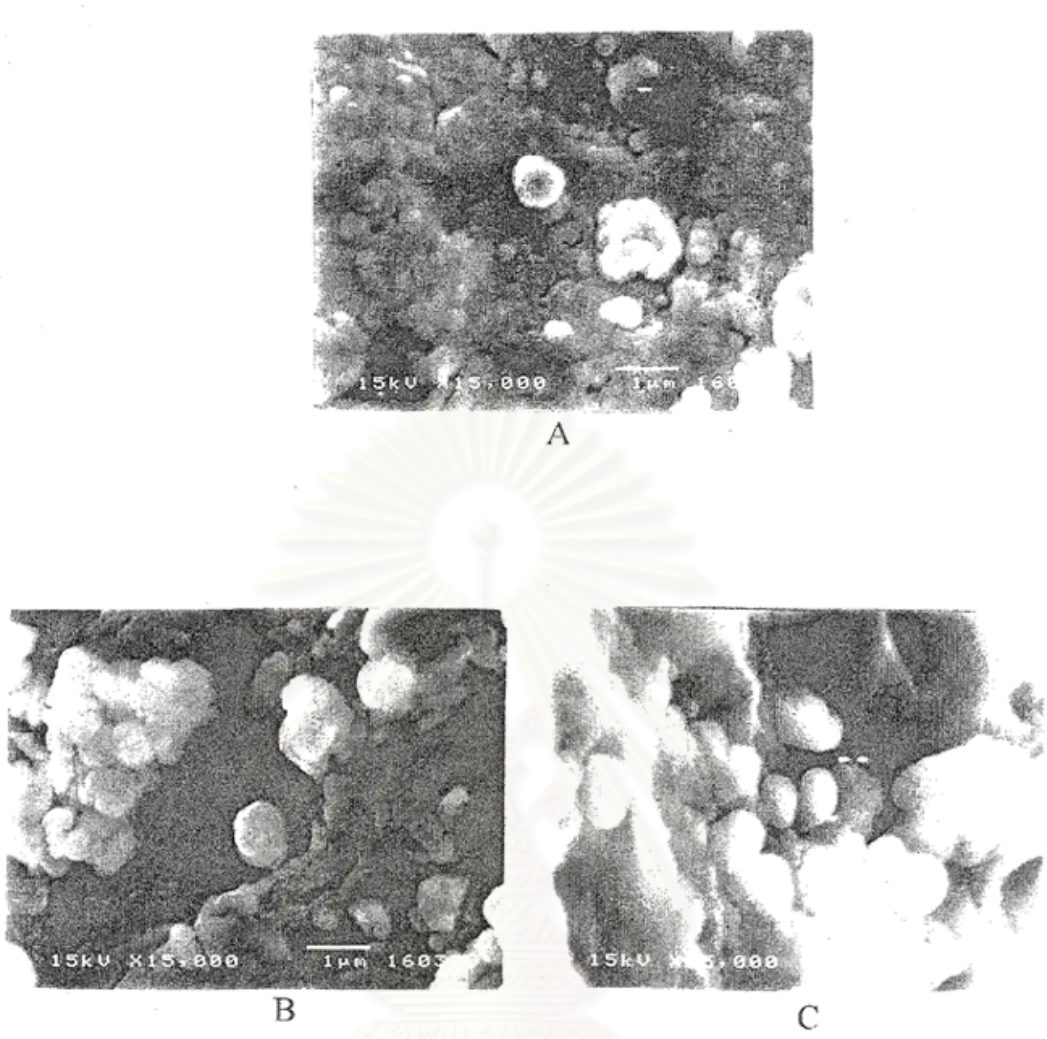


Figure 28 Photomicrographs from freeze fracture technique of pseudolatex formulations (×15000) after one month of storage, containing 2% w/w DTZ HCl, Eudragit RL100 12% w/w, PVP K30 4% w/w, Tween 80 10% w/w and dibutyl phthalate at 4, 6, and 8% w/w, respectively (A-C) in order to observe effect of plasticizer concentration on particle appearance.

Table 18 Physicochemical properties of various formulations of DTZ HCl pseudolatex in this study*.

Formula	pH	viscosity cps	Particle size (nm) distributed by		
			intensity	volume	number
F#1	4.18	497.66	414.6	441.3	432.3
F#2	4.11	595.97	560.5	546.7	539.5
F#3	4.07	614.40	537.6	497.1	479.0
F#4	4.00	602.11	535.8	507.7	500.7
F#5	3.92	497.80	497.8	470.2	466.4
F#6	4.00	485.38	446.3	443.6	436.3
F#7	3.91	552.96	632.4	614.0	608.9
F#8	3.89	602.11	705.2	735.0	725.2
F#9	3.85	568.93	621.3	628.9	656.1
F#10	3.80	565.25	580.9	572.8	564.2
F#11	3.90	430.08	268.7	269.0	269.0
F#12	4.10	473.09	294.1	294.5	294.5
F#13	4.20	715.16	504.1	478.4	468.7
F#14	3.79	411.65	307.3	308.8	308.8
F#15	3.90	448.51	394.3	395.1	395.1
F#16	3.80	675.84	514.7	482.5	471.2
F#17	4.20	399.36	230.1	230.5	230.5
F#18	4.10	421.48	290.2	291.6	291.6
F#19	4.00	485.38	301.8	305.3	292.0
F#20	4.30	602.11	322.1	322.5	322.5
F#21	3.80	363.72	250.4	250.7	250.7
F#22	3.90	405.50	295.0	295.1	295.1

Table 18 (Continued)

Formula	pH	viscosity cps	Particle size (nm) distributed by		
			intensity	volume	number
F#23	3.93	430.08	318.3	318.7	318.7
F#24	3.80	589.82	414.6	441.3	432.3
F#25**	-	-	-	-	-
F#26**	-	-	-	-	-
F#27	4.00	417.79	233.5	233.8	233.8
F#28	4.30	423.94	301.3	301.7	301.7
F#29	3.90	407.35	336.2	337.0	337.0
F#30	4.10	419.02	356.3	356.7	356.7
F#31**	-	-	-	-	-
F#32	4.05	421.48	312.2	313.0	313.0
F#33	4.00	423.94	387.2	388.2	388.2
F#34	3.95	411.65	347.5	360.8	351.6
F#35	3.90	407.35	402.2	402.6	402.4
F#36	3.79	737.28	324.2	325.1	325.1
F#37	3.76	884.74	554.7	558.7	558.7
F#38	3.69	1044.48	578.2	579.0	587.4
F#39**	-	-	-	-	-

* DTZ HCl is stable in pH value as indicated above.

** these formulas do not occurred pseudolatex system

The results from Table 18, In the formulation number 25, 26, 31 and 39 remarked with "**" indicated that there was no pseudolatex system formed in these formulations. When took consideration for F#25 and F#26, which had PVP K30 in high level (8% and 10% respectively), it was found that these two formulas were high viscosity, and not possible to prepare. For F#39 that a quantity of drug was

effected by increasing viscosity of pseudolatex formulation. For F#31, due to percent of plasticizer (i.e. 2% w/w) was not suitable and correspondence to percent of polymer used in the formula, in this case pseudolatex system could not form.

Therefore, physicochemical characteristics in Table 18 were further clarified by independent sample statistic t test with 95% confident level to determine effect of PVP K30 and drug on pH value, viscosity and particle size of pseudolatex formulations as shown in Table 19. Together with Fig.29 shows comparative chart of particle size distribution by intensity, volume and number, including size difference of pseudolatex formulations, when used size distribution by volume in order to determine effect of PVP K30 and drug components on particle size of pseudolatex

Table 19 Comparative mean value of physicochemical properties of pseudolatex formulations.

properties	Mean±SD					
	PVP K30			DTZ HCl		
	6%	4%	p-value	drug	No drug	p-value
pH	3.96(0.13)	3.97(0.17)	0.88	3.87(0.09)	4.09(0.12)	0.00*
viscosity	552.04(87.13)	439.37(65.63)	0.00*	547.98(181.49)	507.34(97.96)	0.43
particle size by volume	475.99(126.12)	319.86(57.46)	0.00*	441.13(137.35)	363.96(102.20)	0.07

* p-value<0.05 (statistic significance with confident level 95%)

() standard deviation

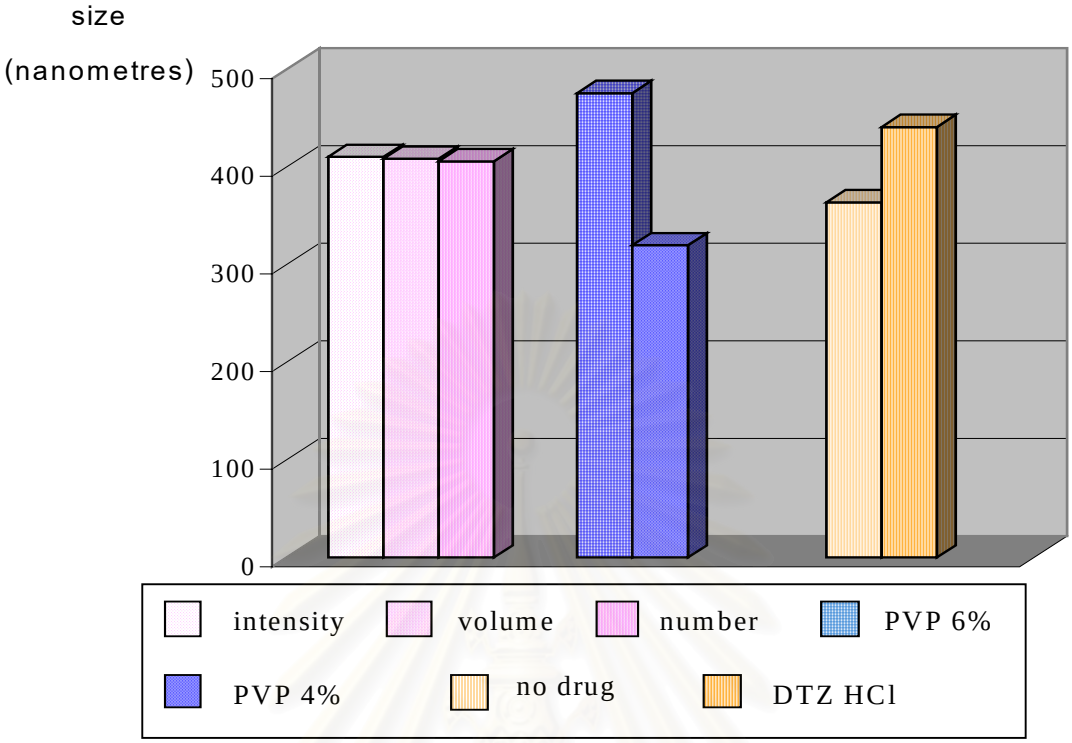


Figure 29 Comparative chart of particle size in size distribution of DTZ HCl pseudolatex by intensity, volume and number, including effect of PVP K30 and drug on particle size of various formulations.

From comparative mean value, particle size distribution by intensity of light scattering from particles, volume and number were insignificant difference among three groups of distribution ($p>0.05$). Moreover effect of PVP K30 on viscosity and particle size were determined by statistical analysis, the results showed that formulations with 6% of PVP K30 had greater mean value of viscosity and particle size than formulations containing 4% w/w of PVP K30 at the 5% significance level. As while DTZ HCl in formulations would effect on pH value, pseudolatexes with DTZ HCl had lower mean value of pH than pseudolatexes without drug ($p<0.05$).

2.3 Effects of Various Components on the Release Profile

2.3.1 The Elucidation of Drug Release Kinetic Model

In order to determine the effect of type of polymer, other components and formulation difference on the model of drug release. Therefore, an analysis of the release profiles were carried out in order to elucidate suitable model (i.e. zero order, first order and Higuchi's model), which could be fitted by the data. The plots between percent release of drug as a function of time (zero order), log of drug remained versus time (first order) and amount of drug versus square root of time (Higuchi's model) were constructed, and determined the one which was the most linear in order to accepted as a model of drug release. Then regression analysis and correlation coefficient values (r) for release data of different formulations according to various kinetic models are shown in Table 20. Moreover Figs.30-41 showed release profiles of different formulations followed kinetic model that mentioned before.

The pattern of delivery achieves by a controlled release system can vary over a wide range, but most release profiles categorized in to three types: (1) Zero order release pattern ; (2) First order release pattern ; (3) Square root time release pattern.

Zero order model, an ideal controlled release device is one which can deliver the drug at a constant rate until the device is exhausted of active agent. The mathematical formula of zero order kinetic is presented below.

$$Q = kt \quad (2)$$

where Q is cumulative amount or extent of drug release, k is drug release constant, and t is time.

First order model, the release rate in this case was proportional to the mass of active agent contained within the device. The relationship of this model is exhibited as a mono exponential declination of drug release. The rate was then given as in equation (3) (Benita et al. 1982 and Pillay et al. 1999).

$$B = Q_0 e^{-kt}$$
$$\text{or } \ln B = \ln Q_0 - kt \quad (3)$$

where B is quantity of drug remaining in device at time t, Q_0 is initial drug content, k and t is mean as previously described.

Square root of time model (Higuchi's model), was discovered by Higuchi (1963) and Schwartz (1963). In contrast to first order release, the release rate here remained finite as the device approached exhaustion. The model equation was derived from drug release throughout continuous ointment base experiment. This type can be described by Higuchi equation.

$$Q = \sqrt{D\varepsilon/\tau\,(2C_0 - \varepsilon C_s)\,C_s t} \quad (4)$$

where Q is amount of drug release per unit area, D is diffusion coefficient, ε is porosity of device, τ is tortuosity of device, C_0

is initial concentration of active ingredient in device, C_s is saturated solubility of drug in device material, and t is time.

The assumptions made deriving equation (4) are as follows:

1. A pseudo-steady state is maintained during release.
2. $C_0 >> C_s$, i.e., excess solute is present.
3. The system is in perfectly sink condition in which C, is approximately zero at all time.
4. Drug particles in porosity of device are much smaller than those in the matrix.
5. The diffusion coefficient remains constant.
6. No interaction between the drug and the matrix occurs.

For purpose of data treatment, equation (4) is usually reduced to

$$Q = k_H t^{1/2} \qquad (5)$$

where k_H is Higuchi's constant. Q, and t is mean as previously described.

Table 20 Regression analysis and correlation coefficient values for dissolution data of different formulations according to various kinetic models.

Formulas	Zero order		First order		Higuchi's model	
	r	k_0	r	k_1	r	k_H
F#6	0.9623	1.36	0.9659	3.60×10^{-3}	0.9963	5.64
F#7	0.9696	1.20	0.9724	3.10×10^{-3}	0.9978	4.94
F#8	0.9830	1.15	0.9862	3.00×10^{-3}	0.9995	4.68
F#9	0.9857	0.80	0.9868	2.10×10^{-3}	0.9969	3.24
F#10	0.9803	1.00	0.9808	2.56×10^{-3}	0.9987	4.07
F#14	0.9779	1.85	0.9818	4.90×10^{-3}	0.9977	7.52
F#15	0.9857	1.68	0.9874	4.50×10^{-3}	0.9974	6.79
F#16	0.9910	2.15	0.9932	5.70×10^{-3}	0.9974	8.62
F#21	0.9958	2.46	0.9975	6.70×10^{-3}	0.9938	9.82
F#22	0.9938	2.24	0.9963	6.00×10^{-3}	0.9959	8.94
F#23	0.9847	1.81	0.9884	4.80×10^{-3}	0.9990	7.33
F#24	0.9886	1.69	0.9916	4.50×10^{-3}	0.9984	6.83
F#29	0.9951	2.52	0.9969	6.90×10^{-3}	0.9935	10.06
F#30	0.9823	1.75	0.9842	4.60×10^{-3}	0.9990	7.09
F#34	0.9901	1.87	0.9922	5.00×10^{-3}	0.9975	7.52
F#35	0.9901	1.69	0.9957	4.50×10^{-3}	0.9930	6.75

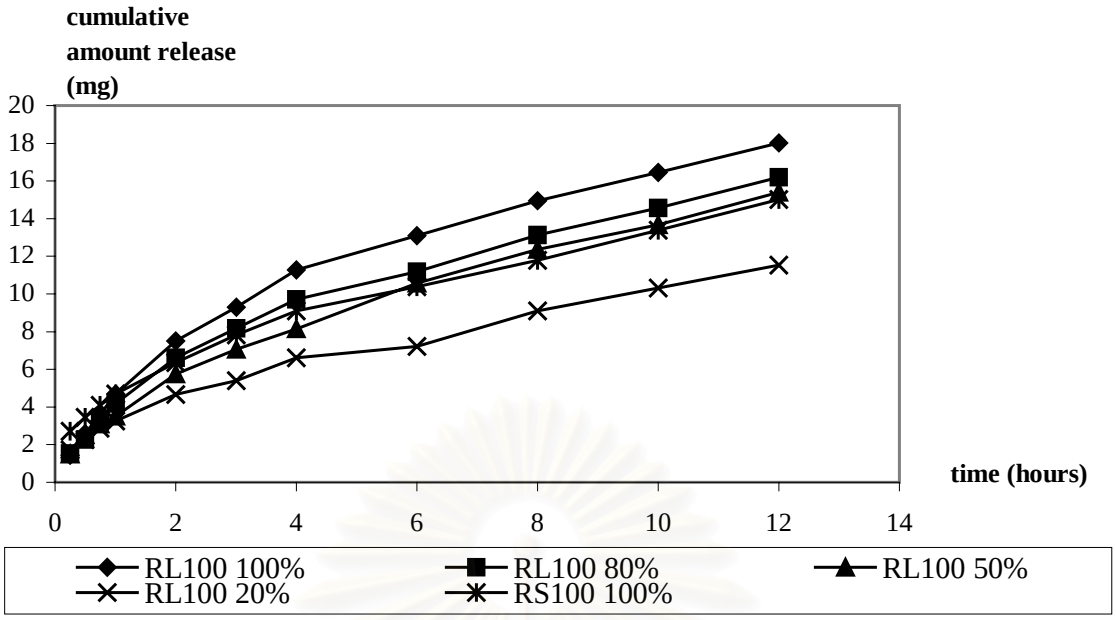


Figure 30 Average release time profiles of DTZ HCl pseudolatex formulations total % of polymer = 14% w/w in the formula at various ratios of polymer between RL100 and RS100 (n=5).

