การประยุกต์ใช้พอลิไวนิลอะซิเทตเป็นสารควบคุมการปลดปล่อยของ 17เบตา-เอสทราไดออลในระบบเมทริกซ์ที่ใช้เป็นยาฝัง

นายสุพจน์ วิทยานุกูลลักษณ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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APPLICATION OF POLYVINYL ACETATE AS RELEASE CONTROLLING AGENT IN 17-β ESTRADIOL IMPLANT MATRICES

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy
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สุพจน์ วิทยานุกูลลักษณ์: การประยุกต์ใช้พอลิไวนิลแอซิเตตเป็นสารควบคุมการปลดปล่อยของ17-บีตาเอสตราไดออลในระบบเมทริกซ์ที่ใช้เป็นยาฝัง. (APPLICATION OF POLYVINYL ACETATE AS RELEASE CONTROLLING AGENT IN 17β-ESTRADIOL IMPLANT MATRICES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. พจน์ กุลวานิช 120หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อการประยุกต์ประยุกต์ใช้ พอลิไวนิล แอซิเตต ที่มีน้ำหนักโมเลกุลที่แตกต่างกัน เป็นสารควบคุมการปลดปล่อยของ 17บีตา-เอสตราไดออล 2 เปอร์เซ็นต์โดยน้ำหนัก โดยใช้โพลิไวนิลไพโรลิโดน(พีวีพีเค30)ในการปรับการปลดปล่อยยา และใช้พลาสติไซเซอร์ 2 ชนิด คือ ไทรเอทิลซิเทรต (พลาสติไซเซอร์ที่ละลายในน้ำ) และ ไดเอทิลพทาเลท(พลาสติไซเซอร์ไม่ละลายน้ำ)เพื่อปรับความยืดหยุ่นของพอลิเมอร์ในระบบเมทริกซ์ เตรียมสารกระจายตัวของของแข็ง 17เบตา-เอสตราไดออลในพอลิเมอร์ โดยใช้วิธีการระเหยสารละลายออก และนำมาตอกอัดเป็นยาฝังที่มีเส้นผ่านศูนย์กลาง 2 มิลลิเมตร ยาวประมาณ 10 มิลลิเมตร ทำการศึกษาคุณลักษณะทางเคมีกายภาพโดยใช้การวิเคราะห์ทางความร้อน การเลี้ยวเบนรังสีเอ็กซ์ อินฟราเรดสเปกโทรสโคปีชนิดฟูเรียร์ทรานสฟอร์มพบว่าการปลดปล่อยยาเพิ่มขึ้นเมื่อเพิ่มเปอร์เซ็นต์พลาสติไซเซอร์และพีวีพีเค 30 ยาฝังที่ประกอบด้วย ไดเอทิลพทาเลทและพีวีพีเค 30 ร้อยละ 20 และ 30 ปลดปล่อยตัวยาร้อยละ 48.13 เปอร์เซ็นต์ ในระยะเวลา 28 วัน จลนศาสตร์การปลดปล่อยยาสามารถอธิบายได้ด้วยสมการฮิกูชิ ซึ่งแสดงว่าเป็นการปลดปล่อยโดยการแพร่ พอลิไวนิลแอซิเตต สามารถนำมาประยุกต์ใช้ในระบบยาฝังของยา17เบตา-เอสตราไดออลสำหรับการนำส่งยาในระยะเวลายาวนาน

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SUPOT WITTAYANUKULLUCK: APPLICATION OF POLYVINYL ACETATE AS RELEASE CONTROLLING AGENT IN 17-β ESTRADIOL IMPLANT MATRICES. THESIS ADVISOR: ASSOC. PROF. POJ KULVANICH, Ph.D., 120 pp.

The aim of the present study is to apply low and high molecular weight polyvinyl acetate (PVAc) as the release controlling agent in 2% 17β-estradiol (E_2) implant matrix system, polyvinylpyrrolidone (PVP K30) was used as releasing modifier. Two plasticizers, triethyl citrate (TEC, a water-soluble plasticizer) or diethyl phthalate (DEP, a water-insoluble plastcizer) was incorporated to alter the flexibility of the matrix. Solid dispersion of E_2 in polymer was prepared by solvent evaporation method and compressed in a mold to have an implant matrix of 2 mm in diameter and 10 mm in length. Physicochemical characterization of implants was performed using differential scanning calorimetry (DSC), X-ray diffractometry and FTIR spectroscopy. It was observed that E_2 released from implants increased with increasing weight percent of plasticizers and PVP K30. E_2 matrix implant with 20% DEP and 30% PVP K30 released 48.13% of drug in 28 days. Released kinetic of the PVAc implant matrix was best described by Higuchi model which indicated drug release by diffusion process. PVAc could be applied in E_2 matrix implant for long term delivery.

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LISTS OF ABBREVIATIONS

ANOVA	=	analysis of variance
BAC	=	benzalkonium
°C	=	degree celsius
C_0	=	initial drug concentration
C_p	=	solubility of drug in polymer
C_s	=	solubility of drug release medium
C_w	=	solubility of drug in water
cm	=	centimeter
d	=	diameter
D_p	=	diffusivity of drug in polymer
D_w	=	diffusion coefficient of drug in water
CV	=	coefficient of variation
DSC	=	differentials scanning calorimetry
E_2	=	17β-estradiol
f_2	=	similarity factor
g	=	gram
HPLC	=	high performance liquid chromatography
hr	=	hour
HRT	=	hormone replacement therapy
k_0	=	zero-order rate constant
k_1	=	first-order rate constant
k_H	=	Higuchi dissolution rate constant
l	=	length
mcg/d	=	microgram per day
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
MW	=	molecular weight

PB	=	phosphate buffer
R^2	=	coefficient of determination
RSD	=	Relative standard deviation
s	=	second
SD	=	standard deviation
t	=	time
T_g	=	glass transition temperature
µg	=	microgram
µl	=	microliter
µm	=	micrometer
USP/NF	=	The United States Pharmacopoeia/National Formulary
UV	=	ultraviolet
w/v	=	weight by volume
w/w	=	weight by weight

CHAPTER I
INTRODUCTION

17β-esreadiol (E_2) is most potent natural estrogen and mainly prescribed in case of postmenopausal symptoms as a part of hormone replacement therapy (HRT), either alone or in combination with another female hormone (Mittal et al., 2007). Furthermore, long-term therapy can prevent cardiovascular disease and osteoporosis (Anderson et al., 2000). Estradiol has good oral absorption but poor bioavilability, because of first pass metabolism. As a result, oral route leading to undesired side effects due to increased levels of active metabolites like estrone and estradiol in the blood circulation (Paoletti et al 2001). Transdermal patch of estradiol offers a number of advantages over oral route but if patch becomes detached then patient will not receive optimum treatment. Subcutaneous implant delivery system may be favorable choice for HRT. E_2-implant protect first pass metabolism of tranditional oral route and improving the patient compliance.

Matrix implant containing a poorly water-soluble drug and using an inert polymer as the matrix implant has been widely investigated. The polymer using carrier in controlling drug release form matrix implant such as silicone elastomer and poly (ethylene-co-vinyl acetate) (EVA) are the delivery levonorgrestrel and etonogestrel, respectively. Furthermore, several studies attempted to acrylate polymer i.e Eudragit RS and Eudragit RL as release controlling agent in 17-β estradiol and norethindrone implant (Chutima, 2549). However, it has been continuously reported that an application of polymer using carrier in controlling drug release form matrix implant.

Vinyl polymer such as Polyvinylacetate (PVAc) and polyvinylpyrrolidone (PVP) has widely used as release controlling agents in orally controlled release system. Polyvinylacetate (PVAc) is water-insoluble polymer. It is slightly hydrophilic and able to absorb water to a slight extent. PVAc has been reported to be effective in controlling the release of various chemical entities, including theophylline and

chlorpromazine hydrochloride (Feng and James, 2000; Niwa et al., 1994; Novoa et al., 2005). PVP is water soluble polymers often used as tablet matrices. The mechanisms and the extent by which these polymers might affect drug release have subjects of some recent studies. For example, it have been show that PVP increased the dissolution rates of frusemide in both mixed and dispersion systems of this drug with PVP (El-Arini and Leuenberger 1995). The drug release characteristics can be controlled by copolymer ratio. Moreover, the release rate adjusted by varying the chemical or physical properties of the matrix. The plasticizers increase the amount of drug released with increasing chain mobility of the polymer by altering polymer structure (Cheong-Weon et al., 2007).

Plasticizers are incorporated into pharmaceutical polymer to facilitated thermal processing, to modify drug release from polymeric system and to enhance mechanical and physiochemical properties. Plasticizer functions by weakening the intermolecular attractions between polymeric chain. Result in drug release from polymeric system was modifying which reported for film and matrix system. Wu and McGinity (1999) investigated the influence of methylparaben, ibuprofen, chlorpheniramine maleate on the thermal and mechanical properties of polymeric films of Eudragit® RS 30D. The dissolution data demonstrated that increasing the amount of ibuprofen and methylparaben decreased the rate of release of the ibuprofen from coated beads. Zhu et al. (2002) found that the effect of triethyl citrate on drug release rate increase from Eudragit® RSPO was prepared hot melt extrusion method.

The study was aimed to apply PVAc as release controlling agents in development of subcutaneous implant. It is a challenge to fabricate a constant rate of drug released from subcutaneous implant, which is basically controlled by matrix diffusion. Thus, the effect of matrix components on drug release was investigated.

The objectives of this study were as follows:

1. To apply PVAc as release controlling agents in implantable controlled-release drug delivery system. This work indicated the possibility of utilizing these polymers in controlled-release dosage form requiring long-term action.

2. To investigate the effect of plasticizer and PVP on the drug release from matrix implants containing PVAc as release controlling agents.

CHAPTER II
LITERATURE REVIEWS

1. Implants and Implantation therapy

The literature about the implants and implantation therapy was well documented by Lafarge 1861, first introduced the concept of implantable systems for sustained drug administration. The concept was later used to produce solid implants containing steroid hormone, initiating the use of implantable system for long-term delivery. These traditional pharmaceutical pellets consisted of pure drug with no added excipient and were defined as small, rod-shaped or ovoid-shaped, sterile tablets consisting of the highly purified drug, usually compressed without excipients, intended for subcutaneous implantation in body tissue. The devices thus prepared had a high degree of hardness and virtually zero porosity. Since water did not penetrate the matrix, drug release occurred principally by surface dissolution. Due to the inherently poor solubility of steroid drug, this method provided a good form of depot medication. There are still a few of the traditional implants in commercial use, including desoxycorticosterone acetate, estradiol and testosterone.

For incorporation of a variety of therapeutic agents with different physiochemical properties and for better control of drug release, a limited number of excipients are now used. Thus more recent implants usually contain the drug in a rate controlling system. These systems are available in a variety of size and shapes. Some of the recently approved implantation products include leuprolide acetate and nafarelin acetate in biodegradable DL-lactic and glycolic acid copolymer for one month release. Also new on the market is a silicone polymer capsule system containing levonorgestrone for five year contraception (Norplant, Wyeth-Ayerst). In addition, several implantable pumps for prolonged drug delivery are in commercial use. With rapid advances in implantation therapy and excipients to control the release pattern, the USP XXII identifies a much broader definition of implants, recognizing

the presence of excipients and implantation in the body at sites other than subcutaneous.

Not unlike other drug-delivery system, implants have advantages and disadvantages. Some of the potential advantages include the following:

1. Less fluctuation in plasma drug levels during therapy
2. Minimal harmful side effects of systemic administration through local (physically targeted) therapy
3. Administration of drugs with short biological half-life may be greatly facilitated
4. Improved patient compliance
5. Possible reduction in therapy costs because of reduced patient care and the potentially lower drug dose required

Potential disadvantages of implantable delivery system are:

1. Possibilities of tissue and body reaction to implant
2. Potential toxicity of by-products of biodegradable polymers
3. Surgical procedures necessary for implantation of some of the systems
4. Pain and discomfort caused by the presence of the implant
5. Cost of implant therapy
6. Danger of toxic effects in case of leakage or burst release of drug
7. Difficulty in terminating drug release, if so desired

2. Mechanisms of Drug Release from Implantable Devices.

Drug release from most implantable devices is controlled by any one of the six different mechanisms discussed below. Although an attempt is made here to cover the most important implant types, it is not possible to cover all the mechanisms under investigation (Chien et al., 1982).

Diffusion Controlled

These devices are based on Fick's law of diffusion which states that the rate of transfer of a diffusing substance though unit area of a section is proportional to the concentration gradient measured normal to the section. In this case, the rate of release is controlled by diffusion of drug through a polymeric membrane. In general, nonerodible diffusion-controlled drug delivery systems work best for drugs with molecular weights of 1000 Daltons or less. It have been reported show that the essential parameters affecting permeability of peptides though a hydrogel are the volume of solute and the water content of the membrane, which are correlated to pore size. Diffusion-controlled devices can be further classified into membrane-permeation controlled, matrix-controlled, and microreservoir-dissolution controlled.

Membrane-Permeation Controlled

The drug reservoir is surrounded by a membrane and because of the presence of the two distinct drug-reservoir and membrane. These are known as heterogeneous devices. When the device containing a highly hydrophilic drug is placed in the aqueous dissolution medium, water penetrates the coating and dissolves the drug, and the concentrated drug solution diffuses out though the polymeric membrane. The release rate of the drug is controlled by diffusion rate of drug dissolution through the polymeric membrane. The rate of drug release, *dM/dt*, though a spherical membrane-permeation controlled system with saturated reservoir is given by Eq. 1

$$\frac{dM}{dt} = \frac{4\P DK(C1-C2)ab}{b-a} \qquad (1)$$

where D is the diffusivity of drug through unit thickness of polymer, K is the partition coefficient (ratio of solubility of drug in the polymer divided by the solubility of drug in the surrounding medium) of drug across the polymer membrane, C1 is the

concentration of drug inside the sphere, C2 is the concentration of drug in the surroundings, it is the inner radius of the coat, and b is the outer radius of the coat.

Mathematical models describing the effect of device geometry on the release pattern have been developed. An important advantage of the reservoir system, as shown by Eq.1, is that at steady state, zero-order drug release is possible. If the drug elimination rate is constant during therapy, a constant drug-plasma level may be achieved. On the other hand, any disruption or crack in the membrane could lead to the release of a large amount of drug with possible toxic effects in the patient. Furthermore, reservoir systems are generally more expensive to manufacture.

Diffusion-controlled reservoir system can also be based on a biodegradable polymer. In this case, the drug is encapsulated in a biodegradable polymer and the release rate is determined by the principles governing membrane-permeation controlled systems. Only after all drugs are exhausted from the device does the polymer undergo significant erosion and eventually dissolves.

Matrix Controlled

In matrix-controlled devices, the drug is uniformly distributed (dissolved or dispersed) throughout the polymer, and hence these are known as homogeneous devices. In the presence of dissolution medium, drug at the surface dissolves first and is released in the dissolution medium. In many cases, the dissolved drug creates a depletion boundary separating the empty or drug-depleted polymer from the drug-loaded polymer matrix. Water penetrates the channels and pores created by drug depletion and dissolve the drug at the depletion boundary. The drug release rate is controlled by the diffusivity barrier provided by the empty polymer matrix which increased in thickness with time. This increased thickness results in a decrease in drug release rate with time. For a matrix system that is exposed to dissolution medium on all the sides, the surface area of the inward-moving depletion boundary decreases, resulting in a decrease in drug release rate, which depends on device geometry.