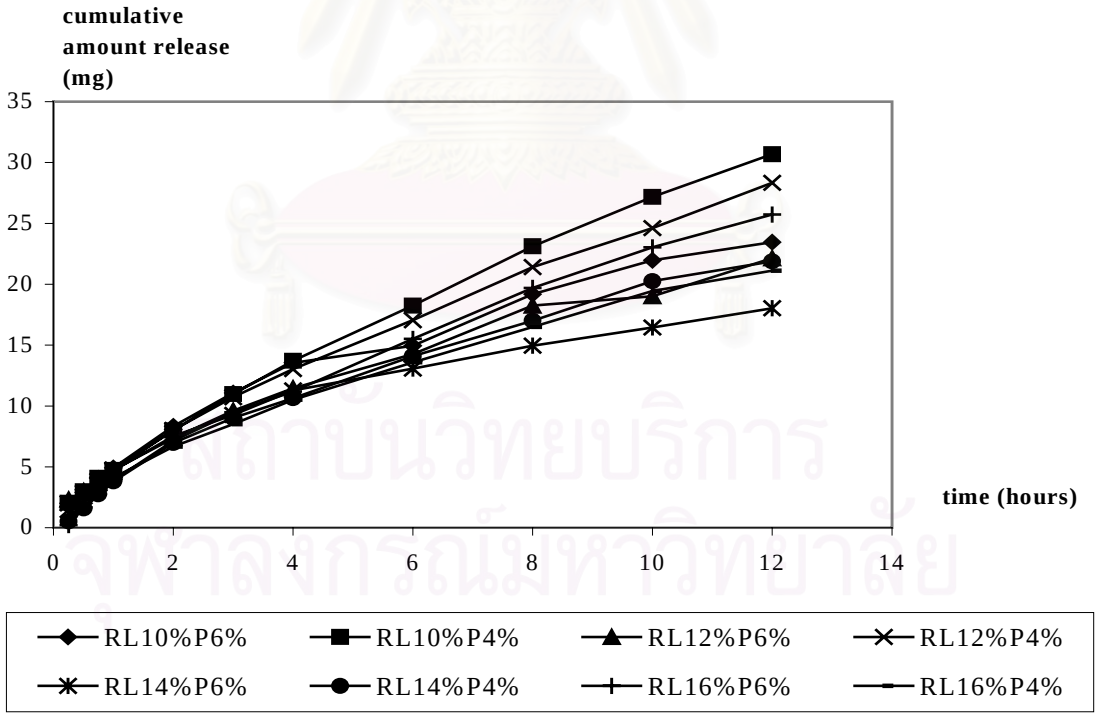


Figure 31 Average release time profiles of DTZ HCl pseudolatex formulations when fixed polymer ratio RL100 100%, Tween 80 10% and plasticizer 4% at various %w/w of polymer RL100 and %w/w of PVP K30 (n=5).

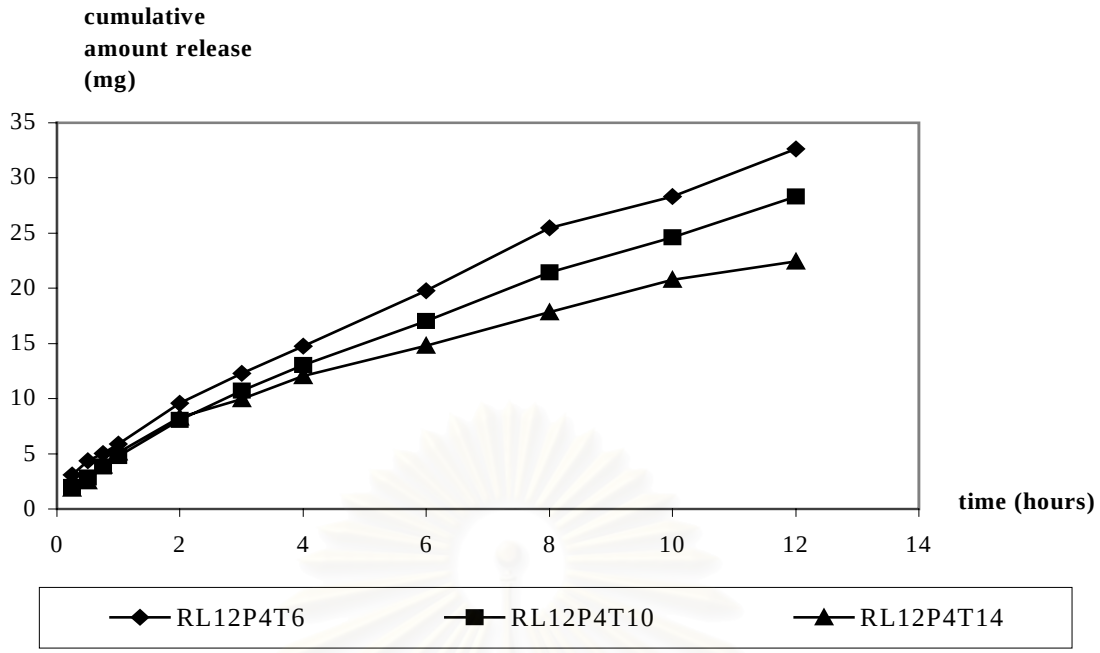


Figure 32 Average release time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and plasticizer 4% w/w at various %w/w of Tween 80. (n=5)

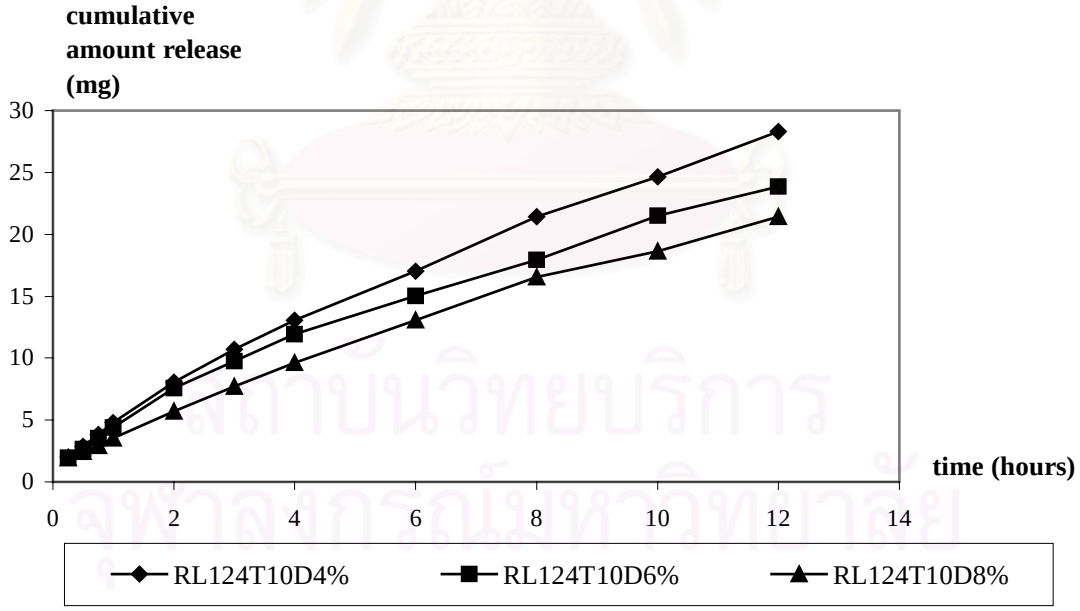


Figure 33 Average release time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and Tween 80 10% at various %w/w of plasticizer (dibutyl phthalate) (n=5).

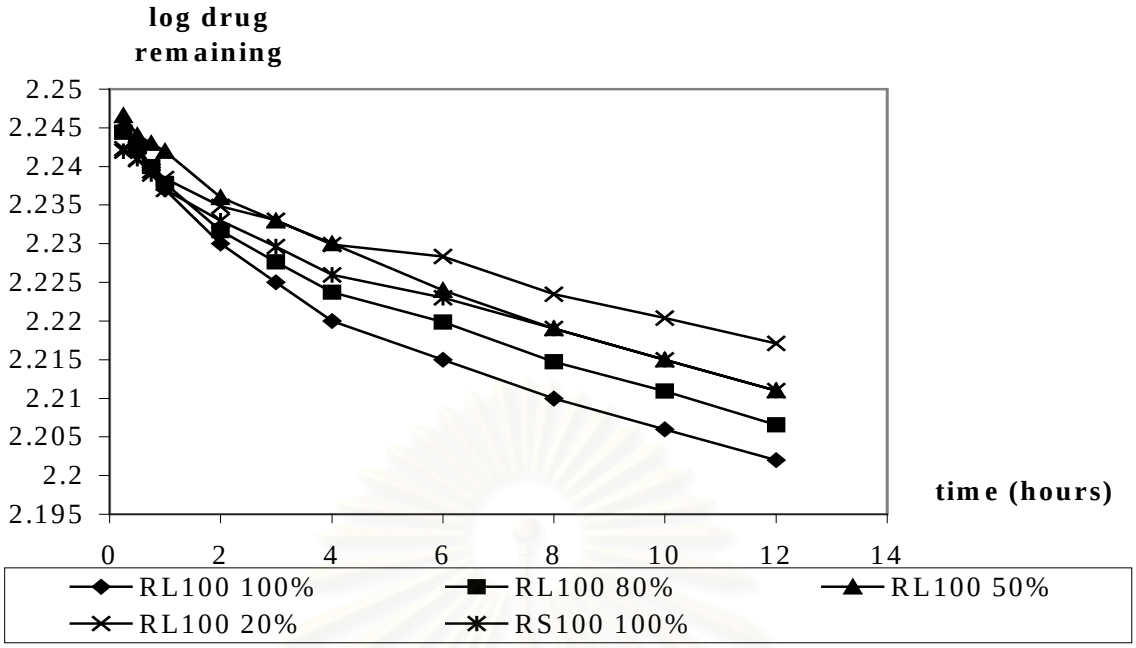


Figure 34 Average logarithm of drug remained time profiles of DTZ HCl pseudolatex formulations total % of polymer = 14% w/w in the formula at various %w/w of polymer between RL100 and RS100.

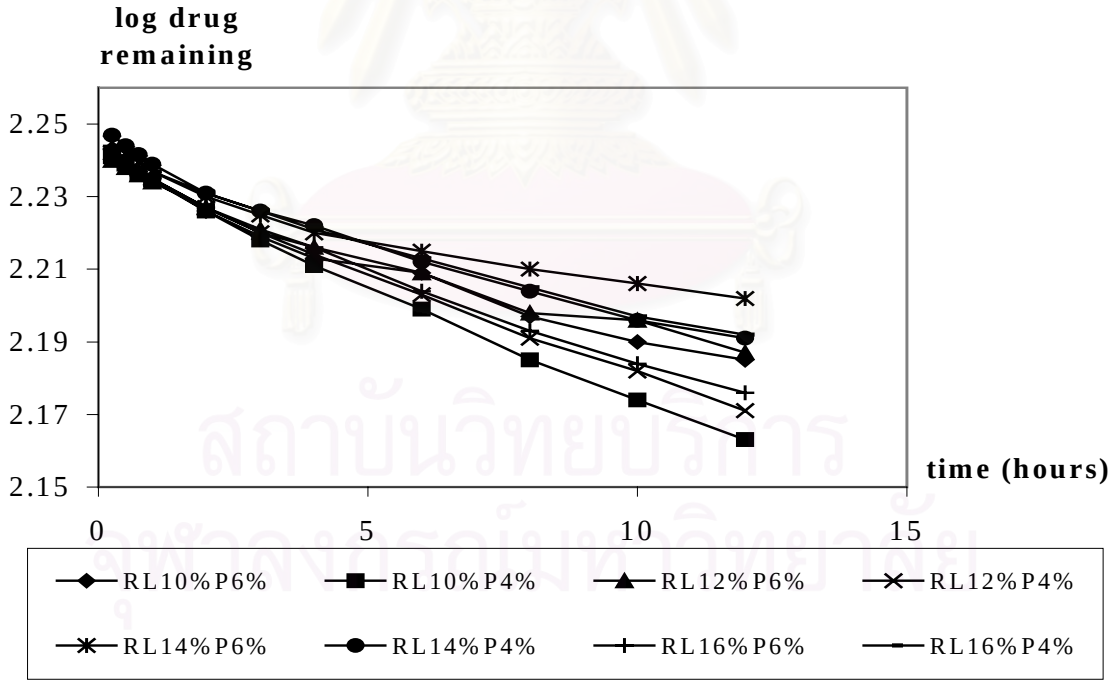


Figure 35 Average logarithm of drug remained time profiles of DTZ HCl pseudolatex formulations when fixed polymer ratio RL100 100%, Tween 80 10%, and plasticizer 4% at various %w/w of polymer RL100 and %w/w of PVP K30.

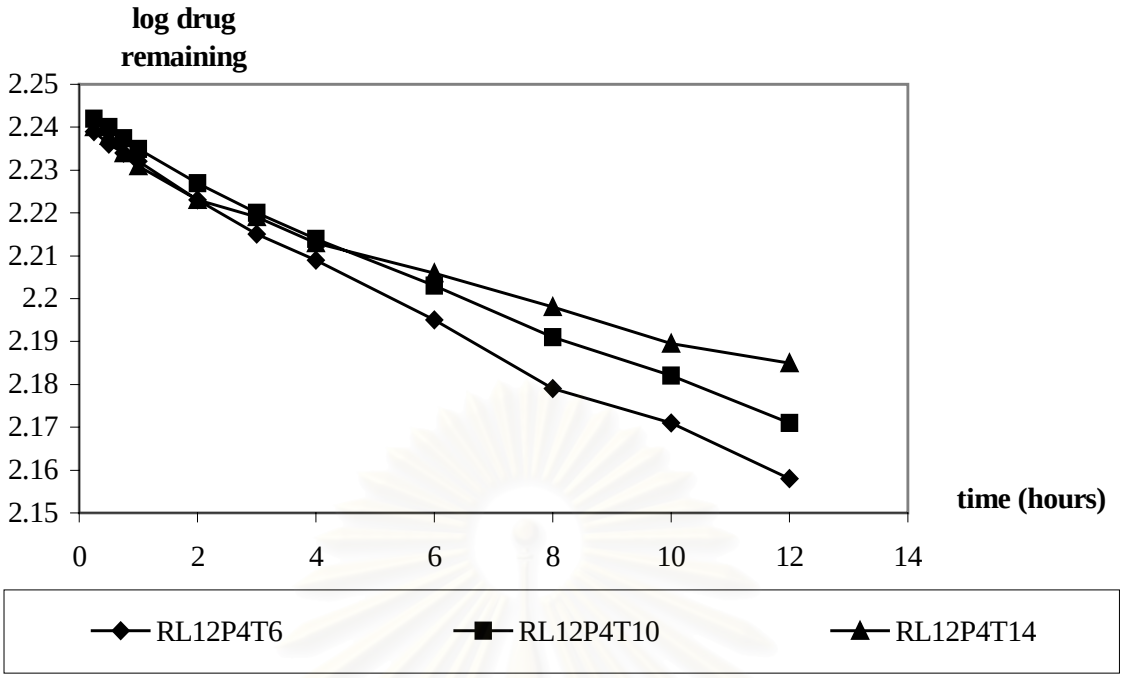


Figure 36 Average logarithm of drug remained time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and plasticizer 4% at various %w/w of Tween 80.

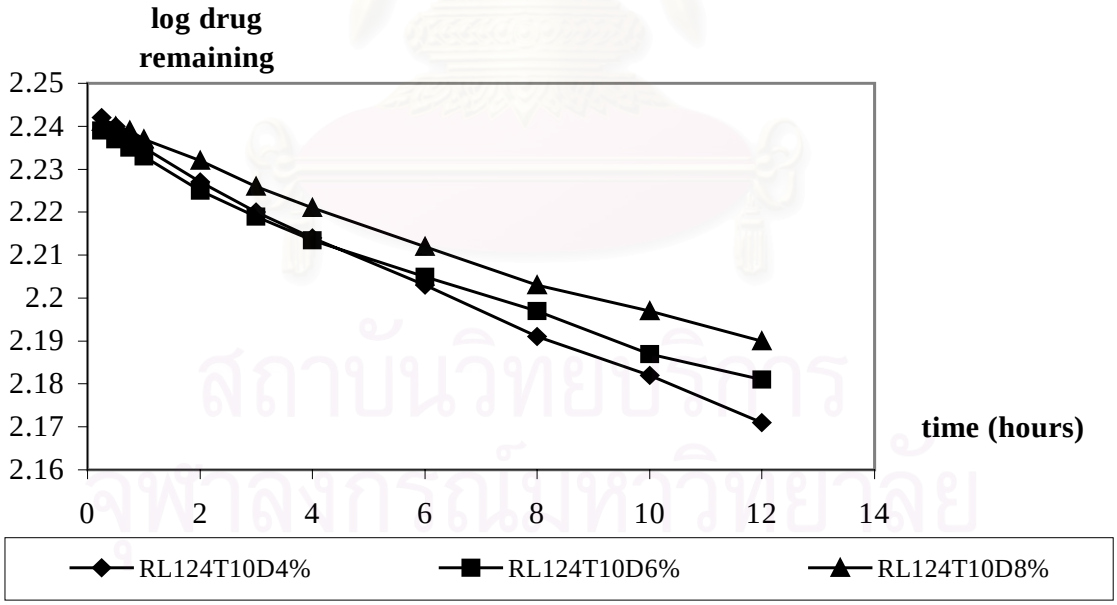


Figure 37 Average logarithm of drug remained time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and Tween 80 10% at various % w/w of dibutyl phthalate.

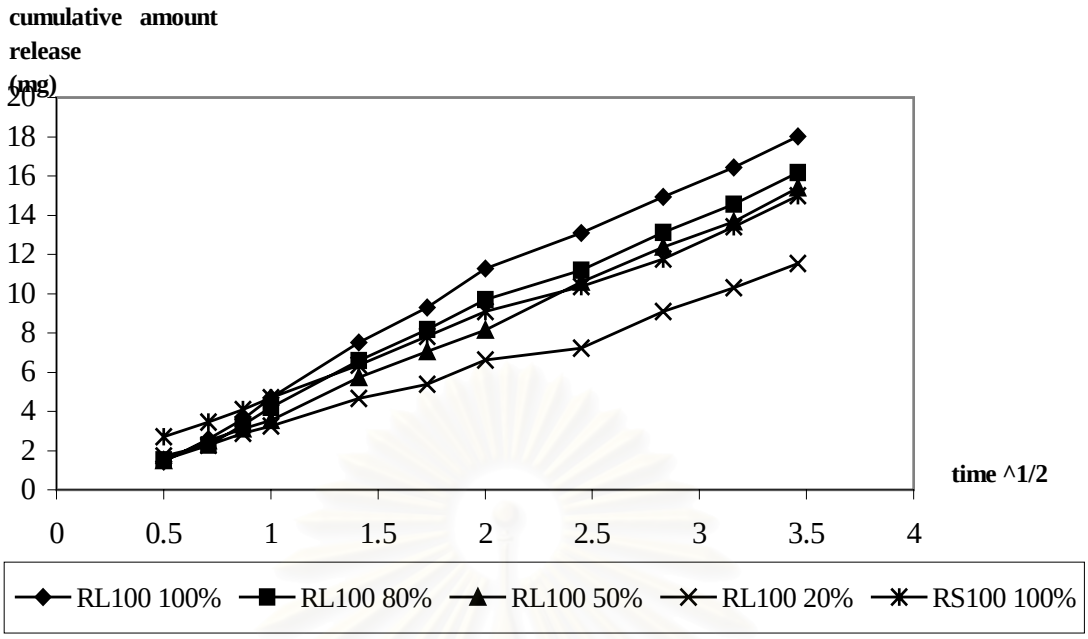


Figure 38 Average release-square root of time profiles of DTZ HCl pseudolatex formulations total % of polymer = 14% w/w in the formula at various %w/w of polymer between RL100 and RS100.

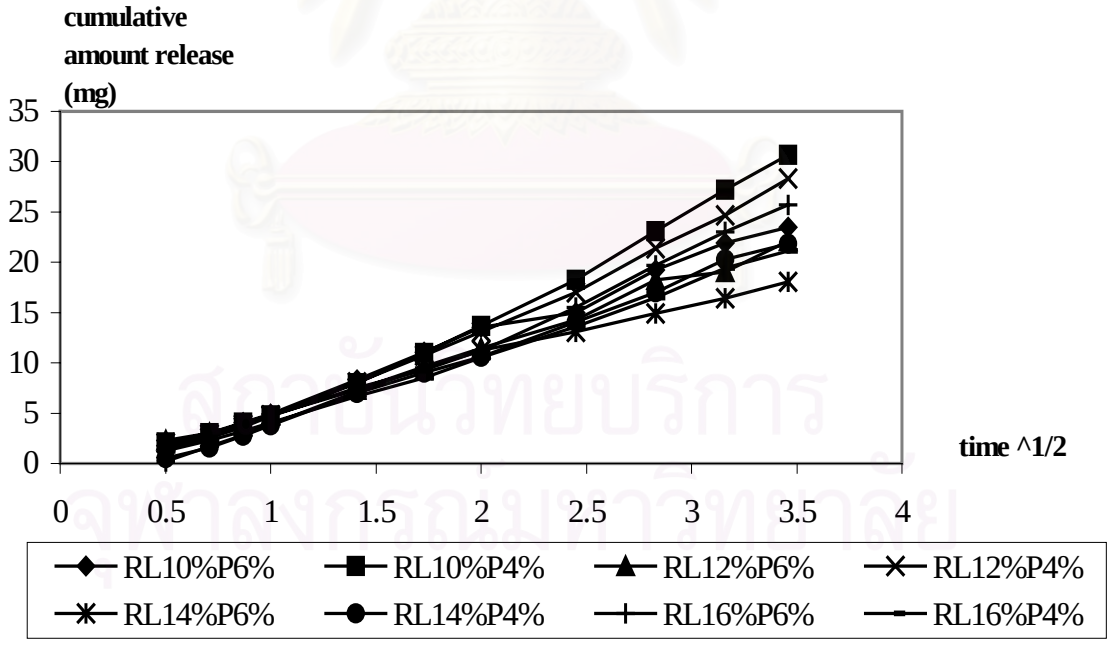


Figure 39 Average release-square root of time profiles of DTZ HCl pseudolatex formulations when fixed polymer ratio RL100 100%, Tween 80 10%, and plasticizer 4% at various % w/w of polymer RL100 and % w/w of PVP K30.

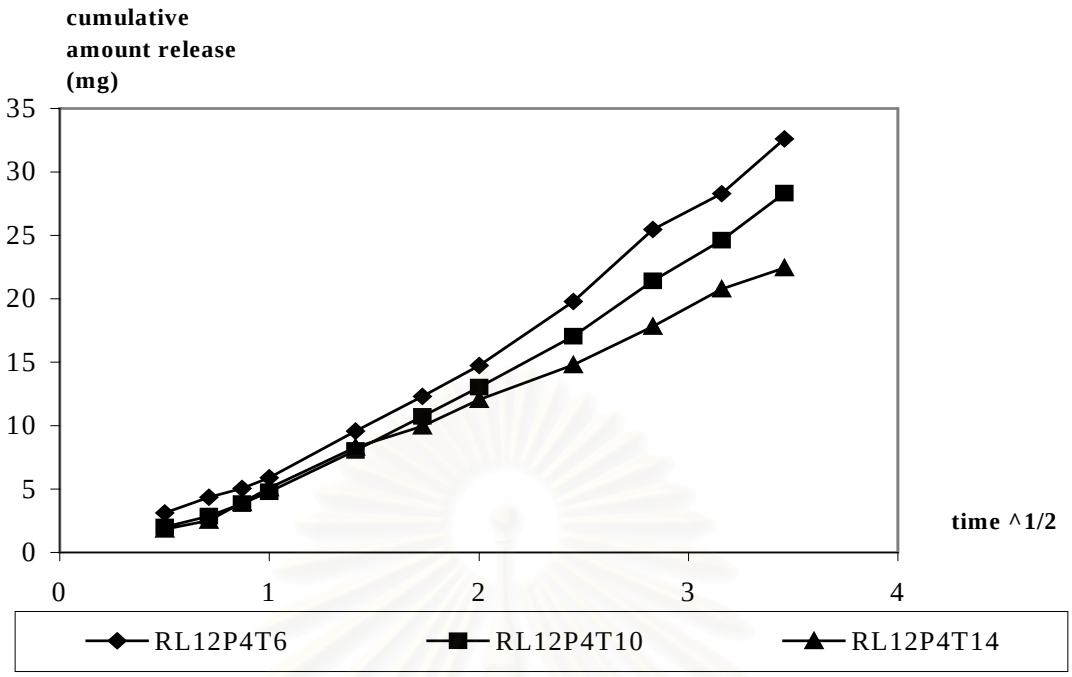


Figure 40 Average release-square root of time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and plasticizer 4% at various % w/w of Tween 80.

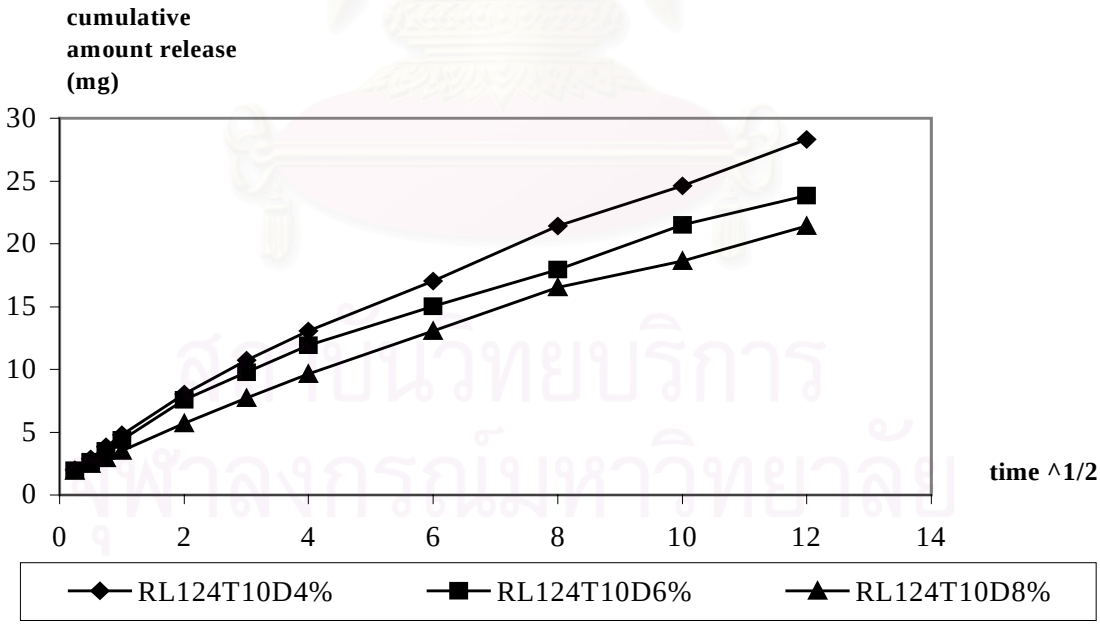


Figure 41 Average release-square root of time profiles of DTZ HCl pseudolatex formulations when fixed Eudragit RL100 12%, PVP K30 4%, and Tween 80 10% at various % w/w of dibutyl phthalate.

From Table 20, since both first order release and the square root of time release plots were linear, as indicated by correlation coefficient then it was necessary to distinguish between the models. The treatment was based upon the differential forms of the first order; equation (3) become to (6) and square root-time order; equation (5) become to (7) (Schwartz, Simonelli and Higuchi, 1968).

The rate predicted by first order model was given by:

$$\frac{dQ'}{dt} = kA_0 - kQ' \qquad (6)$$

where $A = A_0 - Q'$, this indicated that rate will be proportional to Q'. The rates of release were determined by measuring the slopes at different points on the percent of drug release versus times curves.

For Higuchi's model, the rate will be inversely proportional to the total amount of drug release in accordance with equation (7) (Sa, Bandyopadhyay, and Gupta, 1990)

$$\frac{dQ'}{dt} = \frac{k_H^2 S^2}{2Q'} \qquad (7)$$

where $Q' = Q \times S$ (S is the surface area of matrix).

The plots of rates of release versus $1/ Q'$ were linear, indicating that the release was fitted with Higuchi's model. If the plots of rates of release versus Q' were linear, indicating that the first order model was operative. All of formulations was clarified to distinguish model of release between first order and Higuchi's model

by comparison of correlation coefficient of the plots of two equations, and test statistics two groups analysis, indicating difference at 5% significance level as shown in Table 21. About data process of rate of release, 1/ Q and Q of all formulations are given in Appendix D.

Table 21 Comparison of linearity between plots of rate of release against reciprocal amount (1/Q) and amount (Q) of DTZ HCl release from the formulations.

Formulations	Correlation coefficient of rate dQ/dt Mean±SD (n=5)		p-value
	versus Q	versus 1/Q	
F#6 RL100%	$0.9388\pm2.84\times10^{-2}$	$0.8423\pm8.62\times10^{-2}$	0.121
F#7 RL:RS=4:1	$0.8642\pm3.90\times10^{-2}$	$0.8840\pm6.81\times10^{-2}$	0.651
F#8 RL:RS=1:1	$0.7624\pm3.29\times10^{-2}$	$0.9467\pm1.38\times10^{-2}$	0.000*
F#9 RL:RS=1:4	$0.6414\pm3.05\times10^{-2}$	$0.8765\pm2.54\times10^{-2}$	0.000*
F#10 RS100%	$0.6401\pm3.66\times10^{-2}$	$0.8516\pm5.27\times10^{-2}$	0.000*
F#14 RL10P6	$0.8592\pm5.15\times10^{-2}$	$0.9012\pm3.52\times10^{-2}$	0.296
F#15 RL12P6	$0.6578\pm3.03\times10^{-2}$	$0.8488\pm3.36\times10^{-2}$	0.000*
F#16 RL16P6	$0.5434\pm9.65\times10^{-2}$	$0.3387\pm16.7\times10^{-2}$	0.157
F#21 RL10P4	$0.6548\pm8.48\times10^{-2}$	$0.8827\pm4.68\times10^{-2}$	0.000*
F#22 RL12P4	$0.7041\pm2.86\times10^{-2}$	$0.9026\pm3.64\times10^{-2}$	0.000*
F#23 RL14P4	$0.7710\pm3.43\times10^{-2}$	$0.1478\pm12.2\times10^{-2}$	0.000*
F#24 RL16P4	$0.8883\pm3.47\times10^{-2}$	$0.8624\pm14.8\times10^{-2}$	0.735
F#29 RL124T6	$0.5394\pm7.07\times10^{-2}$	$0.8084\pm6.72\times10^{-2}$	0.000*
F#30 RL124T14	$0.7885\pm5.15\times10^{-2}$	$0.8046\pm3.45\times10^{-2}$	0.521
F#34 RL124T10D6	$0.6965\pm3.73\times10^{-2}$	$0.8570\pm6.68\times10^{-2}$	0.001*
F#35 RL124T10D8	$0.5049\pm5.17\times10^{-2}$	$0.7319\pm5.90\times10^{-2}$	0.001*

* The mean difference was significant at the 5% level (p-value<0.05).

The results from Table 21 displayed correlation coefficient between two groups of each formulation which test statistical value by paired t test at 5% significance level (p-value<0.05). Sixteen formulations, ten formulations of them were significant difference, most of all played major role in Higuchi's model. Although six formulas, F#6, F#7, F#14, F#16, F#24 and F#30 were insignificant difference, and they couldn't clarify exactly between first order and Higuchi's model, according to results from Table 20 correlation coefficient of Higuchi's model in every formulations was high value, then Higuchi's model might be fitted to the release data profile. Further analysis the release rate constant followed Higuchi's model among various formulations was determined with ANOVA that provided in Table 22.

Table 22 Comparison of the release rate of Higuchi's model (k_H) among different formulations according to effect of various components.

Effect	N	Subset for alpha = .05			
		1	2	3	4
Ratio of RL and RS					
RL:RS=1:4 (F#9)	5	3.2380			
RS100% (F#10)	5		4.0700		
RL:RS=1:1 (F#8)	5			4.6780	
RL:RS=4:1 (F#7)	5			4.9460	
RL100% (F#6)	5				5.6380

Table 22 Continued

Effect	N	Subset for alpha = .05			
		1	2	3	4
%RL and %PVPK30					
14%RLand6%PVP (F#6)	5	5.63803			
12%RLand6%PVP (F#15)	5		6.7920		
16%RLand4%PVP (F#24)	5		6.8240		
14%RLand4%PVP (F#23)	5		7.3320		
10%RLand6%PVP (F#14)	5		7.5160		
16%RLand6%PVP (F#16)	5			8.6200	
12%RLand4%PVP (F#22)	5			8.9400	8.9400
10%RLand4%PVP (F#21)	5				9.8160
Effect	N	1	2	3	
%Tween in formulations					
14%Tween (F#30)	5	7.0840			
10%Tween (F#22)	5		8.9400		
6%Tween (F#29)	5			10.0560	
Effect	N	1	2	3	
%Plasticizer					
8%plasticizer (F#35)	5	6.7480			
6%plasticizer (F#34)	5		7.5160		
4%plasticizer (F#22)	5			8.9400	

2.3.2 The Evaluation of Drug Release Mechanism

The dissolution data was analyzed to clarify drugs release mechanism using equation of Peppas (1985) given below

$$M_t/M_\infty = kt^n \quad (8)$$

where M_t/M_∞ is the fraction of drug released up to time t
t is the release time, k is a constant incorporating structural and geometric characteristics of the controlled device, n is the diffusion release exponent indicative of the mechanism of release.

The determination of the exponent n is valid for the first 60% of the total released drug ($M_t/M_\infty \leq 0.6$), which also applied only to the early times of release.

Clearly, a desirable mechanism for many applications is that which leaded to n equals 1, which characterized zero order release behavior. For all formulations, the release data profiles were analyzed to determine the exponent n that displayed in Table 23. Table 24 summarized the general dependence of n on the diffusion mechanism.

Table 23 Diffusion exponent of various formulations followed power's law equation.

Formulas	n	r	k
F#6	0.6370	0.9915	0.0236
F#7	0.6081	0.9947	0.0217
F#8	0.5917	0.9990	0.0203
F#9	0.4894	0.9983	0.0185
F#10	0.4456	0.9989	0.0269
F#14	0.6705	0.9968	0.0273
F#15	0.6370	0.9915	0.0276
F#16	1.0619	0.9522	0.0141
F#21	0.7166	0.9989	0.0288
F#22	0.7023	0.9992	0.0277
F#23	0.8958	0.9816	0.0164
F#24	0.7180	0.9982	0.0214
F#29	0.6265	0.9963	0.0367
F#30	0.6576	0.9962	0.0266
F#34	0.6712	0.9986	0.0259
F#35	0.6623	0.9945	0.0222

Table 24 Interpretation of diffusion release mechanisms for drug release data from DTZ HCl pseudolatex.

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous (non-Fickian) transport	t^{n-1}
1.0	Case-II transport	Zero order (time independent) release
$n > 1.0$	Super-Case-II transport	t^{n-1}

Nevertheless, geometry of device was also one of the most important parameter to affluent the analysis and indicates the proper power exponent (n) with power's law equation. Utilizing the geometric knowledge, Peppas and Sahlin (1989) defined the n value for various shapes of device as shown in Table 25.

Table 25 Diffusion exponent for different device geometries.

Diffusion exponent (n)			Mechanism
Film	Cylinder	Sphere	
0.50	0.45	0.43	Fickian diffusion
0.50<n<1.00	0.45<n<0.89	0.43<n<0.85	Anomalous transport
1.00	0.89	0.85	Case II transport

Most of all formulations in Table 23 were identified diffusion exponent as 0.5<n<1.00 which said to be anomalous transport (non fickian). Anomalous case was determined that they have complex mechanism for drug release regulation. Two or more mechanisms were composed in anomalous diffusion. Coupling of both main

mechanism which major influence to its were fickian diffusion and relaxation with unequal strength depend upon different parameters of system. Peppas and Sahlin (1989) proposed the equation for clarified the influence release mechanism in anomalous transport case. When the solvent influxes to polymer matrix, the two phenomena that controlling the drug release were drug diffusion and polymer relaxation. Hence, the equation was consisted two terms of diffusion and relaxation control that might be expressed as:

$$M_t/M_\infty = k_1 t^n + k_2 t^{2n} \quad (9)$$

Where k_1 is diffusion rate controlling constant, k_2 is relaxation rate controlling constant and n is geometrical power exponent.

Due to geometric device, pseudolatex was assumed to be n = 0.5 (Table 25) because SEM pictures showed that this formulation had spherical particles included release kinetic model was diffusion as a major mechanism then equation (9) should be expressed as:

$$M_t/M_\infty = k_1 \sqrt{t} + k_2 t \quad (10)$$

The ratio of k_1 to k_2 could express the strength of main mechanism over supporting mechanism for controlling drug release in anomalous system. If k_1 that related to diffusion control was higher than k_2 (relaxation), it was possible to imply that diffusion control was the major drug release regulation. In order to determine the major mechanism of various formulations, k_1 and k_2 values are showed in Table 26.

Table 26 k_1 and k_2 values of various DTZ HCl pseudolatex formulations.

Formulas	k_1	k_2
F#6	1.381	-0.392
F#7	1.231	-0.238
F#8	0.931	0.070
F#9	0.792	0.209
F#10	0.979	0.020
F#14	1.015	-0.018
F#15	0.803	0.198
F#16	0.668	0.336
F#21	0.450	0.555
F#22	0.556	0.449
F#23	0.875	0.126
F#24	0.757	0.247
F#29	0.460	0.544
F#30	0.935	0.065
F#34	0.697	0.306
F#35	0.437	0.567

Most of formulations played major mechanism as diffusion control while as four formulas; F#21, F#22, F#29 and F#35 couldn't exactly define between diffusion control and polymer relaxation because k_1 and k_2 values of these formulations were a little different.

The results from Table 22 and SEM pictures of different formulations according to effect of various components were used to chose the best formulation step by step for selection suitable formula which continued to study effect of drug concentration, illustrated in Fig.42.