There are two principal categories of matrix device. The active agent is dissolved in the polymer medium, the device called a matrix solution. A device of this is often used the active agent is a liquid; some polymers can easily dissolve up to 20 % or more of these liquids. If the active agent has a more limited solubility in the polymer medium, then only a portion of agent is dissolved in the polymer medium and the remainder is dispersed as small particles throughout the polymer. A device of this type is called matrix dispersion.

Matrix solution

One method of preparing a matrix devices containing dissolved active material is to equilibrate it with the material: for example, the device may be soaked in heat liquid or a concentrated solution. If the active constituent is dissolved homogeneously in the polymer matrix and it is assumed, for simplicity, that one planar surface was available for release, the amount of drug delivered will be obtained by Fick's second law of diffusion.

Matrix dispersion

The second type of matrix system consists of a dispersion of solid active agent in a rate-limiting polymer matrix. The characteristics of matrix dispersion are listed in Table1 (Grass IV and Robinson, 1990). Matrix dispersion systems are of three types; which would be described latter, depending on the volume fraction of agent in matrix.

Table 2.1 Characteristics of matrix diffusion systems

Description	Homogenous dispersion of solid drug in a polymer
Advantages	Easier to produce than reservoir devices Can deliver high molecular weight compounds
Disadvantages	Cannot obtain zero order release Removal of remaining matrix is necessary for implanted system

At low loading levels of agent (0-5 volume percent), the release of the compound involves dissolution of the agent in the polymer medium followed by diffusion to the surface of the device.

At slightly higher loading levels (5-10 volume percent), the release mechanism is more complex since the cavities remaining from the loss of material near the surface are filled with fluid imbibed from the external environment. And these cavities provide preferred pathways for the escape of material remaining within the device.

When the loading of dispersed agent exceed 20 volume percent, the cavities left by the lost of material are sufficiently numerous to form a continuous channel to the surface of the matrix. In this case, the majority of the entire active agent is released by diffusion through these channels.

Mathematical equations describing release have been report which predict that in general, the drug release rate is expected to decrease with time. In case of damage to the device, though the drug release rate may increase slightly, significant dose dumping is not expected. Therefore, these devices have a safety design superior to that of the membrane-controlled systems. Furthermore, matrix systems are less expensive to manufacture. It have been shown that manipulation of the shape or drug distribution may allow a constant delivery rate.

Microreservoir Dissolution-Controlled

In these devices, the drug reservoir is made of a suspension of solid drug particles in an aqueous solution of a water-miscible polymer, forming millions of microscopic drug reservoirs in a polymer matrix. The device is coated with a rate-controlling membrane to further modify the drug release rate. Among the other factor, the release rate is dependent on the solubility of drug in the liquid compartment and on the polymer matrix. Mathematical relationships for the control of drug release have been described.

3. The analysis of dissolution data of controlled release system

3.1 The-release mechanism of controlled release system

In order to analyze the mechanism of the drug from the matrices, the dissolution data may be analyzed using the semi-empirical equation of Peppas given below

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

Where $\frac{M_t}{M_\infty}$ 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 diffusional 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 ($M_t / M_\infty < 0.6$), which also applied only to the early times of release.

Clearly, a desirable mechanism for many applications is that which leaded to n equal to 2, which characterized zero-order release behavior.

In non-swelling metrices, the value of n are 0.45 and 1.00 for Fickian and case II transport, respectively. Case II transport is special case readily identified and characterized by the constant velocity of the moving solvent front and the resulting linear weight gain with time. However, its characteristic are not as well understood, nor are they as fundamental in origin as those of Fickian diffusion when the value of n is > 0.45 and< 1.00, the release was said to be non- Fickian a value of n=1, however,

mean that the drug release is independent of time, regardless of the geometry. Thus, zero-order release can exist for any geometry (Ritger and Peppas, 1987).

Table 2.2 Diffusion exponential and mechanism of diffusional release from various non-swellable controlled release systems

Diffusion exponent,n			Drug release mechanism
Thin film	Cylindrical sample	Spherical sample	
0.5	0.45	0.43	Fickian diffusion
0.5<n<1.00	0.45<n<1.00	0.43<n<1.00	Anomalous(non-fickian) transport
1.00	1.00	1.00	Zero order release

3.2 The release pattern of controlled release system

The pattern of delivery achieves by a controlled release system can vary over a wide range, but most release profiles categorized into three types

1. Zero order release pattern
2. Square root time release pattern
3. First order release pattern

Zero order model

The zero-order model has been used to describe drug release from pharmaceutical dosage form that do not disaggregate and release the drug slowly. The pharmaceutical dosage form following this model release the same amount of drug by unit of time. The zero-order model can be used to describe poorly soluble drug released from matrix tablet. It has been expressed as the following equation;

$$\frac{dM_t}{dt} = k \qquad (3)$$

Where k is a constant, t is time and the mass of active agent released was Mt. This pattern of release is called zero order release models.

Square root of time model (Higuchi model)

The Higuchi model describes drug release as a diffusion process based on the Fick's law, square root time dependent. This relation can be used to describe water soluble drug released from several types of modified release dosage forms. The simplified Higuchi model has been expressed as below;

$$\frac{dM_t}{dt} = \frac{k}{t^{1/2}} \quad (4)$$

In contrast to first order release, the release rate here remained finite as the device approach exhaustion

The release pattern of this type can be described by Higuchi equation

$$Q = \left[\frac{D\varepsilon}{\tau} (2A - \varepsilon Cs) Cst \right]^{1/2} \quad (5)$$

Where Q = weight in grams of drug released per unit surface area

D = diffusion coefficient of drug in the release medium

ε = porosity of matrix

τ = tortuosity of matrix

Cs = solubility of drug in the release medium

A = concentration of drug in the tablet, expressed as g/ml

The assumptions made deriving equation 5 are as follows

1. A pseudo-steady state is maintained during release

2. A >> Cs, i.e., excess solute is present

3. The system is in perfectly sinking condition in which C is approximately to zero at all time.

4. Drugs particles are much smaller than those in the matrix are

5. The diffusion coefficient remains constant.

6. No interaction between the drug and the matrix occurs.

for purposes of data treatment, equation 5 is usually reduced to

$$Q = k_H t^{1/2} \qquad (6)$$

Where k_H was Higuchi constant Therefore, the plot of amount of drug released from matrix versus the square root of time should be increased linearly if drug released from the matrix is diffusion controlled. Although the above equation was based on release from a single face, it may be used to describe diffusion-controlled release from all surface matrix.

First order release model

The first-order model has been originally proposed by Gibaldi and Feldman and later by Wagner. The pharmaceutical dosage form following this model releases drug in a way that is proportional to the amount of drug remaining in its interior, in such way that the amount of drug released by unit of time diminishes (Costa and Lobo 2001). The first-order model can be expressd as the following relationship;

$$\frac{dM_t}{dt} = k(M_o - M_t) \qquad (7)$$

Where M_o is the mass of agent in the device at t=0. On rearrangement, this gave

$$\frac{dM_t}{dt} = kM_o \exp^{-kt} \quad (8)$$

In first order model, therefore, the rate declined exponentially with time, approaching a release rate of zero as the device approached exhaustion.