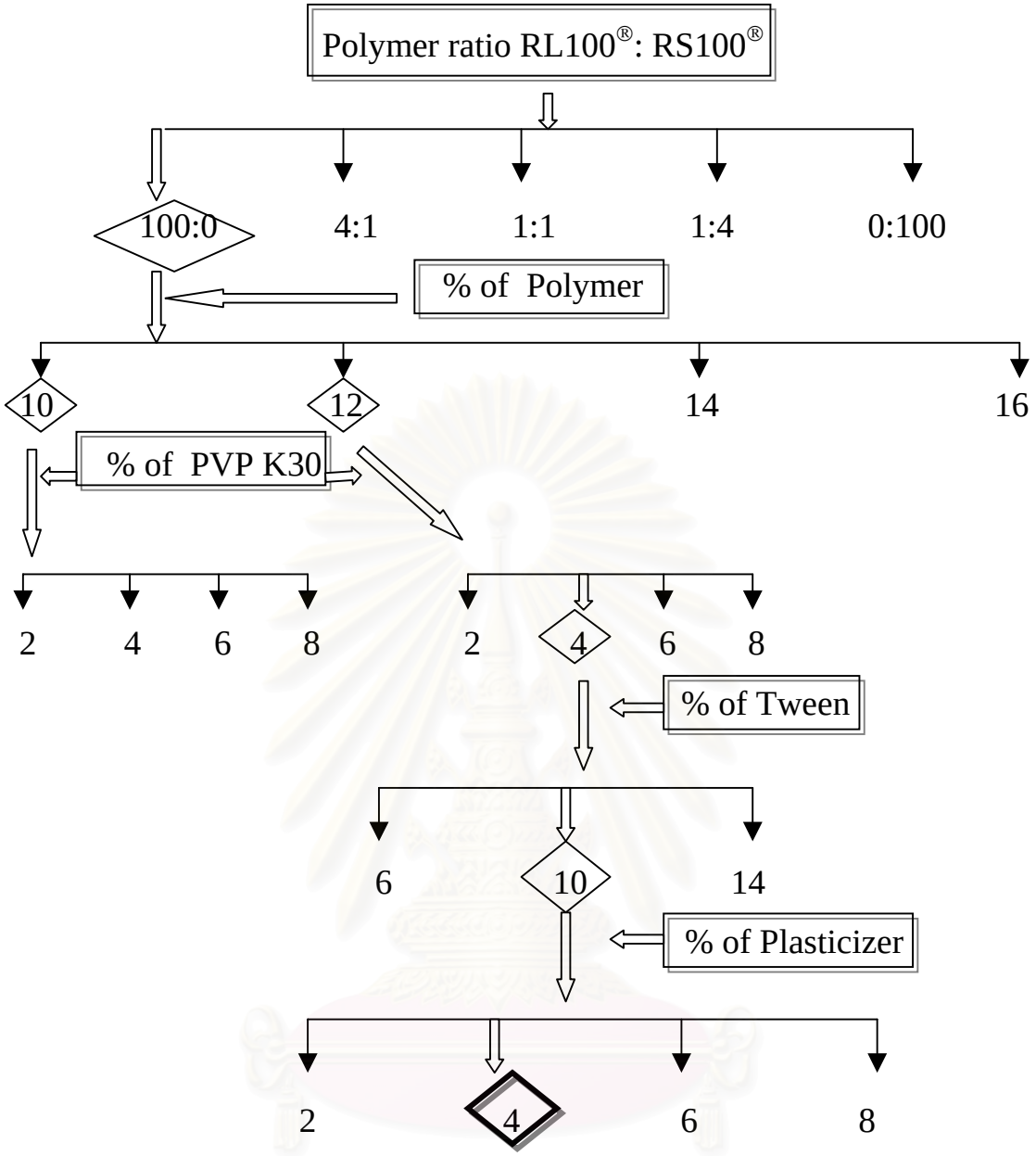


Figure 42 Flow chart of selection suitable formula with step by step using high rate of release and good properties to consider in decision. All of formulations contained DTZ HCl 2% w/w.

A result from Fig 42 showed that a suitable formula, composed of DTZ HCl 2%, RL100® 12%, PVP K30 4%, Tween 80 10% and Plasticizer 4% w/w of formulation, was chosen for further study of the effect of drug concentration.

2.4 Effect of Drug Concentration on Release Skin Profile

2.4.1 Elucidation of Drug Permeation Model

The skin permeation-time profiles of formulas F#22 and F#36 F#38 were determined the effect of drug concentration on the model. In order to clarify what model (zero order, first order and Higuchi's model) could be fitted by the data as shown in Table 27 and Fig.43-45.

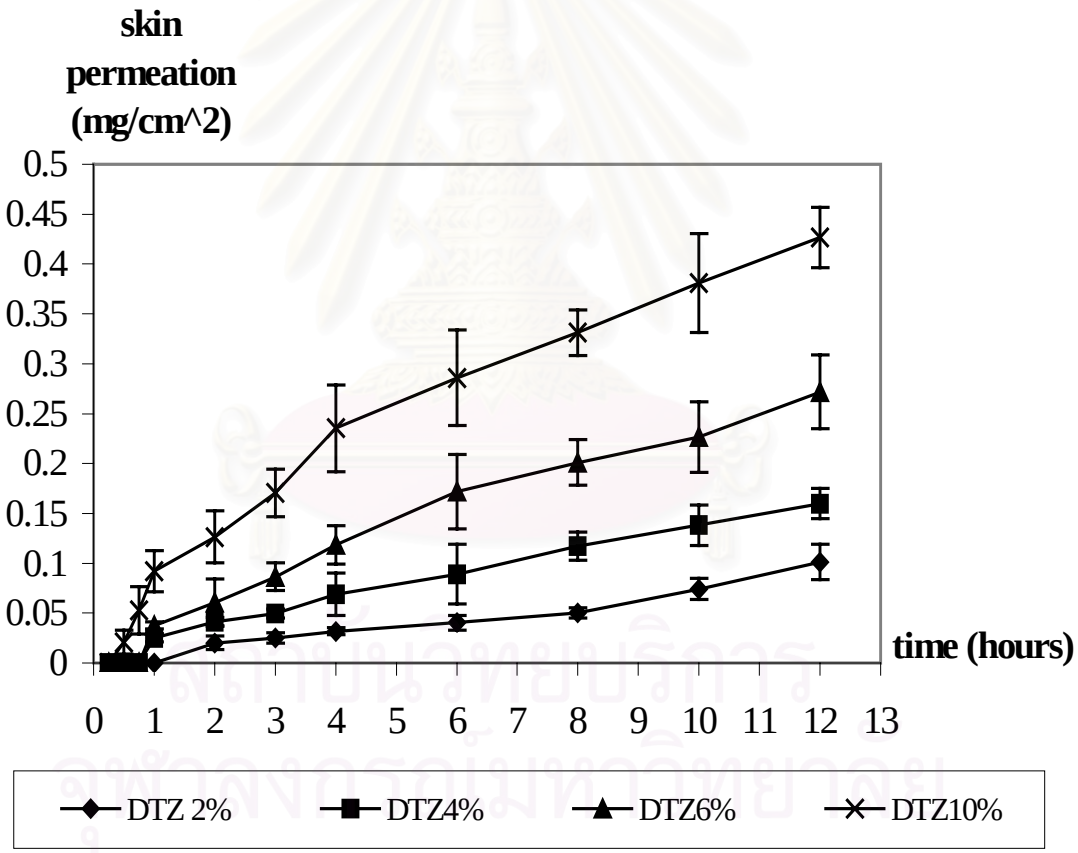


Figure 43 Average skin permeation time profiles of DTZ HCl pseudolatex formulations at various %w/w of drug concentrations. (n=3)

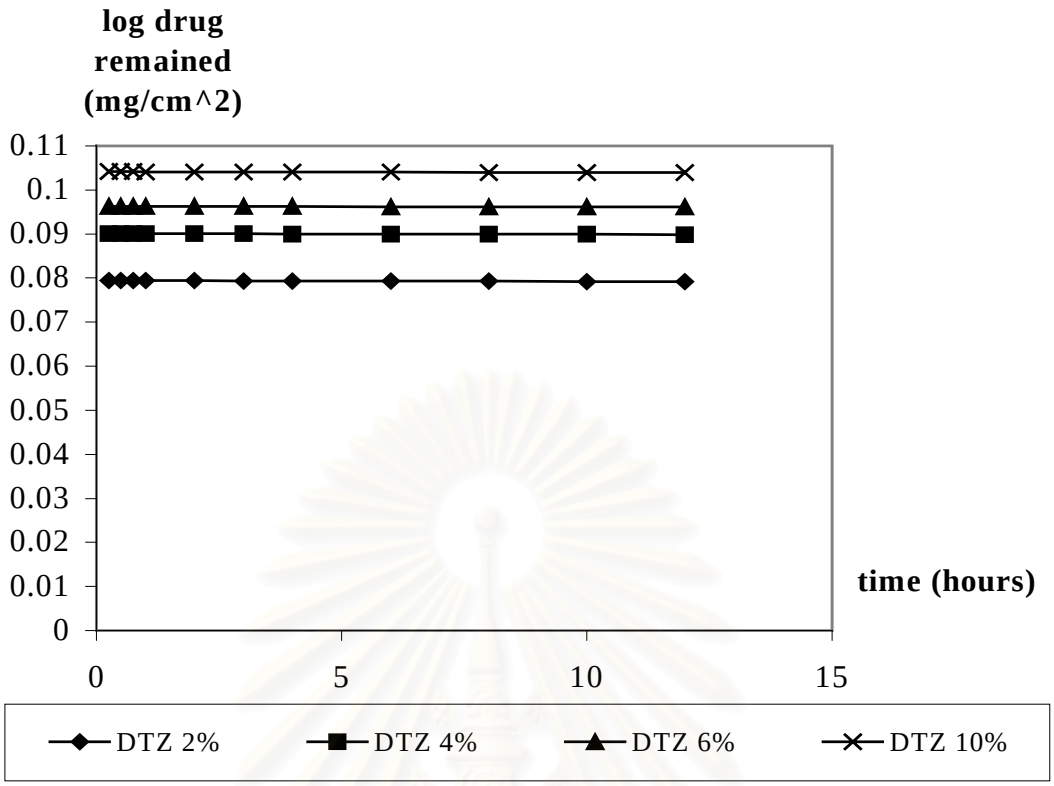


Figure 44 Average logarithm of drug remained time profiles of DTZ HCl pseudolatex formulations at various %w/w of drug concentration.

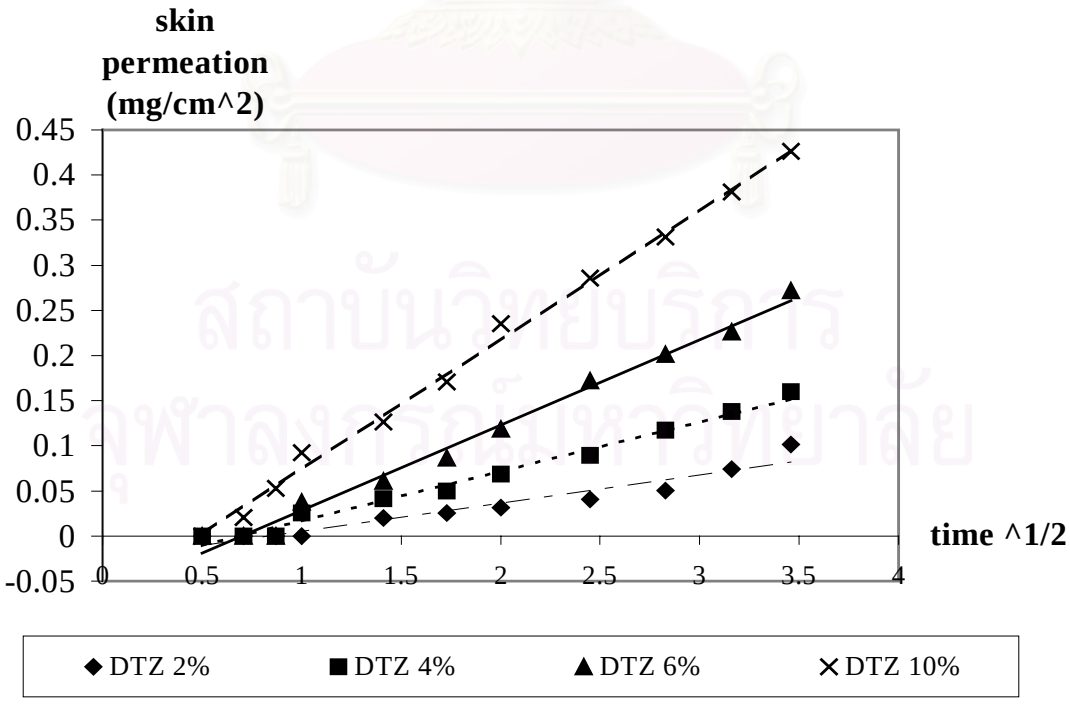


Figure 45 Average release-square root of time profiles of DTZ HCl pseudolatex formulations at various %w/w of drug concentration.

Table 27 Regression analysis and correlation coefficient values for skin permeation of formulations (effect of drug concentration) according to various kinetic model.

Formulas	Zero order		First order		Higuchi's model	
	r	k_0	r	k_1	r	k_H
F#22(2%)	0.9855	8.00×10^{-3}	0.9850	2.00×10^{-5}	0.9636	0.0313
F#36(4%)	0.9884	0.0137	0.9896	2.00×10^{-5}	0.9928	0.0664
F#37(6%)	0.9858	0.0235	0.9866	2.00×10^{-5}	0.9944	0.0945
F#38(10%)	0.9754	0.0352	0.9760	2.00×10^{-5}	0.9978	0.1437

Skin permeation profiles and regression analysis of formulas F#22, F#36, F#37 and F#38 that contained DTZ HCl 2, 4, 6 and 10% w/w, respectively kinetic model assumed to fit zero order rather than first order and Higuchi's model when concentration of drug was at low level. While as a higher concentration of drug inclined correlation coefficient of Higuchi's model became higher too. When considered trend of permeation rate through shed snake skin of zero order and Higuchi's model from Table 27, there were a relation between drug concentration and rate of permeation occurred. For this reason, to identify correlation coefficients of two relations that mentioned above, the plot obtained are shown in Fig 46.

Regression is therefore a modeling tool which can be used to derive the form of relationship between a dependent variable (drug concentrations) and independent variable, the rate of permeation, based on collected experimental data. The relationship between drug concentration and rate of permeation would be probable happening because of the high correlation coefficient of the two relationships.

They composed of relationship between drug concentration and rate of permeation followed by zero order and Higuchi's model which showed r=0.9967 and r=0.9969, respectively. Due to assessment of practical validity is probably the most important aspect of regression analysis to predict value closed to observed value well enough in order to purpose for practical use. Then these results could be used to predict amount of drug in order to get a suitable permeation rate or predict permeation rate in other drug concentrations.

The formula F#38 was chosen to prepare DTZ HCl TDDS for further evaluation drug release and skin permeation, composed of DTZ HCl 10%, RL100®12%, PVP K30 4%, Tween 80 10% and Plasticizer (Dibutyl phthalate) 4% w/w of formulation.

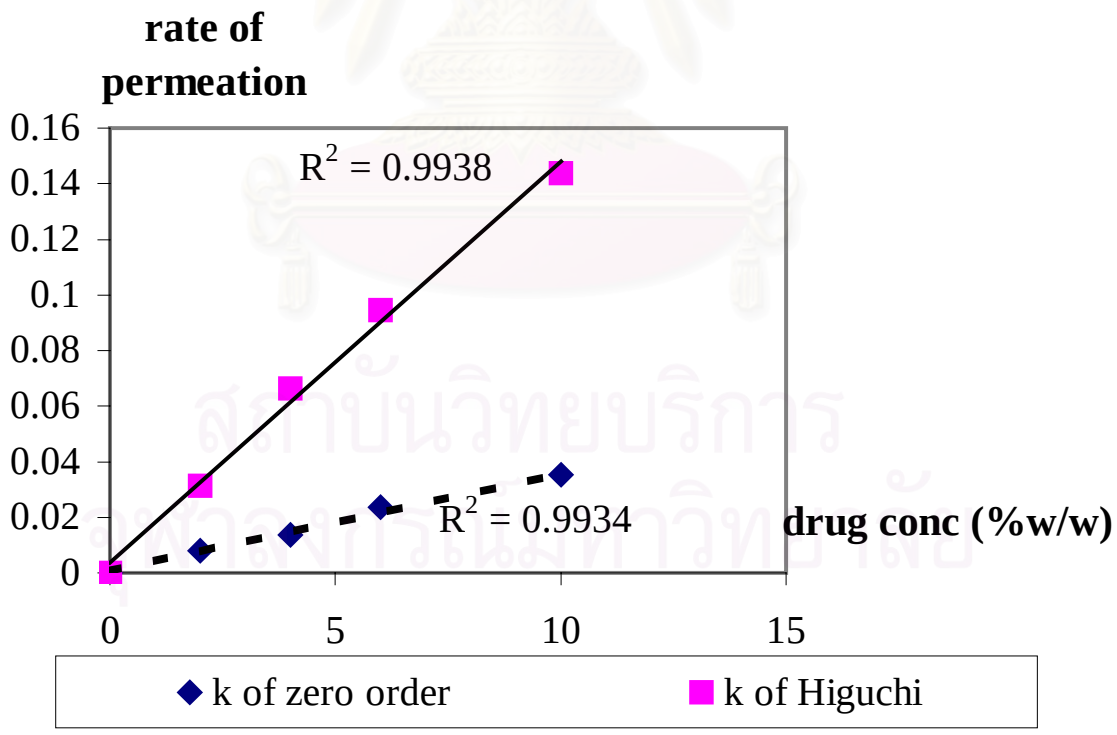


Figure 46 Regression analysis plot between rate of permeation as function to drug concentration (%w/w).

3. Evaluation of DTZ HCl TDDS Formulation

3.1 In Vitro Drug Release of DTZ HCl patch

In order to determine the pattern of release model from patch, what model (zero order, first order and Higuchi's model) was the most linear with high correlation coefficient as shown in Table 28. For release profile was shown in Fig 47.

Due to value of correlation coefficient between first order and Higuchi's model were high, therefore the further treatment was based upon use of the differential forms of the first order and Higuchi's model equations which mentioned before (equation (6) and (7)). The correlation coefficient of rate of release versus Q (r=0.9239) was higher than that of rate versus 1/Q (r=0.8156) with significance different at p-value = 0.01, tested by paired t-test statistics. Then first order model would probably be operative.

3.2 In Vitro Skin Permeation of DTZ HCl patch

Regression analysis of various kinetic models was used to indicate skin permeation, the same as drug release in patch as shown in Table 28. There was the suspecting of a pseudolatex formulation which had drug content as same as DTZ HCl patch was played similarly model of permeation through shed snake skin as patch or not. With this reason, a comparison of skin permeation between two formulations was constructed and illustrated permeation profile in Fig 47, including to correlation coefficient of these were showed in Table 28.

Due to DTZ HCl patch was modified from pseudolatex formulation to matrix-like system when preparing as a patch of TDDS. A loss of water content in DTZ HCl TDDS formulation, although all of components in the formulation was the same as pseudolatex formulation. Then the results of skin permeation model between pseudolatex formulation and DTZ HCl patch were different, indicated by correlation coefficient showed that zero order was the most linear as the accepted model for pseudolatex formulation while as DTZ HCl patch followed by Higuchi's model. Moreover, pseudolatex was high cumulative amount of drug per area at final time more than patch.

A flux calculation of DTZ HCl TDDS which had drug content 101.61mg, was 6.50×10^{-3} $mg/cm^2 \bullet hour^{-1/2}$.

Table 28 Regression analysis and correlation coefficient values for different type of formulations according to various kinetic model.