On the assumption that the exposed surface area of matrix decreased exponential with time, Wanger (1969) suggested that drug release from most controlled release matrices could be described by apparent first order kinetics, thus:

$$Q_t = Q_0 e^{-kt} \quad (9)$$

Where k is first order release constant

Q_0 is initial amount of drug

Q_t is amount of drug remaining in the matrix at time t

4. 17β-estradiol

Estradiol (E_2) is most potent natural estrogen and mainly prescribed in the case of postmenopausal symptoms as a part of hormone replacement therapy (HRT), either alone or in combination with another female hormone, progestin. HRT is given for 2-3 years, if the aim of treatment is symptom control; however, if the main aim is to prevent the long term consequences (eg. osteoporosis) of decreased estrogen levels, then treatment needs tolast for at least 5-10 years. Apart from postmenopausal symptoms, estradiol also has therapeutic use as a contraceptive and hypocholesteremic drug. Also, it has been found that estradiol intake may decrease the risk of Alzheimer's disease by promoting the growth and survival of cholinergic neurons and reducing cerebral amyloid deposition. Estradiol has good oral absorption but poor bioavailability (~10%), because of high gut wall and first pass metabolism. As a result, oral dose is large with conventional delivery system, leading to undesired

side effects due to increased levels of active metabolites like estrone and estriol in the blood circulation. Some of the serious health risk sallied with the use of estradiol are breast cancer and endometrial cancer. All the risk coupled with the estradiol is dose and duration dependent. Although, transdermal patch of estradiol offers a number of advantages over oral route but if patch becomes detached then patient will not receive optimum treatment and patches containing high drug concentrations are often required for obtaining sufficient therapeutic efficiency. A high saturation of the drug in the transdermal drug delivery systems may lead to supersaturated states in the patches which have the tendency to partly recrystallize during storage until a saturated state is achieved. Indeed, one of the main purposes of the controlled release is to improve safety and minimize side effects of drug. Subcutaneous implant delivery system may be favorable choice for HRT. This system offers similar advantages over oral route as transdermal system but it can overcome the limitation of transdermal system.

It have been reported different polymers have been used to prepare matrix implant containing estradiol and combination with another female hormone. For example, poly (ethylene-co-vinyl acetate) (EVA) was the delivery etonogestrel, acrylate copolymer have widely been used as release controlling agents in orally controlled release system was affirmed, the usage in implantable controlled release system, eg. Eudragit RS and Eudragit RL as release controlling agent in 17-β estradiol and norethindrone implant.

4.1 Physicochemical properties

Chemical name: (17β)-estra-1,3,5(10)-triene-3,17-diol

Empirical formula: $C_{18}H_{24}O_2$

Structure formula:

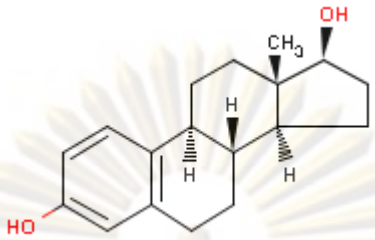


Figure2.1. Chemical structure of 17β-estradiol hemihydrates

Molecular weight:	17β-estradiol hemihydrate 272.38
Description:	White or almost white, crystalline powder or colourless crystals.
Solubility:	Practically insoluble in water, soluble in acetone, sparingly soluble in alcohol, slightly soluble in methylene chloride.
Melting point:	175°C-180°C

5. Polymer

5.1 Polyvinyl acetate (PVAc)

Poly (vinyl acetate) is a thermoplastic polymer obtained by polymerization of vinyl acetate using a suitable starter, without solvent or with water or 2-propanol. The vast majority of the acetate moieties are attached to non-neighbouring carbon atoms of the chain.

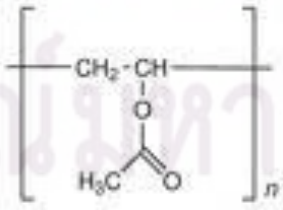


Figure 2.2 Chemical structure of polyvinyl acetate

Description:	White powder or colourless granules or beads.
Solubility:	Practically insoluble in water, freely soluble in ethyl acetate, soluble in alcohol. It is hygroscopic and swells in water. It softens at temperatures above 40-50°C.
Melting point:	175°C-180°C

The index *n* is about 100-17,000. The relative molecular mass lies between 10,000 and 1,500,000. The ester value, which characterizes the degree of hydrolysis, is 615 to 675

Polyvinylacetate has widely used as release controlling agents in orally controlled release system. It is polymer water insoluble, it is slightly hydrophilic and able to absorb water to a slight extent. PVAc has reported to be effective in controlling the release of various chemical entities, including theophylline and chlorpromazine hydrochloride (Feng and James 2000, Niwa et al., 1994, Novoa et al., 2005).

Zhang and McGinity(2000) have investigated the properties of polyvinylacetate as a retardant polymer processed by hot melt extrusion. Due to the low grass transition temperature of the polymer, the melt extrusion process could be conducted at temperature within the range 50°C to 70°C. During the processing, the extrudates was subjected to minimal thermal and mechanical stress. The extrudates had to be ground into fine powder and compressed into tablets with directly compressible. Theophylline was present in the extrudate in its crystalline form and was released from the tablet by diffusion. The Higuchi diffusion model and percolation theories were applied to the dissolution data to explain the drug release of the matrix system.

5.2 Polyvinylpyrrolidone (PVP K30)

Synthetic PVP polymer was consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the differing degree of polymerization of which results in

polymers of various molecular weights. It is characterized by its viscosity in aqueous solution, relative to that of water, expressed as a *K*-value, ranging from 10 to 120.

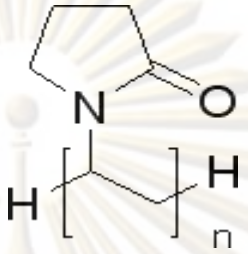


Figure 2.3 Chemical structure of polyvinylpyrrolidone

Description: Fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder.

Solubility: Freely soluble in acids, chloroform, ethanol, ketones, methanol, and water; practically insoluble in ether, hydrocarbons, 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.

Melting point: Softens at 150°C

Povidone (polyvinylpyrrolidone) is a white amorphous hygroscopic powder, soluble in water. It has good binding properties both under dry or wet conditions. Due to its hygroscopicity, povidone promotes water uptake and facilitates diffusion and drug release.

6. Plasticizer

A plasticizer is a liquid that is added to material making that material softer; more flexible (by decreasing the glass-transition temperature *T*g of the polymer), and easier to process. This broad definition encompasses the use of water to plasticize clay for the production of pottery, and oils to plasticize pitch for caulking boats. The development of plasticizer closely follows the development of this commodity

polymer; however, plasticizers are also used with other polymer types (Sears and Darby, 1982).

Mechanism of Plasticizer Action

Four general theories have been proposed to explain external plasticizer action. Some theories involve detailed analysis of polarity, solubility, and interaction parameters and the thermodynamics of polymer behavior, whereas others treat plasticization as a simple lubrication of chains of polymer from each other.

The steps involved in the incorporation of a plasticizer into a polymer product can be divided into five distinct stages:

1. Plasticizer is mixed with polymer (monomer)-adsorption step.
2. Plasticizer penetrates and swells the monomer particles-adhesion step
3. Polar groups in the monomer are freed from each other-absorption step
4. Plasticizer polar groups interact with the polar groups on the monomer-intermolecular plasticizing step.
5. The structure of the monomer is re-established, with full retention of plasticizer-intramolecular plasticizing step.

Steps 1 and 2 can be described as physical plasticization, and the precise details of how this is carried out depends on the applications technology involved the rate at which step 2 occurs depends on the plasticizer viscosity, degree of branching, resin pore size and free volume, and particle size.

Steps 3 and 4, however, can be described as chemical plasticization since the rate at which these processes occur depends on the chemical properties of molecular polarity, molecular volume, and molecular weight. An overall mechanism of plasticizer action must give adequate explanations for this as well as the physical plasticization steps.

The importance of step 5 cannot be stressed too strongly, since no matter how rapidly and easily steps 1-4 occur, if plasticizer is not retained in the final product the product will be rendered useless.

6.1 Theory of Plasticizer Action

6.1.1 The Lubrication Theory. The lubrication theory is based on the assumption that the rigidity of the resin arises from intermolecular friction binding the chains together in a rigid network. On heating, these frictional forces are weakened to allow the plasticizer molecules to lubricate the chains. Once incorporated into the polymer, the plasticizer molecules shield the chains from each other, thus preventing the reformation of the rigid network.