Formulas	Zero order	First order	Higuchi's model
	r	r	r
Release of patch	0.8903	0.9640	0.9590
Permeation of pseudolatex	0.9977	0.9738	0.9847
Permeation of patch	0.9685	no relation	0.9865

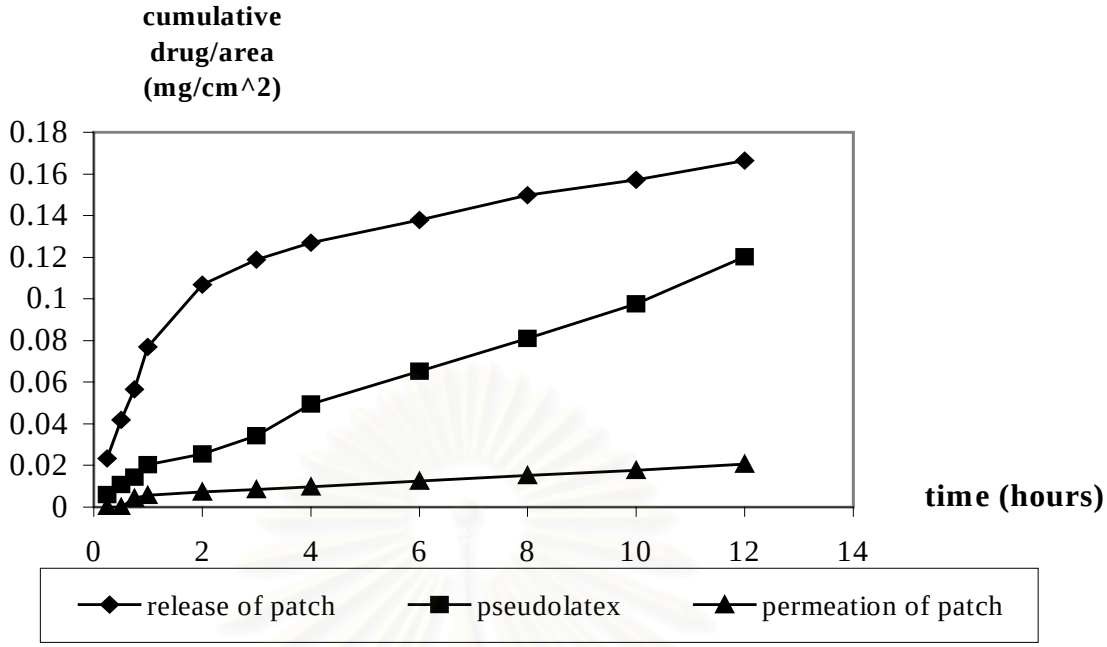


Figure 47 Average release and skin permeation time profiles of various formulation types, patch release, pseudolatex, and patch skin permeation. (n=3)

Fig. 47 showed release of patch through porous membrane ethylene vinyl acetate 9%; a matrix system which modified from pseudolatex formulation with mild condition (evaporated water content at 30 °C and 40% RH) then spherical particles of pseudolatex should be stable, but may be condense because of increasing viscosity dealing with loss of water. For permeation of pseudolatex and patch (matrix system) was used shed snake skin to determine pattern of permeation model kinetic.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

DISCUSSION

Various DTZ HCl pseudolatex formulations will be discussed as follows:

1. Preformulation of drug reservoir for DTZ HCl TDDS

1.1 Determination of water removal time

1.2 Effect of various components on the physicochemical characteristics

1.3 Effect of various components on the release profile

1.4 Effect of drug concentrations on the skin permeation profile

2. Evaluation of DTZ HCl TDDS formulation

2.1 In vitro drug release of DTZ HCl patch

2.2 In vitro skin permeation of DTZ HCl patch

CONCLUSIONS

DISCUSSION

1. Preformulation of Drug Reservoir for DTZ HCl TDDS

1.1 Determination of Water Removal Time

Pseudolatexes are obtained by a process based on emulsification-evaporation technique. The preparation method consists of a polymer solution in organic solvent being emulsified in an aqueous phase containing emulsifiers. This crude emulsion is then passed through homogenizers. After that the emulsification procedure is followed by the removal of the solvent by vacuum steam distillation, producing a fine aqueous dispersion of polymeric particles. Due to water removal time of preparation exerted an influence on particle size and aggregation of polymer then suitable duration time played an important role in the preparation, and stability of colloidal polymer dispersions. The six hours of water removal time was appropriate for pseudolatex system in order to resist sedimentation. There are many steps involved in the process to form pseudolatex formulations, first step; water in oil emulsion is occurred. Thereafter the displacement of water from the internal phase to the external phase by direct vacuum steam distillation, called reverse phase during organic solvent evaporation is got rid of from the preparation. Moreover the drug that chosen for pseudolatex formulation, is a parameter to consider because of physicochemical properties such as solubility and partition coefficient are affected preparing pseudolatex formulation. Therefore, drugs chosen should be compatible with all components in formulation and could be incorporated in the composition of pseudolatex depend upon solubility

value. DTZ HCl, 10% w/w was incorporated at the highest weight to form pseudolatex formulation. Due to a moderate value of partition coefficient between organic solvent to aqueous phosphate buffer pH 7.4 is 2.7 (Illum et al. 1983) that make DTZ HCl pseudolatex using time in order to remove water from preparation as compare with pseudolatex contained no drug. In addition another drugs might be used a different duration for water removal time, this depend upon hydrophilic property of drug, and partition coefficient value.

1.2 Effects of Various Components on the Physicochemical Characteristics

When working on new drug formulations like emulsions, suspension, pseudolatex, etc., formulator need to understand the characteristics of alternate formulations in terms of particle size and its distribution. For these type of dosage forms, particle size must be closely controlled to ensure efficient dosage unit, predictable shelf life, and batch-to-batch consistency. PCS can size particles typically in the submicrometer regime. For pseudolatex systems suitable method to measure particle size is photon correlation spectroscopy (PCS). PCS does this by measuring light intensity fluctuations caused by Brownian motion. Brownian motion is the random movement of particles due to bombardments by the solvent molecules that surround them. The size at which particles become too large for PCS usually depends on the density of the sample rather than the technique itself. Size of particles, the temperature of the system, and the viscosity of the suspending liquid affect the Brownian motion of particles in suspension. The larger the particle, the more slowly it moves. Smaller particles with less inertia receive more of a kick from solvent molecules and,

therefore, move more rapidly. The higher the temperature, the more rapid the movement overall, due to the higher kinetic energy of the solvent molecules. In certain circumstances, the particles in a dispersion may adhere to one another and form aggregates of successively increasing size that may settle out due to gravity. Therefore, one month was used to determine stability of formulations by volume of sedimentation in the experiment.

The SEM pictures illustrated a shape and appearance of pseudolatex particles, which may be one of justify factors for selecting a suitable pseudolatex formulation as a drug reservoir of TDDS. The ratios of polymer between Eudragit RL100® and RS100® were important to drug release and influenced organoleptic properties of pseudolatex with smooth and good shape. Besides these, preparation technique was another factor that should be considered together. With the above reason pure RL100® gave pseudolatex particles a good and smooth shape because it was permitted water permeate from internal phase pass through pieces of polymer to external phase easily, due to its chemical properties. RL100® has ratio between amnonium group and neutral methacrylates of 1:20 while that of RS100® is 1:40 which make different permeability, and make RL100® gave more permeable than RS100®. It might be assumed that, the more ratio of Eudragit RL100® in formulation, the easier preparing of pseudolatex formulations. The results form Table 18 show that formulations with 6% PVP K30 were more viscous than those with 4% w/w. In other words, the higher concentration of PVP K30, the more viscously of formulations, because of a consequence of functional category of PVP K30 to increase viscosity. Furthermore, DTZ HCl had an effect on pH value of

formulations because of its dissociation constant (pK_aSince DTZ HCl is a weak acid with the pK_a of 7.7, its 1% w/w solution in purified water has approximately pH of 4.2. Although DTZ HCl did not incorporate in the pseudolatex formulation, pHs of formulas showed low value (4.09± 0.12). With adding effects of polymer that composed of acrylic acid group and DTZ HCl could lower pH value of formulation when has DTZ HCl together (3.87± 0.09).

Even if DTZ HCl is a large molecule but no significant difference of particle size between pseudolatex formulations with and without DTZ HCl. It might be assumed that DTZ HCl has a suitable conformation to fit in pseudolatex formulation without changing particle size.

1.3 Effects of Various Components on the Release Profile

- Effect of polymer ratio between Eudragit RL100® and RS100®

The effect of polymer type on the model of drug release of formulation number 6, 7, 8, 9, and 10 with composition of Eudragit RL100® and RS100® in the ratio: 100:0, 80:20, 50:50, 20:80 and 0:100, respectively were determined. The analysis of all dissolution data that illustrated in Table 20 and Figs 30, 34, and 38 gave the comparison among the linearization of release rate data by the three models. Both the Higuchi plot and first order plot were linear with the correlation coefficient values of greater than 0.96. However, the Higuchi equation gave consistently higher values for the correlation coefficient than that did the first order equation. Nevertheless, since both models were acceptably linear, a more discriminating test, equation (6) and (7) as well, was utilized to distinguish between two models. The relative

validity of the test was obtained by using the differential forms of the rate equations. The result as shown in Table 21 indicated that the release data would possibly follow Higuchi's model. Main mechanism, which regulated in square root of time model is diffusion controlled.

Although release model of these formulas was identified already, the dissolution data was further analyzed to clarify drug release mechanism using power law equation as shown in equation (8). All results were shown in Table 23 with power exponent value (n), most formulations were non fickian of anomalous diffusion mechanism. This mechanism is determined that they have complex mechanism for drug release regulation. Two or more mechanisms are composed in anomalous diffusion. A couple of main mechanisms which had major influence are fickian diffusion and polymer relaxation with unequal strength depending upon k_1 and k_2 values, diffusion rate controlling constant and relaxation rate controlling constant respectively. All of the formulations played major mechanism as drug diffusion because of high value of diffusion rate controlling constant. Moreover, a comparison among Higuchi rate constants (k_H) as shown in Table 22, these indicated that formulation which composed of only Eudragit RL100® gave the highest rate constant (5.64 mg $hr^{-1/2}$), and therefore it was selected to continue studying of other effects.

- Effects of the percent of polymer and PVP K30

The drug release model of formulation number 14, 15, 6, and 16 with composition of Eudragit RL100® 10, 12, 14, and 16% w/w, respectively, together with 6% w/w of PVP K30, and formulation number 21, 22, 23, and 24 with the same composition of Eudragit

RL100® mentioned above, but different amount of PVP K30, 4% w/w of PVP K30, were determined. Table 20 and Figs 31, 35, and 39 gave the comparison for the linearization of release rate data for the three models. Both of them, the Higuchi plot and first order plot provided linearity with the correlation coefficient values of greater then 0.96. Furthermore the Higuchi equation had higher value of correlation coefficient than the first order equation. The result from Table 21 pointed out that Higuchi's model would possible be operative.

The release exponent (n), as shown in Table 23, indicated that the mechanism was anomalous transport. Since the value of diffusion rate controlling constants were greater than those of the relaxation rate controlling constants, most of these formulations seemed to had drug diffusion mechanism. Formula F#21 and F#22 were exception because the two rate constants were comparable; therefore the two mechanisms were assumed to have effect on the release.

In order to select a good formulation with good physicochemical properties and high rate of release, a comparison among Higuchi rate constants (k_H) as shown in Table 22, found out formula F#21 was the highest release rate. However the characteristics of pseudolatex particles from SEM picture of formula F#21 showed aggregation of particles and had sedimentation occur when settle more than one month while as F#22 had good appearance of particles. In addition, a comparison of release between F#21 and F#22 showed insignificant difference. Therefore F#22 was chosen to clarify effect of surfactant with high release rate constant (8.94 mg $hr^{-1/2}$).

- Effect of surfactant

Formulation number 29, 22, and 30 with composition of Tween 80 at 6, 10, and 14 %w/w, respectively were clarified the model of drug release. Figs 32, 36, and 40 gave the comparison among the linearity of release rate data by the three models. Both of Higuchi plot and first order plot were linear with the high value of correlation coefficient. The result from Table 20 and Table 21 elucidated that Higuchi's model could be fitted by the release data.

The release mechanism of the three formulas implied that anomalous transport seemed suitable to them. A trend of diffusion rate controlling constant of three formulas was higher when increased concentration of surfactant in formulation that F#30 > F#22 > F#29. This result might be assumed that surfactant (Tween 80) influence on mechanism, which acted as drug diffusion mechanism in high concentration.

Nevertheless, Higuchi rate constant value of these formulas was lower when increased concentration of surfactant. Due to chemical property of DTZ HCl, which is a highly soluble drug, the low concentration of surfactant could add effect on helping DTZ HCl diffused from pseudolatex system, while formulation with high concentration of surfactant obstructed DTZ HCl to pass easily through the system. A consideration of choosing good appearance particles from SEM picture (Fig.27), and moderately high value of release rate, was formula F#22.

- Effect of plasticizer

Formulation number 22, 34 and 35 with composition of dibutyl phthalate at 4, 6, and 10 % w/w, respectively were elucidated for the model of drug release. Figs. 33, 37, and 41 gave the comparison among the linearity of release rate data by the three models. As well as considering effect of surfactant, effect of plasticizer displayed model of release pattern as Higuchi's model. Higuchi's model was indicated that major mechanism is diffusion controlled.

Although diffusion controlled was the important mechanism of release, from anomalous transport equation, value of diffusion rate controlling constant was not differ form value of relaxation rate controlling constant that meant two mechanisms played an effect together on the release of drug from pseudolatex. This result might be expected that function of plasticizer could make polymer flexible that influence on relaxation rate constant of polymer as shown in Table 26.

In this step of selection a suitable pseudolatex formulation with high rate of release and good appearance physical properties in order to study further effect of drug concentration on skin permeation, formula F#22 was chosen.

- Effect of drug concentration on the skin permeation profile

Formulation number 22, 36, 37, and 38 with drug concentration at 2, 4, 6, and 10 % w/w, respectively were elucidated for the model of drug permeation. At the low concentration of DTZ HCl (2 % w/w), skin permeation was determined as zero order model while as the

higher concentration, Higuchi's model seemed to be operative. In addition, Fig.43 showed skin permeation of 10% drug w/w gave a lot of drug amount more than other formulas. It said that "burst effect" might affect skin permeation model. Burst effect can be explained that in formulation with high concentration of drug will occur drug crystal, it must be embedded on the surface of device and rapidly dissolved when touching dissolution medium.

As the relationship between permeation rate constant and drug concentration from Fig.46 that the effect of the changing the drug concentration on the permeation rate constant followed by zero order and Higuchi's model, was tested, using weight by weight drug concentration of DTZ HCl. Both permeation rate constants versus drug concentration plots were linear relationship with high value of correlation coefficient. These conclude that the permeation rate constant increased with the increase in drug concentration which applying to predict data in practical use in the range of 2-10 % w/w of DTZ HCl in pseudolatex formulations.

2. Evaluation of DTZ HCl TDDS Formulation

2.1 In Vitro Drug Release of DTZ HCl Patch

Formulation number 38 was chosen to prepare DTZ HCl TDDS formulation. From the release study as previously illustrated in Fig. 47. This patch formulation was elucidated to be fitted a first order model. It is displayed as a monoexponential declination of drug release.

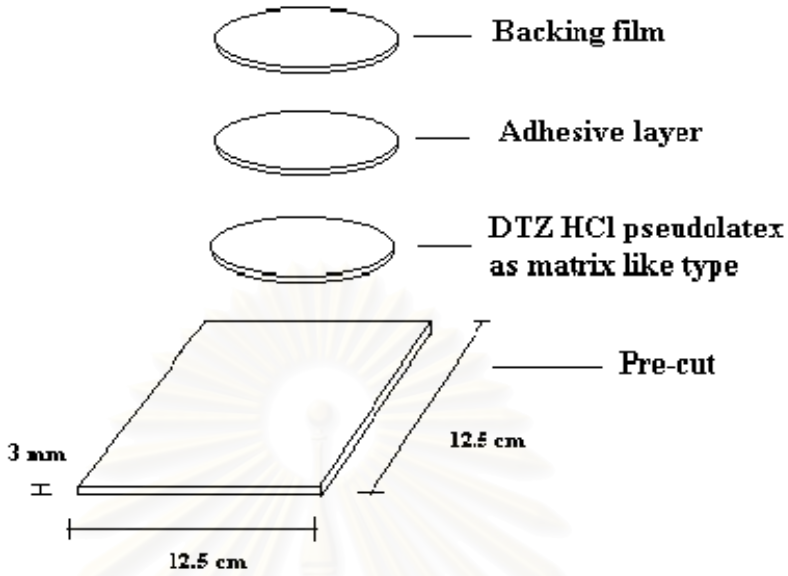


Figure 48 Schematic view of DTZ HCl patch.

The result from Fig.47 which showed release and permeation characteristics among three groups of various formulation types in different models due to pattern of patch release from porous membrane consisted of two phase which first phase had high rate while as second phase, slope was declined. It might be dealing with gradient of drug concentration because at the first phase, drug gradient was strong power, furthermore membrane that used in release of patch was porous membrane that allowed DTZ HCl and water pass through itself easily than shed snake skin. Then, amount of drug release per area was more than other types in the first phase of release pattern. However this type of preparation did not suitable to use membrane for controlling drug from patch due to excessive amount of drug that release in the first part of duration time using to study of release. It might be occur dose dumping when tested in human body.

2.2 In Vitro Skin Permeation of DTZ HCl Patch

A DTZ HCl patch was determined the permeation model kinetic would follow Higuchi's model while as pseudolatex formulation with the drug content of patch indicated to be zero order model. A comparison between two types of formulation revealed different kinetic models even though most of components had the same ratio. However, water constant in formulation was an important factor that effect on permeation kinetic model due to water content in pseudolatex formulation was more than in patch at least 15 % w/w.

For permeation of pseudolatex and patch through shed snake skin; patch had lag time because it used a time for water influx to preparation and can diffuse drug come out of patch while as pseudolatex which had more water content prompted to release drug from formulation with regular pattern. Moreover, the chemical structure of DTZ HCl which had high permeation rate and solubility included it consisted of polar and non polar parts in the molecule. Therefore, pseudolatex formulation that had surfactant acted as enhancer to form DTZ HCl in oil droplet by using non polar part while polar part might be insert between polymer layer and surfactant layer. The conformation of drug in pseudolatex system was so complex depended upon hydrophilicity value and partition coefficient of drug included other effects of components in formulation which be interesting to study in order to clarify a formation of pseudolatex particles.

A TDDS DTZ HCl formulation composed of DTZ HCl 10% Eudragit RL100® 12%, PVP K30 4% Tween 80 10%, and dibutyl phthalate 4% w/w of formulation had a flux of permeation of with drug

concentration 101.61 mg was 6.5 microgram/cm^2 $hr^{-1/2}$ followed by Higuchi's model. It might be possible that matrix system of patch played Higuchi's model because of effect of formulation which contained spherical particles in preparation and effect of surfactant to enhance drug easily to pass through skin included other unknown effects that will be clarify further in the future.