6.1.2 The Gel Theory. This theory extends the lubrication theory by having the plasticizer break the resin-resin attachments of a three-dimensional honeycomb or gel structure and by masking these centers of attachment from each other, preventing their reformation. This gel is formed by loose attachment occurring at intervals along the polymer chain. This facilitates the movement of plasticizer molecules, thus imparting flexibility.

6.1.3 The Free Volume Theory. The Free Volume Theory is a future extension of the lubricity and gel theories and can be used to explain both external and internal plasticization. Free volume is measure of the internal space available in a polymer for the movement of the polymer chain, which imparts flexibility to the resin. Plasticizers increase the free volume of the resin and ensure that free volume is maintained as the resin-plasticizer mixture is cooled form the melt, preventing interactions between neighboring polymer chains. For the plasticized resin, free volume can arise from motion of the chain ends, side chain, or the main chain. The fact that free volume increases with molecular motion is useful in explaining internal plasticization achieved by side-chain addition, where each side chain acts as a small molecule and free volume of the system is increased.

The introduction of a plasticizer, which is a molecule of lower molecular weight than that of the resin, has the ability to impart a greater free volume per volume of material because there is an increase in the proportion of end groups and the plasticizer has a glass-transition temperature (Tg) lower than that of the resin itself.

6.1.4 Thermodynamic or Mechanistic Theory. From the observation of migration of plasticized polymers it is clear that plasticizer molecules are not bound permanently to the polymer, but rather a dynamic equilibrium exists between salvation and desolvation of the polymer chains by plasticizer. Different families of plasticizers are attracted to the polymer by forces of different magnitude but the attraction is not permanent. There is a continuous exchange where a plasticizer molecule becomes attached to an active group on the polymer chain only to be dislodged and replaced by another plasticizer molecule.

6.2 Interaction Parameter .

6.2.1 The Hildebrand Solubility parameter. This parameters, defined by δ (eq.10), can be estimated based on data for a set of additive constants *F*, for the more common groups in organic molecules to account for the observed magnitude of the solubility parameter:

$$\delta = \sum F / V \qquad (10)$$

where V represents molar volume. Solubility parameters can be used to classify plasticizers of a given family in terms of their compatibility with PVC

6.2.2 Polarity Parameter. This parameter, defined by ϕ (eq.11), shows a good correlation with plasticizer activity for nonpolymeric plasticizers. The parameter is defined as

$$\Phi = [M(A_p / P_o)]/1000 \qquad (11)$$

where M is the molar mass of plasticizer, A_p the number of carbon atoms in the plasticizer excluding aromatic and carboxylic acid carbon atoms, and P_o the number of polar (eg, carbonyl) groups present. The 1000 factor is used to produce values of convenient magnitude. Polarity parameters provide useful predictions of the activity of monomeric plasticizers but not activity of plasticizers from different families.

6.2.3 The Solid-Gel Transition Temperature. This temperature or clear point, T_m, is a measure of plasticizer activity and is the temperature at which a single grain of polymer dissolves in excess plasticizer. The more efficient plasticizers show lower values of T_m as a result of their higher solvating power.

6.2.4 The Flory-Huggins Interaction Parameter. These ideas, based on a study of polymer miscibility, have been applied to plasticizer according to the following equation (eq.12) in which V_1 is the molar volume of the plasticizer, obtained from molar mass figures and density values at T_m, and X represents the interaction parameter.

$$1/T_{m} = 0.002226 + 0.1351(1 - X)/V_1 \qquad (12)$$

6.2.5 Specific Interactions. Some mechanism of attraction and interaction between PVC and plasticizer must exist for the plasticizer to be retained in the polymer after processing.

Plasticizers are an important component in a polymeric film coating formulation since pharmaceutical polymers are brittle rather than ductile materials. Plasticizer may be classified as internal and external. The internal plasticizer modify the chemical nature of the basic polymer, thereby altering the physical properties i.e. copolymerization with softening monomers of greater chain length. The external plasticizers change the mechanical and adhesive properties of the film i.e. adding suitable substances to the coating formulations.

The basic requirements of plasticizer are permanence and compatibility. Permanence dictates that plasticizer has a low vapor pressure and low diffusion rate within the polymeric film, a requirement that favors high molecular weight plasticizers. Compatibility, on the other hand, demands that the plasticizer be miscible with the polymer and exhibit similar intermolecular forces to those present within the polymer (Sears and Darby 1982, Wheatley and Steuernagel 1997).

The plasticization of a polymer is generally attributed to the intermolecular secondary valence forces between the plasticizer and the polymer. Different plasticizers at the same concentration will affect the glass transition temperature and hence the mechanical properties to a different extent. The degree of plasticization of the polymer is dependent to a large extent on the amount of plasticization in the film and the interaction between the plasticizer and the polymer. For a plasticizer to be effective, it must be able to diffuse into and interact with the polymer and have minimal or no tendency to migration or exudation from the polymer. The decrease in the *T*g of a polymeric film as the plasticizer concentration increase is a common measure of plasticizer effectiveness. (Lin et al., 2000)

This result allowing the polymer molecular to move more readily which increasing in free film elongation, reduction in elastic modules, tensile strength, polymer melt viscosity, glass transition temperature or softening temperature of the polymer. The polymer toughness and flexibility is improved and lower thermal processing temperature can be employed. For instance, pharmaceutical polymers used in film coating typically require a plasticizer in order to reduce brittleness and enhance polymer coalescence and film formation. The plasticizer reduces both the glass transition temperature and the minimum film formation temperature (MFT) as a result, the temperature requires for film coating is reduced.

The solubility and miscibility of the plasticizer with the retardant polymer are important criteria to consider since, for example, acetyl triethyl citrate will plasticizer HPMC during process but it is immisible with Eudragit L100. Tributyl citrate seems to be the first choice for plasticize the Eudragit E film (Lin et al., 2000). The

efficiency of a plasticizer was related to its functional groups with those of the polymer (Gutierrez- Rocca and McGinity, 1997). A strong interaction between a drug and a polymer has been reported to significant influence drug release through a polymeric film (Bodmeier and Paeratakul, 1989). The physical-mechanical properties of polymers will be influenced by both environment factors and the chemical composition of the polymer. Structural properties of the polymer will include molecular weight, branching, crystal morphology, type and amount of plasticizer, and presence of additives or fillers. Environmental factors influencing polymer properties will include temperature, time and rate of stressing the polymer, pressure, stress and strain amplitude, type of deformation, and the nature-surrounding atmosphere.

Plasticizers are incorporated into pharmaceutical polymers to modify drug released from polymeric system and to enhance the mechanical properties and surface appearance of dosage form. Saettone et al. (1995) demonstrated the type and amount of plasticizer influence the drug release rate of pellet coated with latex aqueous dispersion of ethylcellulose and acrylic polymer by altering the water permeability. Rey et al. (2000) show the slightly lower dissolution rate from minitablet when Triethyl citrate was incorporated. And many research have demonstrated plasticizer modified drug release from polymeric system (Bodmeier and Paeratakul, 1990; Frohoff-Hulsmann et al., 1999; Mulye and Turco, 1994; Okarter and Singla, 2000).