CONCLUSIONS

DTZ HCl is a calcium ion influx inhibitor that has first pass effect. Moreover, it has a short elimination half-life. Pseudolatex base is used to offer an effective approach as drug reservoir of transdermal drug delivery system. Physicochemical study and In vitro experiments were performed to characterize some factors affecting release of DTZ HCl pseudolatex formulations. In addition, in vitro skin permeation of DTZ HCl patch was considered. The results of this preliminary can be summarized as follows:

(1) The six hours of water removal time might be appropriated for pseudolatex system including with DTZ HCl pseudolatex in this experiment.

(2) For pseudolatex formulations, particle size must be closely controlled to ensure efficient formulas and stability, contain spherical solid or semisolid typically less than 1 μm that suitable to resist sedimentation and aggregation.

(3) Ratios of polymer between Eudragit RL100® and RS100® were important influence on forming pseudolatex with good appearance of particles, and easily preparing formulation, could be ranks in the following orders: 100:0 > 80:20 > 50:50 > 20:80

> 0:100 and the ranks order of drug release was obtained as follows : 100:0 > 80:20 > 50:50 > 0:100 > 20:80 indicated that RL100 100% seemed to be the best ratio of polymer for preparing pseudolatex.

(4) In vitro drug release model of all pseudolatex formulas appeared to be Higuchi's model while as in vitro drug release mechanism revealed that non fickian diffusion or anomalous transport would be operative.

(5) Effects of % polymer and PVP K30 on release rate of formulations indicated that increasing polymer concentration probably decreasing DTZ HCl release.

(6) Effect of surfactant on the release mechanism with anomalous transport, a trend of diffusion rate controlling constant was higher when increased concentration of surfactant in formulation. This result might be assumed that Tween 80 affected drug diffusion mechanism of the release.

(7) Function of plasticizer could make polymer flexible that influenced relaxation rate constant of polymer, which was one of mechanisms, played an effect on the release of drug from pseudolatex.

(8) At the low concentration of DTZ HCl (2% w/w), skin permeation was determined as zero order model while as the higher concentration, Higuchi's model seemed to be operative because of burst effect.

(9) The relationships between permeation rate constants followed by Higuchi and zero order model, and drug concentration were high value of correlation coefficient. These could be used to predict amount of drug in order to get a suitable permeation rate or

predict permeation rate in other drug concentrations in the range of 2-10 % w/w of formulation.

(10) Formulation number 38 composed of DTZ HCl 10%, Eudragit RL100® 12%, PVP K30 4% Tween 80 10%, and dibutyl phthalate 4% w/w of formulation was chosen to prepare DTZ HCl TDDS.

(11) The release model of DTZ HCl patch was elucidated to fit as a first order. For the skin permeation; a comparison between two types of formulations (DTZ HCl patch and pseudolatex) revealed different kinetic model which patch followed by Higuchi's model while as pseudolatex followed by zero order model due to water content in formulation. Furthermore, a flux of permeation of DTZ HCl TDDS formulation with drug content 101.61 mg was 6.5 microgram/ cm^2 $hr^{-1/2}$.

REFERENCES

Ansel H.C. Popovich N.G. and Allen L.V.,Jr. 1995. Transdermal drug deliery systems ointment, cream, lotion, and other preparation. Pharmaceutical Dosage Forms and Drug Delivery Systems 6th ed. pp.357-372 Penseylvania : CRC Press.

American Pharmaceutical Association Staff 1994. In Ainley Wade and Paul J Weller (eds.), Handbook of Pharmaceutical Excipients 2nd ed. Washington : The American Pharmaceutical Association.

Baker, R.W. 1987. Diffusion controlled systems. In Richard W. Baker (ed.), Controlled release of biologically active agents. pp.39-80. New York : Wiley-Interscience publication.

Balasubranmanian V. Iyer and Ravindra C. Vasavada 1979. Evaluation of lanolin alcohol films and kinetics of triamcinolone acetonide release. J. Pharm. Sci. 68(6): 782-787.

Benita, S., and Donbrow, M. 1982. Release kinetics of sparingly soluble drugs from ethylcellulose-walled microcapsules: theophylline microcapsules. J. Pharm. Pharmacol. 34: 77-82.

Bhatt, P.P., Rytting, J.H., and Topp E. M. 1991 Influence of Azone® and lauryl alcohol the transport of acetaminophen and ibuprofen through shed snake skin. Int. J. Pharm. 72: 219-226.

Bhattachar, S.N., Rytting, J. H., Itoh, T., and Nishihada, T. 1992. The effects of complexation with hydrogenated phospholipid on the transport of salicylic acid, diclofenac and indomethacin across snake stratum corneum. Int. J. Pharm. 79: 263-271.

Bronough, R.L., and Stenart, R.F. 1986. Method for in vitro percutaneous absorption study VI: preparation of the barrier layer. J. Pharm. Sci. 75: 487-491.

Chandrasekaran, S.K., and Paul, D.R. 1982. Dissolution controlled transport form dispersed matrixes. J. Pharm. Sci. 71(12):1399-1403.

Chien, Y.W. 1987. Drug delivery systems of tomorrow. Drugs of Todays 23: 31-45.

Chien, Y.W. 1992. Novel drug delivery systems (2nd ed.) Drugs and the Pharmaceutical sciences Vol.50. New York: Marcel Dekker.

Chien, Y.W., and Valia, K.H. 1984. Development of dynamic skin permeation system for long-term permeation studies. Drug Dev. Ind. Pharm. 10: 575-599.

David J., Mazzo, Obetz, C.L., and Shuster, J. 1994. Diltiazem hydrochloride. In Harry G. Brittain (eds.), Analytical Profiles of Drug substances and Excipient Vol.23. pp.53-98. USA.: Academic Press.

David R. Friend 1992. In vitro skin permeation techniques. J. Contr. Release. 18: 235-248.

Dawid, A.W. 1984. Polymeric Materials Used in Drug Delivery Systems. In Florence, A.T. (eds.), Material Used in Pharmaceutical Formulation. Frome and London: Buttler&Tanner Ltd.

Deepak Thassu and S.P. Vyas 1991. Controlled transdermal mucolytic delivery systems. Drug Dev.& Ind. Pharm. 17(4): 561-576.

Dehghan, M.H.G., Parakh, S.R., and Deshpande S.G. 1993. Studies on polymeric systems for transdermal drug delivery. Indian Drugs 30 (12): 616-621.

Efentakis, M., Buckton, G. 1990. Modeling drug release from hydrophobic matrices by use of thermodynamic activation parameters. Int. J. Pharm. 60: 229-234.

F.Bottari, G.D.Colo, E. Nannipieri, M.F. Saettone, and M.F. Serafini. 1974 Influence of drug concentration on in vitro release of salicylic acid from ointment bases. J.Pharm.Sci. 63: 1779-1783

Franz, T.J. 1975. Percutaneous absorption on the relevance of in vitro data. J. Invest. Dermatol. T4: 190-193.

Gummer, C.L., Hinz, R.S., and Maibach, H.I. 1987. The skin penetration cell: a design update. Int. J. Pharm. 40: 101-104.

Harada, K., and others 1992. In-vitro permeability to salicylic acid of human, rodent, and shed snake skin. J. Pharm. Pharmacol. 45: 414-418.

Heller J. 1984. Recent advances in drug delivery systems. In JM. Anderson and SW. Kim (eds.), pp. 101-121. New York: T Lenum.

Higuchi, T. 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed on solid matrices. J. Pharm. Sci. 52(12): 1145-1149.

Hopfen Derg HB. 1976. Controlled release polymeric formulations. In Dr.Paul and FW. Ham's (eds.), pp. 26-32. Washington DC:ACS Symposium, series 33.

Itoh, T., Xia, J.,Magavi, R., Nishihata, and Rytting, J.H. 1990. Use of shed snake skin as a model membrane for in-vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. 7 (10): 1042-1047.

Itoh, T., Wasinger, L., Turunen, T.M., and Rytting, J.H. 1992. Effects of transdermal penetration enhancers on the permeability of shed snake skin. Pharm. Res. 9(9): 1168-1172.

Jain, S.K. Vyas S.P., and Dixit V.K. 1990. Effective and controlled transdermal delivery of ephedrine. J. Contr. Release. 12: 257-263.

Jain, S.K. Vyas S.P., and Dixit V.K. 1994. Development and evaluation of transdermal salbutamol delivery systems. Drug Dev. & Ind. Pharm. 17(4): 1991-2003.

James, M. Anderson and Sung Wan Kim 1986. Advances in Drug Delivery systems (Controlled Released Series,1). pp.89-120. USA. : Elsevier science publishing Com, Inc.

Keshary, P.R., and Chien, Y.W. 1984. Mechanisms of transdermal controlled nitroglycerin administration (I): development of a finite-dosing skin permeation system. Drug Dev. & Ind. Pharm. 10: 883-913.

Kydonieus, A.F., and Berner, B. 1987. Transdermal Delivery of Drugs Vol.1-3. Florida:CRC Press.

Mann, P.S. 1995. Statisticsl business ans economics : Multiple regression. pp.722-765. USA.: John Wilely & Sons, Inc.

M. Kittgen, M. Durrani and K. Lehmann. 1997. Acrylic polymers: a review of pharmaceutical applications. S.T.P. Pharma.Sci. 7(6): 403-437.

Micaela M.-T. Buckley, Susan M. Grant, Karen L. Goa, Donna Mctavish and Ugene M. Sorkin. 1990. Diltiazem: a reappraisal of its pharmacological properties and therapeutic use. Drugs 39(5): 757-806.

Mills, S.N., and Davis, S.S. 1987. Controlled drug delivery. In Lisbeth Illum and Stanley S.Davis (eds.), Polymer in Controlled drug delivery. Great Britain: IOP Publishing Limited.

M.M. Feldstein, V.N. Tohnakhchi, L.B. Malkhazov, A.E. Vasiliev, N.A. Plat′e 1996. Hydrophillic polymeric matrices for enhanced transdermal drug delivery. Int. J. Pharm. 131: 229-242.

Peppas, N.A. 1985. Analysis of fickian and non-fickian drug release from polymers. Pharm. Acta Helv. 60(4): 110-111.

Peppas, N.A., and Sahlin, J.J. 1989. A simple equation for the description of solute release III. Coupling of diffusion and relaxation. Int. J. Pharm.. 57(4): 169-172.

Pierfrancesco Morganti, Alessio Fionda, Licia Tiberi and Mavi Sud 1999. The transdermal cosmetic delivery system. Allured's Cosmatic & Toiletries magazine Vol. 114(2): 53-58.

Pillai, J.C., Baber, A., and Plakogiannis, F.M. 1988. Polymers in cosmetic and pharmaceutical industries. Pharm. Acta Helv. 63: 46-53.

Pillay, V., and Fassihi, R. 1998. Evaluation and comparison of dissolution data derived from different modified release dosage forms : an alternative method. J. Contr Release. 55:45-55.

P. Rama Rao and Prakash V. Diwan 1998. Formulation and in vitro evaluation of polymeric films of diltiazem hydrochloride and indomethacin for transdermal administration. Drug Dev. & Ind. Pharm. 24(4): 327-336.

Quintanar-Guerrero, E. All′emann, H. Fessi, E. Doelker 1999. Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. Int. J. Pharm. 188: 155-164.

Rande, V.V., and Hollinger, M.A. 1996. Drug delivery systems. Florida:CRC press, Inc.

Reynolds, J.E.F. 1993. Martindale the Extra Pharmacopoeia 30th ed. London: The Pharmaceutical Press.

Rong-Kun Chang, Jame C. Price and Charles Hsiao 1989. Preparation and preliminary evaluation of eudragit RL and RS pseudolatices for controlled drug release. Drug Dev. & Ind. Pharm. 15(3): 361-372.

S.P. Vyas, C.R. Jain, S. Gupta and A. Uppadhayay 1994. Polymeric pseudolatices dispersion bearing isosorbide dinitrate for transdermal application. Drug Dev. & Ind. Pharm. 17(8): 1041-1058.

S.P. Vyas, P.J. Gogoi and S.K. Lain 1991. Development and Characterization of pseudolatex based transdermal drug delivery systems of diclofenac. Drug Dev. & Ind. Pharm. 17(8): 1041-1058.

Sa, Bandyopadhyay A.K., and Gupta, D.K. 1990 Development and in vitro evaluation of ethylcellulose micropellets as a controlled release dosage form theophylline. Drug Dev. & Ind. Pharm. 16: 1153-1163.

Schwartz, J.B., Simonelli, A.P., and Higuchi, W.T. 1968. Drug release from wax matrices I: analysis of data with first-order kinetics and with the diffusion controlled model. J. Pharm. Sci. 57(2): 274-277.

Sood, A., and Panchagunula, R. 1998. Drug release evaluation of diltiazem CR preparations. Int. J. Pharm. 175: 95-107.

Steven M. Wick, R. Ph., 1995. Developing a drug-In-Adhesive design for transdermal drug delivery. Adhesives Age September.

Triggle, D.J. 1990. Calcium Antagonists. In M. Antonaccio (ed.), Cardiovascular Pharmacology 3rd ed. New York: Raven.

Vanderhoff, J.W., El-Aasser, M.S., Ugelstad, J. 1979. Polymer emulsification process. U.S. Pat. 4,177,177.

Yamaguchi Yukiya, Sato Hiroshi, Sugibayashi Kenji and Morimoto Yasunori. 1996. Drug release test to assess quality of topical formulations in Japanese market. Drug Dev & Ind. Pharm. 22(7): 569-577.

Zatz, J.L. 1990. Scratching the surface: skin permeation. Cosm & Toilet. 105: 229-241.

APPENDICES

Appendix A

Standard calibration curve

A data and profile of relationship between drug concentration and absorbency at appropriate wavelength of DTZ HCl in various media were presented below in Tables 29-30 and Figs.46-47.

Suitable wavelength of DTZ HCl in phosphate buffer pH 7.4 and mixed solvent between methylenechloride and methanol = 1:1 were 236 nm and 241 nm, respectively.

Table 29 Concentration and absorbency data for DTZ HCl in phosphate buffer pH 7.4.

Concentration (mcg/ml)	Absorbency
0	0
4	0.240
6	0.358
10	0.574
12	0.695
14	0.801
16	0.906
18	1.019

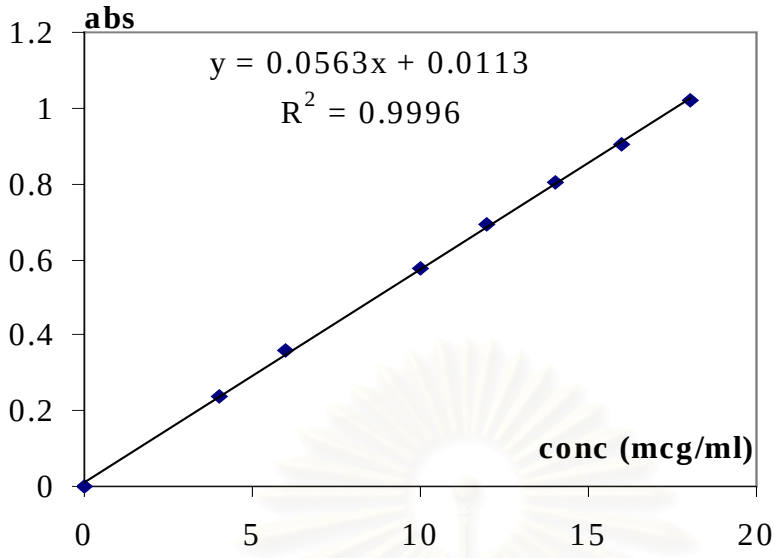


Figure 46 Standard calibration curve of DTZ HCl in phosphate buffer pH 7.4.

Table 30 Concentration and absorbency data for DTZ HCl in mixed solvent.

Concentration (mcg/ml)	Absorbency
0	0
2.20	0.118
4.40	0.240
8.80	0.464
11.00	0.570
13.20	0.689
15.40	0.810

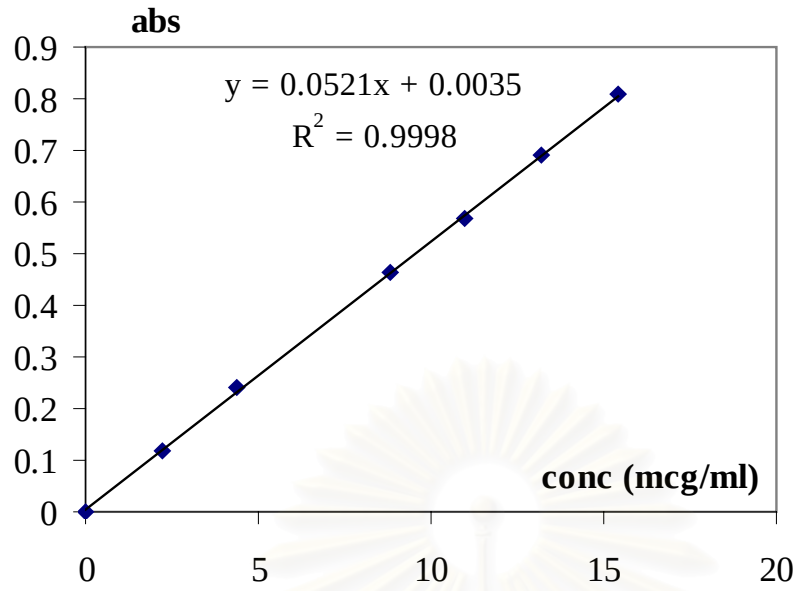


Figure 46 Standard calibration curve of DTZ HCl in mixed solvent

Appendix B

Determination of DTZ HCl content in pseudolatex

weigh of formulas		gram of pseudolatex to analyze			quantity of drug in pseudolatex			% content of drug			Mean	SD
	gram	1	2	3	1	2	3	1	2	3		
F#6 (2%)	81.98	0.5101	0.5021	0.5170	0.0122	0.0120	0.0124	98.20	98.10	98.45	98.25	0.1472
F#7 (2%)	81.38	0.5207	0.5250	0.5188	0.0123	0.0124	0.0121	95.77	96.00	95.10	95.62	0.3818
F#8 (2%)	78.22	0.5022	0.5000	0.5023	0.0121	0.0121	0.0121	94.50	94.69	94.50	94.56	0.0896
F#9 (2%)	78.42	0.5088	0.5172	0.5253	0.0122	0.0124	0.0127	93.79	94.22	94.95	94.32	0.4788
F#10 (2%	74.72	0.5034	0.5106	0.5158	0.0123	0.0124	0.0126	90.98	91.01	91.42	91.14	0.2007
F#14(2%)	82.02	0.5215	0.5162	0.5135	0.0112	0.0109	0.0108	87.94	86.56	86.45	86.98	0.6780
F#15 (2%	81.25	0.5099	0.5182	0.5352	0.0114	0.0117	0.0121	91.11	91.87	92.22	91.73	0.4633
F#16 (2%	81.42	0.5226	0.5263	0.5300	0.0122	0.0123	0.0124	94.65	95.26	95.58	95.16	0.3858
F#21 (2%	80.36	0.5118	0.5162	0.5117	0.0123	0.0125	0.0123	96.63	97.07	96.62	96.77	0.2098
F#22 (2%	81.73	0.5035	0.5212	0.5074	0.0120	0.0125	0.0121	97.57	98.00	97.77	97.78	0.1757
F#23 (2%	80.78	0.5163	0.5129	0.5105	0.0118	0.0117	0.0116	92.42	92.12	92.02	92.19	0.1700
F#24 (2%	80.47	0.5175	0.5198	0.5298	0.0113	0.0114	0.0118	88.17	88.28	89.59	88.68	0.6450
F#29 (2%	81	0.5229	0.5250	0.5114	0.0123	0.0124	0.0120	95.57	95.70	95.05	95.44	0.2808
F#30 (2%	80.45	0.5100	0.5140	0.5160	0.0118	0.0119	0.0119	93.08	93.10	93.13	93.10	0.0205
F#34 (2%	83.08	0.5180	0.5211	0.5177	0.0111	0.0113	0.0111	88.93	90.00	88.92	89.28	0.5068
F#35 (2%	80.2	0.5200	0.5195	0.5101	0.0125	0.0124	0.0121	96.61	95.95	95.24	95.93	0.5594
F#36 (4%	81.4	0.2505	0.2611	0.2559	0.0120	0.0127	0.0123	97.57	98.89	98.19	98.22	0.5392
F#37 (6%	79.6	0.2498	0.2504	0.2510	0.0179	0.0181	0.0182	95.25	96.16	96.16	95.86	0.4290
F#38(10%	78.87	0.2544	0.2580	0.2535	0.0315	0.0321	0.0314	97.67	98.18	97.70	97.85	0.2337

Example of calculated % content of drug in pseudolatex

Concentration of drug with suitable dilution for UV determination was calculated by absorbance value in the equatio

$$y = 0.0521x + 0.0035$$

Quantity of drug in pseudolatex with dilution ratio 1:25 & 1:50 in gram of pseudolatex to analyze by UV such as F#6 has quantity of drug =(9.78 mcg * 25*50)/1000 = 12.22 mg in 0.5101 g of pseudolatex to analyze.