However, most pharmaceutical grade plasticizers are in a liquid state and homogeneous blend of plasticizer with the powder blend containing the active ingredient must be obtained after the process. An incomplete mixing for a polymer powder with a liquid additive has been show to result in an unstable mass flow when feeding mixture (Tate et al, 1996). Several reports have focus on the evaporation and loss of plasticizer during a high temperature operation, thus causing stability problem in a finished dosage form. (Gutierrez- Rocca and McGinity, 1993; Skultety and Sims, 1987)

Zhu et al. (2002) shows the effect of triethyl citrate levels on drug release rates were dependent on the thermal processing method used to prepared the solid

composite. As triethyl citrate levels increased the drug release rate decreased for tablet prepared by either direct compression or from granule made by high shear hot melt granulation. In contrast, drug release rates increased with increasing triethyl citrate levels for the hot melt extruded tablet. Fujimori et al (2002) were successfully developed the polymer materials having temperature-sensitive and high biological safety, Eudragit® RS and PEG400 blend polymers were prepared. The Eudragit® RS and PEG400 blend polymers that have the Tg around the body temperature were prepared by the addition of 5-13% PEG400 into Eudragit® RS. The acetaminophen release rate and release mechanism from the matrix tablet changed slightly below the Tg of the tablet and then changed markedly above the Tg. Incorporation of Diethyl Phthalate or Triethyl citrate into solid dispersion system of the Propanolol and Eudragit® RS affected drug release profiles and increase in crushing strength of matrices and provided better control of drug release. The effect was more pronounced for higher concentrations of plasticizers, which helped matrices to retain their shape throughout the dissolution test. (Sadeghi et al., 2004) The plasticizer was used in this study are generally classified as water-soluble and water insoluble. For the organic system, dibutyl phthalate was previously selected by many researchers to investigate effect of drug release. Triethyl citrate, water-soluble plasticizer is one of the most popular plasticizer that was used in organic system and hot melt process.

6.3 Properties of plasticizer in experiment

6.3.1 Triethyl citrate

USP/NF : Triethyl citrate

Formular : $C_{12}H_{20}O_7$

Molecular weight : 276.29

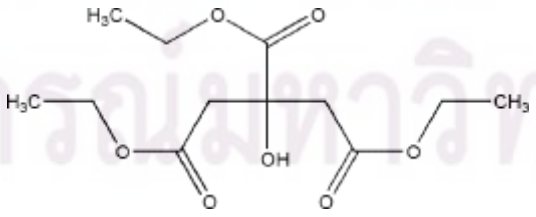


Figure 2.4 Chemical structure of triethyl citrate

Appreance : Clear, odorless, practically colorless, oily liquid

Boiling point : 288 °C

Solubility : Soluble 1 in 125 of peanut, 1 in 15 of water, miscible with ethanol (95%), acetone and propan-2-ol

Toxicity : LD 50 dermal rabbit > 5000 mg/kg

6.3.2 Dietyl phthalate

USP/NF : Dietyl phthalate

Formular : $C_{12}H_{14}O_4$

Molecular weight : 222.24

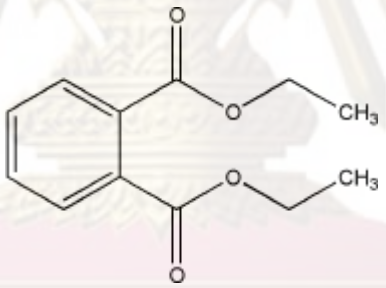


Figure 2.5 Chemical structure of dietyl phthalate

Appearance : A clear, colorless, oily liquid. It is practically odorless, or with a very slight aromatic odor and a bitter, disagreeable taste.

Boiling point : 295°C

Solubility : Miscible with ethanol (95%), ether, and many other organic solvents; practically insoluble in water.

Toxicity : Maximum 170 µg/kg body weight per day

CHAPTER III
EXPERIMENTAL

1. Materials

The following materials obtained from commercial sources were used.

1.1 Model drug

- 17β-estradiol (E_2) (Fluka Chemica, Germany, Lot.No.120667)

1.2 Excipients

- Polyvinyl acetate molecular weight 500,000 (Sigma-Aldrich Chemical Co.,Inc. USA., Lot.No.08310AD)
- Polyvinyl acetate molecular weight 113,000 (Sigma-Aldrich Chemical Co.,Inc. USA., Lot.No. 14521MB)
- Polyvinylpyrrolidone K30 (Seinghai Chemical Industrial, China, Lot. No. 00087527)
- Diethyl phthalate (Fluka Chemica, Germany, Lot.No.407073/1)
- Triethyl citrate (Fluka Chemica, Germany, Lot.No. 445571/1)

1.3 Chemicals

- Acetonitrile, HPLC grade (Lab Scan Co., Ltd., Thailand)
- Benzalkonium chloride (Sigma-Aldrich Chemical, USA, Lot.No. 1298263)
- Ethanol absolute (Lot no. K37461883726, Merck, Darmstadt, Germany)
- Methanol HPLC grade (Batch no.HAVG3H, Honeywell Berdick & Jackson, Ulsan, Korea)
- Potassium dihydrogen phosphate (Ajax Finechem, Australia Lot.No. 3A2822631)
- Sodium hydroxide (Lot no. B131198214, Merck KGaA, Damstadt, Germany)

2. Equipments

- Analytical balance (Model PB3002, Mettler Toledo, Schwerzenbach, Switzerland and Model A200S, Satorious GmBh, Goettingen, Germany)
- Differential scanning calorimeter (Model 822^e, Mettler Toledo, Schwerzenbach, Switzerland)
- Fourier transformed infrared spectrometer (Model SP2000, Perkin-Elmer Ltd., England)
- High performance liquid chromatograph (Model SCL-10A VP, Shimadzu, Kyoto, Japan) assembled with
  - System controller (Model SCL-10A VP, Shimadzu, Japan)
  - Liquid chromatograph (Model LC-10AD VP, Shimadzu, Japan)
  - Degasser (Model DGU-14A, Shimadzu, Japan)
  - Auto injector (Model SIL-10AD VP, Shimadzu, Japan)
  - Column oven (Model CTO-10AS VP, Shimadzu, Japan)
  - UV-VIS detector (Model SPD-10A VP, Shimadzu, Japan)
- Hot air oven (Model UL80, Memmert, Germany)
- Hotplate magnetic stirrer (Model M6, CAT, Germany)
- Hydraulic press equipment (Model 240, CAT, Germany237)
- pH meter (Model 210A+, Thermo orion, Germany)
- Ultrasound transonic digital sonicator (Model T680/H, Elma, Singen, Germany)
- X-ray diffractometer (Bruker AXS, model D8 Discover)

3. Methods

3.1 Preparation of E_2 implant using PVAc as the controlling agent containing PVP with plasticizer

3.1.1 Preparation of E_2 in Solid Dispersion

3.1.1.1 Preparation of 2-6% w/w E_2 using low and high MW PVAc as the controlling agent

Solid dispersions of E_2 in low and high MW PVAc at concentration range of 2-6 %w/w were prepared by solvent evaporation. Specific weight of E_2 and low and high MW PVAc were dissolved in 10 ml of ethanol to get clear solution and then poured onto glass plate. The ethanol was allowed to evaporate off overnight at 30°C and dried samples were kept in desiccators over silica bead.

3.1.1.2 Preparation of 2% w/w E_2 using low and high MW PVAc as the controlling agent with plasticizer

Solid dispersions of 2% w/w E_2 in low and high MW PVAc with plasticizer were prepared by solvent evaporation. The solid dispersion compositions are presented in Table 3.1. Accurately weigh E_2 and low and high MW PVAc were dissolved in 10 ml of ethanol to get clear solution. Then the plasticizer 10-20% were added and mixed to the clear solution. The method of evaporation and stored of the matrices was the same as 3.1.1.2.

3.1.1.3 Preparation of 2% w/w E_2 using low and high MW PVAc as the controlling agent containing various weight percent of PVP and plasticizer

Solid dispersions of E2 using low and high MW PVAc as described in 3.1.1.1 were prepare but containing various weight percent of PVP and plasticizers. The solid dispersion compositions are presented in Table 3.1. The method of preparation was the same as described in 3.1.1.2.