Total weight of pseudolatex of F#6 was 81.98 g then total quantity of drug = (0.01222/0.5101)*81.98 = 1.964 g

% content for F#6 which had 2% w/w of DTZ HCl in formulation = (1.964/2)*100 = 98.20

Appendix C

Drug release data from dissolution study

Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα	Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα
			Mean *	SD						Mean *	SD		
F#6	0.00	0.00	0.00	0.00	2.2485	0.0000	F#7	0.00	0.00	0.00	0.00	2.2482	0.0000
	0.25	0.50	1.44	0.44	2.2450	0.0081		0.25	0.50	1.53	0.40	2.2444	0.0086
	0.50	0.71	2.58	0.42	2.2420	0.0146		0.50	0.71	2.27	0.40	2.2426	0.0128
	0.75	0.87	3.60	0.49	2.2395	0.0203		0.75	0.87	3.32	0.51	2.2400	0.0187
	1	1.00	4.68	0.59	2.2370	0.0264		1	1.00	4.19	0.54	2.2378	0.0237
	2	1.41	7.51	0.73	2.2300	0.0424		2	1.41	6.60	0.81	2.2317	0.0373
	3	1.73	9.29	0.88	2.2250	0.0524		3	1.73	8.18	1.05	2.2276	0.0462
	4	2.00	11.27	0.91	2.2200	0.0636		4	2.00	9.70	1.04	2.2237	0.0548
	6	2.45	13.09	0.88	2.2150	0.0739		6	2.45	11.18	1.44	2.2198	0.0631
	8	2.83	14.94	1.32	2.2100	0.0843		8	2.83	13.13	1.32	2.2147	0.0742
	10	3.16	16.44	1.28	2.2060	0.0928		10	3.16	14.56	1.74	2.2109	0.0822
	12	3.46	18.03	1.19	2.2020	0.1017		12	3.46	16.19	1.58	2.2065	0.0914
F#8	0.00	0.00	0.00	0.00	2.2497	0.000	F#9	0.00	0.00	0.00	0.00	2.2465	0.0000
	0.25	0.50	1.49	0.32	2.2466	0.0084		0.25	0.50	1.71	0.07	2.2423	0.0097
	0.50	0.71	2.52	0.39	2.2440	0.0142		0.50	0.71	2.26	0.15	2.2409	0.0128
	0.75	0.87	3.09	0.40	2.2430	0.0174		0.75	0.87	2.86	0.20	2.2394	0.0162
	1	1.00	3.53	0.47	2.2420	0.0198		1	1.00	3.25	0.21	2.2384	0.0184
	2	1.41	5.74	0.52	2.2360	0.0323		2	1.41	4.66	0.23	2.2349	0.0264
	3	1.73	7.06	0.58	2.2330	0.0397		3	1.73	5.39	0.27	2.2330	0.0306
	4	2.00	8.16	0.59	2.2300	0.0459		4	2.00	6.61	0.20	2.2299	0.0375
	6	2.45	10.58	0.60	2.2240	0.0595		6	2.45	7.23	0.31	2.2283	0.0410
	8	2.83	12.37	0.64	2.2190	0.0696		8	2.83	9.11	0.44	2.2235	0.0516
	10	3.16	13.68	0.61	2.2150	0.0770		10	3.16	10.31	0.55	2.2204	0.0584
	12	3.46	15.39	0.82	2.2110	0.0866		12	3.46	11.53	0.58	2.2171	0.0654

* Mean of five determinations.

Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα	Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα
			Mean *	SD						Mean *	SD		
F#10	0.00	0.00	0.00	0.00	2.2491	0.0000	F#14	0.00	0.00	0.00	0.00	2.2472	0.0000
	0.25	0.50	2.70	0.53	2.2420	0.0152		0.25	0.50	1.77	0.36	2.2430	0.0100
	0.50	0.71	3.45	0.50	2.2410	0.0194		0.50	0.71	2.89	0.43	2.2400	0.0164
	0.75	0.87	4.10	0.54	2.2390	0.0231		0.75	0.87	4.08	0.39	2.2370	0.0231
	1	1.00	4.70	0.55	2.2370	0.0265		1	1.00	4.92	0.42	2.2350	0.0278
	2	1.41	6.35	0.60	2.2330	0.0358		2	1.41	8.33	0.57	2.2260	0.0471
	3	1.73	7.82	0.58	2.2296	0.0441		3	1.73	11.08	0.67	2.2190	0.0627
	4	2.00	9.10	0.57	2.2260	0.0513		4	2.00	13.56	0.83	2.2130	0.0767
	6	2.45	10.37	1.05	2.2230	0.0584		6	2.45	14.97	0.98	2.2090	0.0847
	8	2.83	11.78	0.62	2.2190	0.0664		8	2.83	19.21	1.16	2.1970	0.1087
	10	3.16	13.40	0.68	2.2150	0.0755		10	3.16	21.95	1.19	2.1900	0.1242
	12	3.46	15.01	0.62	2.2110	0.0845		12	3.46	23.46	1.45	2.1850	0.1327
F#15	0.00	0.00	0.00	0.00	2.2454	0.000	F#16	0.00	0.00	0.00	0.00	2.2446	0.0000
	0.25	0.50	2.29	0.24	2.2400	0.0130		0.25	0.50	0.19	0.21	2.2440	0.0011
	0.50	0.71	2.98	0.23	2.2380	0.0170		0.50	0.71	1.68	0.21	2.2400	0.0096
	0.75	0.87	3.91	0.23	2.2360	0.0222		0.75	0.87	2.80	0.24	2.2380	0.0159
	1	1.00	4.75	0.16	2.2340	0.0270		1	1.00	3.88	0.30	2.2350	0.0221
	2	1.41	7.33	0.19	2.2270	0.0417		2	1.41	7.13	0.25	2.2270	0.0406
	3	1.73	9.63	0.57	2.2210	0.0547		3	1.73	9.50	0.44	2.2200	0.0541
	4	2.00	11.44	0.59	2.2160	0.0650		4	2.00	11.19	0.38	2.2160	0.0637
	6	2.45	14.26	0.68	2.2090	0.0811		6	2.45	15.51	0.64	2.2040	0.0883
	8	2.83	18.26	1.58	2.1980	0.1038		8	2.83	19.68	0.35	2.1930	0.1121
	10	3.16	19.03	1.80	2.1960	0.1082		10	3.16	23.04	0.73	2.1840	0.1312
	12	3.46	22.13	1.28	2.1870	0.1258		12	3.46	25.71	0.88	2.1760	0.1464

* Mean of five determinations.

Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα	Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα
			Mean *	SD						Mean *	SD		
F#21	0.00	0.00	0.00	0.00	2.2465	0.0000	F#22	0.00	0.00	0.00	0.00	2.2470	0.0000
	0.25	0.50	2.07	0.42	2.2410	0.0117		0.25	0.50	2.00	0.11	2.2420	0.0113
	0.50	0.71	2.99	0.45	2.2390	0.0170		0.50	0.71	2.86	0.19	2.2400	0.0162
	0.75	0.87	4.08	0.50	2.2360	0.0231		0.75	0.87	3.84	0.27	2.2375	0.0217
	1	1.00	4.76	0.57	2.2345	0.0270		1	1.00	4.78	0.32	2.2350	0.0270
	2	1.41	7.97	0.72	2.2260	0.0452		2	1.41	8.05	0.50	2.2270	0.0456
	3	1.73	11.00	0.85	2.2180	0.0623		3	1.73	10.73	0.62	2.2200	0.0607
	4	2.00	13.70	0.95	2.2110	0.0777		4	2.00	13.05	0.71	2.2140	0.0739
	6	2.45	18.25	1.17	2.1990	0.1034		6	2.45	17.04	1.02	2.2030	0.0965
	8	2.83	23.11	1.39	2.1850	0.1310		8	2.83	21.42	1.47	2.1910	0.1213
	10	3.16	27.19	1.53	2.1740	0.1542		10	3.16	24.63	1.29	2.1820	0.1395
	12	3.46	30.69	1.94	2.1630	0.1740		12	3.46	28.32	1.43	2.1710	0.1604
F#23	0.00	0.00	0.00	0.00	2.2484	0.000	F#24	0.00	0.00	0.00	0.00	2.2475	0.0000
	0.25	0.50	0.51	0.10	2.2470	0.0029		0.25	0.50	1.24	0.28	2.2440	0.0070
	0.50	0.71	1.57	0.07	2.2440	0.0089		0.50	0.71	2.31	0.24	2.2420	0.0131
	0.75	0.87	2.75	0.09	2.2416	0.0155		0.75	0.87	3.19	0.28	2.2400	0.0180
	1	1.00	3.81	0.11	2.2390	0.0215		1	1.00	4.08	0.31	2.2370	0.0231
	2	1.41	6.97	0.16	2.2310	0.0393		2	1.41	6.67	0.43	2.2310	0.0377
	3	1.73	9.02	0.24	2.2260	0.0509		3	1.73	8.51	0.34	2.2260	0.0481
	4	2.00	10.62	0.30	2.2220	0.0599		4	2.00	10.52	0.38	2.2210	0.0595
	6	2.45	14.10	0.66	2.2120	0.0796		6	2.45	13.60	0.52	2.2130	0.0769
	8	2.83	17.00	0.77	2.2040	0.0960		8	2.83	16.49	0.57	2.2050	0.0933
	10	3.16	20.26	0.84	2.1960	0.1144		10	3.16	19.45	0.82	2.1970	0.1100
	12	3.46	21.86	1.20	2.1910	0.1234		12	3.46	21.13	0.72	2.1920	0.1195

* Mean of five determinations.

Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα	Formulas	Time	Time^1/2	mg cumulative		log drug remaining	Mt/Mα
			Mean *	SD						Mean *	SD		
F#29	0.00	0.00	0.00	0.00	2.2467	0.0000	F#30	0.00	0.00	0.00	0.00	2.2443	0.0000
	0.25	0.50	3.10	0.50	2.2390	0.0176		0.25	0.50	1.84	0.15	2.2400	0.0105
	0.50	0.71	4.35	0.65	2.2360	0.0247		0.50	0.71	2.53	0.09	2.2380	0.0144
	0.75	0.87	5.02	0.54	2.2340	0.0285		0.75	0.87	3.92	0.16	2.2340	0.0223
	1	1.00	5.88	0.58	2.2320	0.0333		1	1.00	5.11	0.29	2.2310	0.0291
	2	1.41	9.56	0.27	2.2230	0.0542		2	1.41	8.28	0.49	2.2230	0.0472
	3	1.73	12.29	0.44	2.2150	0.0696		3	1.73	9.98	0.56	2.2190	0.0568
	4	2.00	14.74	0.66	2.2090	0.0835		4	2.00	12.05	0.82	2.2130	0.0686
	6	2.45	19.78	0.54	2.1950	0.1121		6	2.45	14.78	0.90	2.2060	0.0842
	8	2.83	25.45	1.73	2.1790	0.1442		8	2.83	17.81	1.23	2.1980	0.1015
	10	3.16	28.29	0.76	2.1710	0.1603		10	3.16	20.77	0.94	2.1895	0.1183
	12	3.46	32.61	1.78	2.1580	0.1848		12	3.46	22.42	1.52	2.1850	0.1278
F#34	0.00	0.00	0.00	0.00	2.2441	0.000	F#35	0.00	0.00	0.00	0.00	2.2460	0.0000
	0.25	0.50	1.94	0.13	2.2390	0.0111		0.25	0.50	1.91	0.15	2.2410	0.0109
	0.50	0.71	2.66	0.19	2.2370	0.0152		0.50	0.71	2.45	0.12	2.2400	0.0139
	0.75	0.87	3.53	0.25	2.2350	0.0201		0.75	0.87	2.91	0.08	2.2390	0.0165
	1	1.00	4.39	0.37	2.2330	0.0250		1	1.00	3.51	0.10	2.2370	0.0199
	2	1.41	7.58	0.28	2.2250	0.0432		2	1.41	5.72	0.21	2.2320	0.0325
	3	1.73	9.76	0.30	2.2190	0.0556		3	1.73	7.70	0.26	2.2260	0.0437
	4	2.00	11.93	0.48	2.2135	0.0680		4	2.00	9.63	0.18	2.2210	0.0546
	6	2.45	15.00	0.53	2.2050	0.0855		6	2.45	13.05	0.37	2.2120	0.0741
	8	2.83	17.93	0.38	2.1970	0.1022		8	2.83	16.54	0.76	2.2030	0.0939
	10	3.16	21.53	0.64	2.1870	0.1227		10	3.16	18.65	0.35	2.1970	0.1058
	12	3.46	23.86	1.49	2.1810	0.1360		12	3.46	21.41	0.68	2.1900	0.1215

* Mean of five determinations.

Formulas	Time	Time^1/2	mg cum permeation		log drug	Formulas	Time	Time^1/2	mg cum permeation		log drug
			Mean*	SD	remaining				Mean*	SD	remaining
F#22	0.00	0.00	0	0	0.0794	F#36	0.00	0.00	0	0	0.0901
	0.25	0.50	0	0	0.0794		0.25	0.50	0	0	0.0901
	0.50	0.71	0	0	0.0794		0.50	0.71	0	0	0.0901
	0.75	0.87	0	0	0.0794		0.75	0.87	0	0	0.0901
	1	1.00	0	0	0.0794		1	1.00	0.7137	0.0043	0.0900
	2	1.41	0.5716	0.0069	0.0794		2	1.41	1.1691	0.0041	0.0900
	3	1.73	0.7133	0.0052	0.0794		3	1.73	1.4024	0.0045	0.0900
	4	2.00	0.8961	0.0036	0.0794		4	2.00	1.9448	0.0211	0.0900
	6	2.45	1.1444	0.0074	0.0793		6	2.45	2.5182	0.0297	0.0900
	8	2.83	1.4210	0.0053	0.0793		8	2.83	3.3125	0.0143	0.0899
	10	3.16	2.0994	0.0104	0.0792		10	3.16	3.9057	0.0202	0.0899
	12	3.46	2.8619	0.0176	0.0792		12	3.46	4.5153	0.0150	0.0899
F#37	0.00	0.00	0	0	0.0963	F#38	0.00	0.00	0	0	0.1042
	0.25	0.50	0	0	0.0963		0.25	0.50	0	0	0.1042
	0.50	0.71	0	0	0.0963		0.50	0.71	0.5864	0.0119	0.1041
	0.75	0.87	0	0	0.0963		0.75	0.87	1.4863	0.0238	0.1041
	1	1.00	1.0631	0.0036	0.0963		1	1.00	2.6022	0.0207	0.1041
	2	1.41	1.7119	0.0238	0.0963		2	1.41	3.5711	0.0261	0.1041
	3	1.73	2.4444	0.0139	0.0962		3	1.73	4.8248	0.0238	0.1041
	4	2.00	3.3447	0.0194	0.0962		4	2.00	6.6506	0.0435	0.1040
	6	2.45	4.8572	0.0372	0.0962		6	2.45	8.0810	0.0480	0.1040
	8	2.83	5.6799	0.0230	0.0961		8	2.83	9.3599	0.0225	0.1040
	10	3.16	6.4000	0.0353	0.0961		10	3.16	10.7698	0.0496	0.1040
	12	3.46	7.6851	0.0370	0.0961		12	3.46	12.0564	0.0303	0.1039

*Mean of three determinations

Formulas	time	mg cum*	SD	Formulas	time	mg cum*	SD	Formulas	time	mg cum*	SD
release of	0.00	0	0	pseudolatex	0.00	0	0	permeation	0.00	0	0
patch	0.25	0.0233	0.0028		0.25	0.0061	0.0012	of patch	0.25	0	0
	0.50	0.0419	0.0045		0.50	0.0109	0.0018		0.50	0	0
	0.75	0.0566	0.0075		0.75	0.0144	0.0042		0.75	0.0045	0.0014
	1.00	0.0769	0.0084		1.00	0.0205	0.0047		1.00	0.0056	0.0012
	2.00	0.1068	0.0059		2.00	0.0255	0.0055		2.00	0.0073	0.0013
	3.00	0.1188	0.0047		3.00	0.0341	0.0062		3.00	0.0085	0.0017
	4.00	0.1270	0.0099		4.00	0.0494	0.0120		4.00	0.0097	0.0022
	6.00	0.1378	0.0155		6.00	0.0654	0.0126		6.00	0.0124	0.0029
	8.00	0.1497	0.0223		8.00	0.0810	0.0142		8.00	0.0152	0.0038
	10.00	0.1573	0.0283		10.00	0.0976	0.0145		10.00	0.0177	0.0041
	12.00	0.1664	0.0310		12.00	0.1203	0.0161		12.00	0.0207	0.0045

* Mean of three determinations.

Appendix D

Data process of rate of release, 1/Q and Q

Formulas	Mean time	dQ/dt*	1/Q*	Q*	Formulas	Mean time	dQ/dt*	1/Q*	Q*
F#6	0.125	3.2370	1.3174	0.8093	F#7	0.125	3.4475	1.2267	0.8619
	0.375	2.5866	0.6987	1.4559		0.375	1.6660	0.8015	1.2784
	0.625	2.2956	0.4987	2.0298		0.625	2.3791	0.5438	1.8732
	0.875	2.4446	0.3828	2.6410		0.875	1.9715	0.4279	2.3660
	1.500	1.5932	0.2377	4.2342		1.500	1.3589	0.2715	3.7250
	2.500	1.0071	0.1920	5.2413		2.500	0.8917	0.2194	4.6167
	3.500	1.1183	0.1579	6.3596		3.500	0.8567	0.1843	5.4734
	5.000	0.5134	0.1358	7.3864		5.000	0.4184	0.1604	6.3103
	7.000	0.5201	0.1193	8.4266		7.000	0.5510	0.1359	7.4124
	9.000	0.4258	0.1082	9.2781		9.000	0.4020	0.1230	8.2164
	11.000	0.4460	0.0986	10.1701		11.000	0.4598	0.1102	9.1359
F#8	0.125	3.3535	1.2353	0.8384	F#9	0.125	3.8843	1.0311	0.9711
	0.375	2.3047	0.7196	1.4146		0.375	1.2494	0.7818	1.2834
	0.625	1.3044	0.5815	1.7407		0.625	1.3619	0.6182	1.6239
	0.875	0.9703	0.5109	1.9832		0.875	0.8830	0.5439	1.8447
	1.500	1.2434	0.3117	3.2266		1.500	0.7975	0.3790	2.6421
	2.500	0.7463	0.2529	3.9730		2.500	0.4126	0.3279	3.0548
	3.500	0.6159	0.2187	4.5888		3.500	0.6902	0.2671	3.7450
	5.000	0.6808	0.1685	5.9504		5.000	0.1769	0.2442	4.0988
	7.000	0.5033	0.1440	6.9570		7.000	0.5313	0.1940	5.1614
	9.000	0.3697	0.1301	7.6964		9.000	0.3399	0.1715	5.8411
	11.000	0.4807	0.1157	8.6579		11.000	0.3486	0.1532	6.5384

*Mean of five determinations.

Formulas	Mean time	dQ/dt*	1/Q*	Q*	Formulas	Mean time	dQ/dt*	1/Q*	Q*
F#10	0.125	6.0907	1.7368	0.6768	F#14	0.125	3.9944	1.0294	0.9986
	0.375	1.6864	0.7490	0.5232		0.375	2.5524	0.6204	1.6367
	0.625	1.4586	0.6369	0.4391		0.625	2.6789	0.4363	2.3064
	0.875	1.3644	0.5722	0.3815		0.875	1.9093	0.3611	2.7838
	1.500	0.9296	0.4072	0.2815		1.500	1.9278	0.2129	4.7116
	2.500	0.8282	0.3460	0.2279		2.500	1.5569	0.1599	6.2685
	3.500	0.7202	0.2989	0.1957		3.500	1.4007	0.1307	7.6692
	5.000	0.3587	0.2074	0.1724		5.000	0.4003	0.1184	8.4698
	7.000	0.3970	0.1995	0.1510		7.000	1.1975	0.0922	10.8648
	9.000	0.4561	0.1969	0.1327		9.000	0.7758	0.0807	12.4163
	11.000	0.4519	0.1842	0.1184		11.000	0.4265	0.0755	13.2693
F#15	0.125	5.2148	0.7732	1.3037	F#16	0.125	0.7732	-20.7553	0.1093
	0.375	1.5707	0.5923	1.6964		0.375	0.5923	1.0585	0.9549
	0.625	2.1084	0.4510	2.2235		0.625	0.4510	0.6314	1.5918
	0.875	1.9089	0.3707	2.7007		0.875	0.3707	0.4550	2.2066
	1.500	1.4663	0.2401	4.1670		1.500	0.2401	0.2467	4.0569
	2.500	1.3049	0.1833	5.4719		2.500	0.1833	0.1851	5.4094
	3.500	1.0295	0.1542	6.5014		3.500	0.1542	0.1571	6.3679
	5.000	0.8032	0.1236	8.1077		5.000	0.1236	0.1134	8.8316
	7.000	1.1365	0.0969	10.3806		7.000	0.0969	0.0893	11.2057
	9.000	0.2189	0.0931	10.8184		9.000	0.0931	0.0763	13.1165
	11.000	0.8803	0.0797	12.5789		11.000	0.0797	0.0684	14.6375

*Mean of five determinations.

Formulas	Mean time	dQ/dt*	1/Q*	Q*	Formulas	Mean time	dQ/dt*	1/Q*	Q*
F#21	0.125	4.6981	0.8740	1.1745	F#22	0.125	4.5350	0.8843	1.1338
	0.375	2.0922	0.5982	1.6976		0.375	1.9328	0.6206	1.6170
	0.625	2.4703	0.4364	2.3151		0.625	2.2188	0.4622	2.1717
	0.875	1.5260	0.3746	2.6966		0.875	2.1286	0.3711	2.7038
	1.500	1.8224	0.2226	4.5190		1.500	1.8549	0.2200	4.5587
	2.500	1.7152	0.1611	6.2342		2.500	1.5136	0.1651	6.0723
	3.500	1.5358	0.1291	7.7700		3.500	1.3173	0.1356	7.3896
	5.000	1.2878	0.0969	10.3456		5.000	1.1289	0.1039	9.6473
	7.000	1.3773	0.0765	13.1001		7.000	1.2407	0.0828	12.1288
	9.000	1.1587	0.0650	15.4176		9.000	0.9086	0.0719	13.9459
	11.000	0.9905	0.0576	17.3985		11.000	1.0432	0.0625	16.0323
F#23	0.125	1.1571	3.5458	0.2893	F#24	0.125	2.8161	1.4986	0.7040
	0.375	2.3975	1.1271	0.8886		0.375	2.4072	0.7735	1.3058
	0.625	2.6575	0.6445	1.5530		0.625	1.9884	0.5588	1.8029
	0.875	2.3806	0.4659	2.1482		0.875	2.0298	0.4351	2.3104
	1.500	1.7856	0.2544	3.9337		1.500	1.4642	0.2659	3.7746
	2.500	1.1573	0.1966	5.0910		2.500	1.0374	0.2081	4.8120
	3.500	0.9044	0.1669	5.9955		3.500	1.1393	0.1682	5.9512
	5.000	0.9817	0.1259	7.9588		5.000	0.8721	0.1301	7.6954
	7.000	0.8201	0.1044	9.5991		7.000	0.8166	0.1073	9.3285
	9.000	0.9206	0.0876	11.4403		9.000	0.8365	0.0910	11.0015
	11.000	0.4515	0.0813	12.3432		11.000	0.4738	0.0838	11.9491

*Mean of five determinations.

Formulas	Mean time	dQ/dt*	1/Q*	Q*	Formulas	Mean time	dQ/dt*	1/Q*	Q*
F#29	0.125	7.0342	0.5817	1.7585	F#30	0.125	4.2048	0.9565	1.0512
	0.375	2.8333	0.4132	2.4669		0.375	1.5700	0.6933	1.4437
	0.625	1.5142	0.3547	2.8454		0.625	3.1585	0.4484	2.2333
	0.875	1.9440	0.3025	3.3314		0.875	2.7037	0.3445	2.9092
	1.500	2.0861	0.1847	5.4175		1.500	1.8100	0.2125	4.7192
	2.500	1.5441	0.1438	6.9616		2.500	0.9666	0.1763	5.6858
	3.500	1.3875	0.1200	8.3491		3.500	1.1798	0.1462	6.8656
	5.000	1.4292	0.0893	11.2075		5.000	0.7792	0.1191	8.4239
	7.000	1.6037	0.0696	14.4149		7.000	0.8641	0.0989	10.1521
	9.000	0.8069	0.0624	16.0286		9.000	0.8415	0.0846	11.8351
	11.000	1.2224	0.0542	18.4734		11.000	0.4721	0.0785	12.7794
F#34	0.125	4.4336	0.9058	1.1084	F#35	0.125	4.3426	0.9260	1.0856
	0.375	1.6399	0.6616	1.5184		0.375	1.2318	0.7191	1.3936
	0.625	1.9864	0.4983	2.0150		0.625	1.0331	0.6058	1.6519
	0.875	1.9595	0.4015	2.5048		0.875	1.3543	0.5028	1.9904
	1.500	1.8178	0.2316	4.3226		1.500	1.2573	0.3083	3.2478
	2.500	1.2395	0.1799	5.5620		2.500	1.1217	0.2291	4.3695
	3.500	1.2388	0.1472	6.8008		3.500	1.0959	0.1830	5.4654
	5.000	0.8755	0.1171	8.5518		5.000	0.9727	0.1351	7.4107
	7.000	0.8351	0.0979	10.2220		7.000	0.9906	0.1067	9.3920
	9.000	1.0250	0.0815	12.2721		9.000	0.5975	0.0945	10.5869
	11.000	0.6640	0.0738	13.6000		11.000	0.7843	0.0823	12.1555

*Mean of five determinations.

Appendix E

Data Processing by SPSS Statistical Program

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	measurement	Statistic	df	Sig.	Statistic	df	Sig.
particle size (nm)	intensity	.148	35	.051	.922	35	.025
	volume	.144	35	.064	.937	35	.063
	number	.138	35	.091	.933	35	.049

a. Lilliefors Significance Correction

Oneway

Descriptives

			N	Mean	Std. Deviation	Std. Error
particle size (nm)	measurement	intensity	35	410.6171	129.8730	21.9526
		volume	35	408.5114	127.2667	21.5120
		number	35	405.8543	126.9525	21.4589
		Total	105	408.3276	126.8155	12.3759

Descriptives

			95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
particle size (nm)	measurement	intensity	366.0042	455.2301	230.10	705.20
		volume	364.7938	452.2291	230.50	735.00
		number	362.2446	449.4640	230.50	725.20
		Total	383.7857	432.8695	230.10	735.00

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
particle size (nm)	.068	2	102	.935

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
particle size (nm)	Between Groups	398.758	2	199.379	.012	.988
	Within Groups	1672146	102	16393.588		
	Total	1672545	104			

Post Hoc Tests

Homogeneous Subsets

particle size (nm)

Duncan[a]

measurement	N	Subset for alpha = .05
		1
number	35	405.8543
volume	35	408.5114
intensity	35	410.6171
Sig.		.885

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 35.000

T-Test

Group Statistics

	formulars	N	Mean	Std. Deviation	Std. Error Mean
viscosity	pseudolatex without drug	16	507.3412	97.9613	24.4903
	pseudolatex with drug	19	547.9800	181.4856	41.6357

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means			
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference
viscosity	Equal variances assumed	2.731	.108	-.802	33	.429	-40.6388
	Equal variances not assumed			-.841	28.514	.407	-40.6388

Independent Samples Test

		t-test for Equality of Means		
		Std. Error Difference	95% Confidence Interval of the Mean	
			Lower	Upper
viscosity	Equal variances assumed	50.7013	-143.7913	62.5138
	Equal variances not assumed	48.3043	-139.5053	58.2278

T-Test

Group Statistics

	pvpK30	N	Mean	Std. Deviation	Std. Error Mean
VISCO2	pvpK30 6%	16	552.0381	87.1334	21.7834
	pvpK30 4%	16	439.3731	65.6296	16.4074

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means			
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference
VISCO2	Equal variances assumed	2.420	.130	4.131	30	.000	112.6650
	Equal variances not assumed			4.131	27.876	.000	112.6650

Independent Samples Test

		t-test for Equality of Means		
		Std. Error Difference	95% Confidence Interval of the Mean	
			Lower	Upper
VISCO2	Equal variances assumed	27.2712	56.9698	168.3602
	Equal variances not assumed	27.2712	56.7913	168.5387

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	correlation coef of Q RL100%	.938780	5	2.84E-02	1.27E-02
	correlation coef of 1/Q RL100%	.842260	5	8.62E-02	3.86E-02
Pair 2	corre coef of Q RL80%	.864180	5	3.90E-02	1.74E-02
	corre coef of 1/Q RL80%	.884040	5	6.81E-02	3.05E-02
Pair 3	corre coef of Q RL50%	.762380	5	3.29E-02	1.47E-02
	corre coef of 1/Q RL50%	.946700	5	1.38E-02	6.17E-03
Pair 4	corre coef of Q RL20%	.641360	5	3.05E-02	1.36E-02
	corre coef of 1/Q RL20%	.876520	5	2.54E-02	1.13E-02

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	correlation coef of Q RL100% & correlation coef of 1/Q RL100%	5	-.790	.112
Pair 2	corre coef of Q RL80% & corre coef of 1/Q RL80%	5	-.399	.506
Pair 3	corre coef of Q RL50% & corre coef of 1/Q RL50%	5	-.143	.819
Pair 4	corre coef of Q RL20% & corre coef of 1/Q RL20%	5	.586	.299

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	correlation coef of Q RL100% - correlation coef of 1/Q RL100%	9.65E-02	.110077	4.92E-02	-4.0E-02	.233198	1.961	4	.121
Pair 2	corre coef of Q RL80% - corre coef of 1/Q RL80%	-2.0E-02	9.10E-02	4.07E-02	-.132852	9.31E-02	-.488	4	.651
Pair 3	corre coef of Q RL50% - corre coef of 1/Q RL50%	-.184320	3.75E-02	1.68E-02	-.230849	-.137791	-10.999	4	.000
Pair 4	corre coef of Q RL20% - corre coef of 1/Q RL20%	-.235160	2.58E-02	1.16E-02	-.267234	-.203086	-20.356	4	.000

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	correlation coef of Q RS100%	.640100	5	3.66E-02	1.64E-02
	correlation coef of 1/Q RS100	.851560	5	5.27E-02	2.36E-02
Pair 2	corre coef of Q RL10P6	.859160	5	5.15E-02	2.30E-02
	corre coef of 1/Q RL10P6	.901200	5	3.52E-02	1.57E-02
Pair 3	corre coef of Q RL12P6	.657800	5	3.03E-02	1.35E-02
	corre coef of 1/Q RL12P6	.848760	5	3.36E-02	1.50E-02
Pair 4	corre coef of Q RL16P6	.543400	5	9.65E-02	4.31E-02
	corre coef of 1/Q RL16P6	.338740	5	.167202	7.48E-02

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	correlation coef of Q RS100% & correlation coef of 1/Q RS100	5	.972	.006
Pair 2	corre coef of Q RL10P6 & corre coef of 1/Q RL10P6	5	-.622	.262
Pair 3	corre coef of Q RL12P6 & corre coef of 1/Q RL12P6	5	.835	.079
Pair 4	corre coef of Q RL16P6 & corre coef of 1/Q RL16P6	5	-.996	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	correlation coef of Q RS100% - correlation coef of 1/Q RS100	-.211460	1.92E-02	8.60E-03	-.235351	-.187569	-24.574	4	.000
Pair 2	corre coef of Q RL10P6 - corre coef of 1/Q RL10P6	-4.2E-02	7.83E-02	3.50E-02	-.139307	5.52E-02	-1.200	4	.296
Pair 3	corre coef of Q RL12P6 - corre coef of 1/Q RL12P6	-.190960	1.86E-02	8.34E-03	-.214115	-.167805	-22.897	4	.000
Pair 4	corre coef of Q RL16P6 - corre coef of 1/Q RL16P6	.204660	.263387	.117790	-.122378	.531698	1.737	4	.157

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	correlation coef of Q RL10P4	.654840	5	8.48E-02	3.79E-02
	correlation coef of 1/Q RL10P4	.882680	5	4.68E-02	2.09E-02
Pair 2	corre coef of Q RL12P4	.704140	5	2.86E-02	1.28E-02
	corre coef of 1/Q RL12P4	.902600	5	3.64E-02	1.63E-02
Pair 3	corre coef of Q RL14P4	.771000	5	3.43E-02	1.53E-02
	corre coef of 1/Q RL14P4	.147780	5	.122258	5.47E-02
Pair 4	corre coef of Q RL16P4	.888300	5	3.47E-02	1.55E-02
	corre coef of 1/Q RL16P4	.862420	5	.148181	6.63E-02

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	correlation coef of Q RL10P4 & correlation coef of 1/Q RL10P4	5	.926	.024
Pair 2	corre coef of Q RL12P4 & corre coef of 1/Q RL12P4	5	.730	.161
Pair 3	corre coef of Q RL14P4 & corre coef of 1/Q RL14P4	5	.935	.020
Pair 4	corre coef of Q RL16P4 & corre coef of 1/Q RL16P4	5	-.219	.724

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	correlation coef of Q RL10P4 - correlation coef of 1/Q RL10P4	-.227840	4.50E-02	2.01E-02	-.283754	-.171926	-11.313	4	.000
Pair 2	corre coef of Q RL12P4 - corre coef of 1/Q RL12P4	-.198460	2.50E-02	1.12E-02	-.229467	-.167453	-17.771	4	.000
Pair 3	corre coef of Q RL14P4 - corre coef of 1/Q RL14P4	.623220	9.10E-02	4.07E-02	.510257	.736183	15.318	4	.000
Pair 4	corre coef of Q RL16P4 - corre coef of 1/Q RL16P4	2.59E-02	.159410	7.13E-02	-.172054	.223814	.363	4	.735

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	correlation coef of Q RL124T6	.539440	5	7.07E-02	3.16E-02
	correlation coef of 1/Q RL124T6	.808440	5	6.72E-02	3.00E-02
Pair 2	corre coef of Q RL124T14	.788480	5	5.15E-02	2.30E-02
	corre coef of 1/Q RL124T14	.804580	5	3.45E-02	1.54E-02
Pair 3	corre coef of Q RL124D6	.696460	5	3.73E-02	1.67E-02
	corre coef of 1/Q RL124D6	.856980	5	6.68E-02	2.99E-02
Pair 4	corre coef of Q RL124D8	.504920	5	5.17E-02	2.31E-02
	corre coef of 1/Q RL124D8	.731920	5	5.90E-02	2.64E-02

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	correlation coef of Q RL124T6 & correlation coef of 1/Q RL124T6	5	.886	.045
Pair 2	corre coef of Q RL124T14 & corre coef of 1/Q RL124T14	5	.342	.573
Pair 3	corre coef of Q RL124D6 & corre coef of 1/Q RL124D6	5	.841	.075
Pair 4	corre coef of Q RL124D8 & corre coef of 1/Q RL124D8	5	.518	.371

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	correlation coef of Q RL124T6 - correlation coef of 1/Q RL124T6	-.269000	3.30E-02	1.48E-02	-.310001	-.227999	-18.216	4	.000
Pair 2	corre coef of Q RL124T14 - corre coef of 1/Q RL124T14	-1.6E-02	5.12E-02	2.29E-02	-8.0E-02	4.75E-02	-.703	4	.521
Pair 3	corre coef of Q RL124D6 - corre coef of 1/Q RL124D6	-.160520	4.08E-02	1.83E-02	-.211201	-.109839	-8.794	4	.001
Pair 4	corre coef of Q RL124D8 - corre coef of 1/Q RL124D8	-.227000	5.47E-02	2.45E-02	-.294919	-.159081	-9.279	4	.001

VITAE

Miss. Chomchan Amnuaikit was born on November 11, 1974. She graduated from Chiangmai University, Chiangmai, Thailand and got degree in Bechalor of Pharmacy with second class honor. In 1997-1998, She worked as a lecturer in Faculty of Pharmaceutical Science at Prince of Songkhla University, Songkhla, Thailand.