Table 3.1.Formulations of implants containing E_2 2% with various weight percents of polymer and various types and percent by weight of plasticizer used in components

Formulation	Plasticizer (TEC, DEP) (%)				Ratio of PVAc(low and high MW):PVP			
	0	10	15	20	100:0	90:10	80:20	70:30
E_2-implant	✓				✓			
E_2-implant 10TEC		✓			✓			
E_2-implant 10TEC-10PVP		✓				✓		
E_2-implant 10TEC-20PVP		✓					✓	
E_2-implant 10TEC-30PVP		✓						✓
E_2-implant 15TEC			✓		✓			
E_2-implant 15TEC-10PVP			✓			✓		
E_2-implant 15TEC-20PVP			✓				✓	
E_2-implant 15TEC-30PVP			✓					✓
E_2-implant 20TEC				✓	✓			
E_2-implant 20TEC-10PVP				✓		✓		
E_2-implant 20TEC-20PVP				✓			✓	
E_2-implant 20TEC-30PVP				✓				✓
E_2-implant 10DEP		✓			✓			
E_2-implant 10DEP-10PVP		✓				✓		
E_2-implant 10DEP-20PVP		✓					✓	
E_2-implant 10DEP-30PVP		✓						✓

Table 3.1.Formulations of implants containing E_2 2% with various weight percents of polymer and various types and percent by weight of plasticizer used in components (cont.)

Formulation	Plasticizer (TEC, DEP) (%)				Ratio of PVAc(low and high MW):PVP			
	0	10	15	20	100:0	90:10	80:20	70:30
E_2-implant 15 DEP			✓		✓			
E_2-implant 15 DEP -10PVP			✓			✓		
E_2-implant 15 DEP -20PVP			✓				✓	
E_2-implant 15 DEP -30PVP			✓					✓
E_2-implant 20 DEP				✓	✓			
E_2-implant 20 DEP -10PVP				✓		✓		
E_2-implant 20 DEP -20PVP				✓			✓	
E_2-implant 20 DEP -30PVP				✓				✓

3.1.2 Preparation of Implants

E_2 implant was produced by compressing solid dispersion into a mold of 2 mm in diameter (see Figure 3.1 for mold implant assembly), at a constant pressure for 60 seconds using hydraulic press with a compression force of 1,000 to 2,000 psi and heat mold at temperature 50°C for 1 hour.

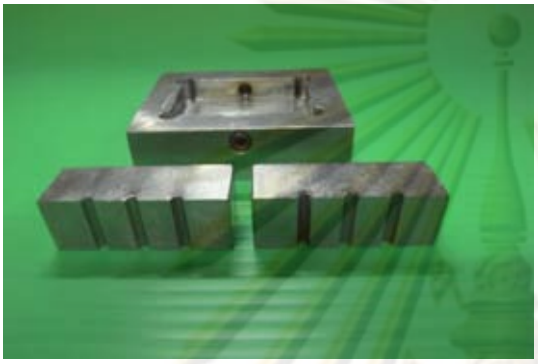


Figure 3.1 Mold implant assembly used in production of E2 implants with 2 mm in diameter

3.2 Evaluation of Physicochemical Characteristics

3.2.1 Water Uptake and erosion

The matrix implants sample, accurately weighed and then soaked in dissolution medium at 37°C. At measured intervals, the sample was carefully wiped with filter paper and tested. The percentage of water absorption was monitored gravimetrically from the difference between the initial and the periodic weight of the sample divided by the initial weight of the matrix implants sample.

$$\% \text{ Water Absorption} = \frac{\text{Weight of sample at time} - \text{Weight of sample at initial}}{\text{Weight of sample at initial}} \times 100$$

3.2.2 Differential scanning calorimetry and thermogravimetric analysis

Thermal analyses were performed using differential scanning calorimetry (DSC; Model 822^e, Mettler Toledo) and thermogravimetric analysis (TGA; Model SDTA 851^e, Mettler Toledo). Weight loss profile of sample was studied using a TGA with a refrigerated cooling system and nitrogen as purge gas was used. E_2 of approximately 3.0 mg was added to cover pan and scanned from 0°C to 250°C at 5°C/min. A DSC with a refrigerated cooling system and nitrogen as purge gas was

used. The calorimeter was calibrated using indium. E_2 of approximately 3.0-5.0 mg was added to standard aluminium pan 40µl with cover, sealed and scanned from 0°C to 200°C at scanning rate of 5°C/min; cooled down to 0°C at 20°C/min; heat up again to 200°C at rate of 5°C/min.

3.2.3 X-ray Powder Diffractometry

Crystallinity of E_2 in solid dispersion was examined using X-ray powder diffractometry. Samples were filled in zero-background quartz holder and exposed to CuK radiation (40 kV, 40 mA) by a wide angle X-ray diffractometer (D8 Discover, Bruker AXS). The instrument was operated using the step-scan mode, in increments of 0.02° 2θ/step. The angular range was 5° to 40° 2θ and counts were accumulated for 0.2 s at each step.

3.2.4 Fourier Transformed Infrared spectrometry

FTIR spectra of low and high MW PVAc, E_2 and solid dispersions containing 2 % w/w E_2 in low and high MW PVAc were prepared with a Perkin-Elmer FTIR Spectrum One using potassium bromide disks.

3.3 Evaluation of Drug Release

3.3.1 Content of E_2 and Plasticizer in Solid Dispersions

The E_2 and plasticizer content in the solid dispersions was quantitatively determined by mean of absorption peak area using high performance liquid chromatography (HPLC) method.

3.3.1.1 Content of E_2 in Solid Dispersions

E_2 was analyzed by reversed phase HPLC. The design chromatographic conditions were previously mentioned (Ye and Chien,1996)

HPLC analysis

HPLC chromatographic condition:

Column	: Inersil® BDS (C18) column (250x4.6mm) 5 µm (Thermohypersil, UK) equipped with guard column packed with BDS(C18), 5µm particle size
Mobile phase	: Acetronitrile:water 50:50
Flow rate	: 1 ml/min
Injection volume	: 50 µl
Detector	: UV detector at 280 nm
Temperature	: Ambient
Runtime	: 20 min
Internal standard	: prednisolone

Validation of the HPLC method

The typical analytical parameters to be considered for assay validation are specificity, linearity, accuracy and precision.

Preparation of internal standard solution for validation:

An accurately weighed 0.20 mg of prednisolone was placed into a 100 ml volumetric flask and diluted with methanol to volume. The final concentration of internal standard was 0.02 mg/ml

Preparation of standard solutions for validation:

An accurately weighed 50.00 mg of E2 was placed into a 100 ml volumetric flask then diluted with methanol to volume. This solution was used as the standard solution and final concentration was 0.5 mg/ml. the standard stock solution of 0.1, 0.2, 0.3, 0.4, 1.0 and 2.0 ml were transferred into 25 ml volumetric flask. Then 50 µl of internal standard solution was added and dilute to volume with mobile phase. Five dilutions were prepared as standard solution in the concentration range of 2-40 µg/ml

Assay preparation:

An accurately weighed 25 mg of matrix implants was placed into a 25 ml volumetric flask. The solution for E_2 content analysis was prepared by dissolving the matrix implants with methanol 10 ml and 50 µl of internal standard solution was added. The solution was adjusted to volume with mobile phase. Content of E_2 in matrix implants was calculated from the linear regression equation obtained calibration curve of standard solutions.

All solutions were filtered through 0.45 µm membrane filter before analysis and injected on column.

Linearity:

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the concentration of drugs in samples within a given range. Triplicate of each concentration of standard solutions in various concentrations range from 2 to 40 µg/ml were analyzed. The linear regression analysis of the peak area ratio versus the concentrations was calculated.

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatly to multiple samplings of homogenous sample. The percentage of coefficient of variation (%CV) or relative standard deviation (%RSD) values of peak area of standard solutions both within run and between run less than 2.00% which indicates that HPLC methods can be used to determine the amount of E_2 over period of time studied.

Within run precision

The within run precision was determined by analyzing the standard solution at 100% of the test concentration (35µg/ml). Repeatability was assessed using a minimum of six determinations. The percentage of relative standard deviation (%RSD) value of peak area of E_2 was determined.

Between run precision

The between run precision was determined by analyzing the standard solution at 100% of the test concentration which prepared and injected on different days. The percentage of relative standard deviation (%RSD) value of peak area of E_2 was determined.

Accuracy:

The accuracy of an analytical method is the closeness of the test results obtained by that method to the true value. Three concentration levels of drug solution (80, 100 and 120% of assay concentration). Accuracy was calculated as the percentage of recovery of each drug solution. The mean percentage of recovery of 95-105% with percent of coefficient of variation (%RSD) < 2.00% indicates the high accuracy of the method.

Specificity:

The specificity of an analytical method is the ability to assess the peak of drug from the sample without interfered by other components, presented in the sample. To determine the specificity of the method, the contents consisting excipients without active ingredient present in final formulation was prepared in 25 ml of mobile phase. This solution was injected on column after filtration through 0.45 µm nylon filter and peak response was recorded. The chromatogram of excipients blend was compared with the chromatogram of the drug solution.

Actual E_2 content in matrix implant was determined by HPLC. The percentage of E_2 content in these matrices implant was calculated using the following equation:

$$\%\ E_2\ \text{content} = \frac{\text{Actual } E_2 \text{ content}}{\text{Theoretical } E_2 \text{ content}} \times 100$$

3.3.1.2 Content of DEP in Solid Dispersions

DEP was analyzed by reversed phase HPLC. The design chromatographic condition was the same as described in 3.3.1.1.

Validation of the HPLC method

The typical analytical parameters to be considered for assay validation are linearity.

Preparation of standard solutions for validation:

An accurately weighed 100.00 mg of was DEP placed into a 100 ml volumetric flask then diluted with methanol to volume. This solution was used as the standard solution and final concentration was 1.0 mg/ml. the standard stock solution of 1.0, 2.0, 4.5, 9.0 and 20.0 ml were transferred into 100 ml volumetric flask. Then 50 µl of internal standard solution was added and dilute to volume with mobile phase. Five dilutions were prepared as standard solution in the concentration range of 10-200 µg/ml

Assay preparation:

An accurately weighed 25 mg of matrix implants was placed into a 25 ml volumetric flask. The solution for DEP content analysis was prepared by dissolving the matrix implants with methanol 10 ml and 50 µl of internal standard solution was added. The solution was adjusted to volume with mobile phase. Content of E_2 in matrix implants was calculated from the linear regression equation obtained calibration curve of standard solutions.

All solutions were filtered through 0.45 µm membrane filter before analysis and injected on column.

Linearity:

The linearity of an analytical method is the ability to elicit test results that are directly proportional to the concentration of drugs in samples within a given range. Triplicate of each concentration of standard solutions in various concentrations range from 10 to 200µg/ml were analyzed. The linear regression analysis of the peak area ratio versus the concentrations was calculated.

3.3.2 In vitro E_2 release studies

Release studies of E_2 from matrix implants were conducted in phosphate buffer (PB) pH 7.4 with 3.5% w/v BAC under sink conditions. The matrix implants were individually placed in a screw-capped test tube containing 3.0 ml of release medium. The sample test tubes were constantly shaken at 120 rpm in a shaking incubator at 37°C. The whole release medium was taken out periodically and replaced by fresh release medium. The samples were filtered through 0.45 µm membrane filter and analyzed for E_2 concentration by HPLC method. The E_2 concentration was determined from the calibration curve. The release profiles were plotted as percent of cumulative drug released as opposed to time.

Similarity factor (f_2) test was employed to compare drug release profiles of different implant formulation. f_2 test adopted by the Center for Drug Evaluation and Research (FDA) and by Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicine Products (EMEA) can be defined as the following equation (Costa and Lobo, 2001)

$$f_2 = 50 * \log\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n}|Rj - Tj|^2\right]^{-0.5} * 100\}$$

when *n* is the sampling number, *Rj* and *Tj* are the percent dissolved of two comparative formulations at each time point *j*.

Generally, f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curve and the performance of the test and reference products.

Release rate constants were determined E2 release from different types of implants formulation with the release models and were subjected to ANOVA tests. Scheffe posthoc tests with statistical significance set at $P < 0.01$ were used to examine the differences between pairs of different types of components.

CHAPTER IV
RESULTS AND DISCUSSION

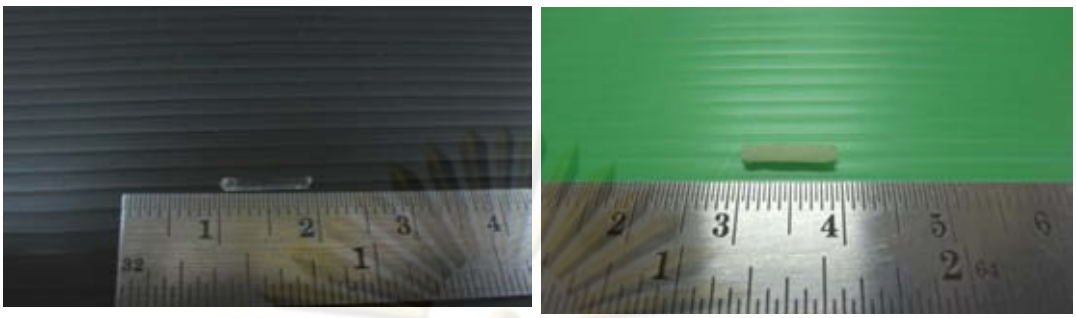
Hormone replacement therapy (HRT) has widely been recognized in controlling early menopausal symptoms. E_2 has been advocated as the estrogen replacement of choice because it is the most potent naturally occurring estrogen and it is the major estrogen secreted during the reproductive years. HRT was available in several forms, such as, tablet, transdermal patch and subcutaneous implant. Previous studies attempted to research and develop a subcutaneous implant by preparing matrix polymers.

This study aimed to formulate the implant using PVAc as the controlling agent prepared by solvent evaporation and the effect of plasticizers and water soluble polymer in matrix implant on drug release profile.

The present study was devide into three parts which were preparation of E_2 implant, evaluation of physiochemical characteristics and evaluation of drug release.

1. Implant Morphology

An E_2 implant produced by solvent evaporation and compress solid dispersion into a mold is shown in Figure 3.1. E_2 implants were rod matrix and translucent with diameter of 2 mm and length of approximately 10 mm. Furthermore, the E_2 implant after in vitro release study was opaque as shown in Figure 4.1



(a) **(b)**

Figure 4.1 .Photo-graphs of E_2-implants using PVAc as a release controlling agent (a) E_2-implants before in vitro release study (b) E_2-implants after in vitro release study

2. Evaluation of Physiochemical Characteristics

2.1 Water Uptake and Erosion

Water uptake and erosion when immersed in the medium (phosphate buffer pH 7.4 in benzalkonium (BAC) 3.5%) of implant using low and high MW PVAc with 0, 10, 15 and 20% plasticizers were investigated. After 28 day in medium, the matrix implant samples were found to maintain their integrity and slight swelling observed. The percent of water uptake and weight loss due to erosion of implant using low and high MW PVAc with and without plasticizer at various time intervals are shown in Figure 4.2 and 4.4. It was noticed that PVAc implant without plasticizer reached the maximum water uptake of about 16 % within 24 hrs, after that the implants weight decreased until 28th day of observation, which might be caused by polymeric erosion. Whereas PVAc implant with 10, 15 and 20% of TEC and DEP had percent of water uptake and weight loss less than PVAc implant without plasticizer and after 28 days the weight of implant declined to be lower weight than the initial weight as shown in Figure 4.3. The profiles of water uptake and weight loss could be distinguished into 4 groups of percentage weight of plasticizer in the following order: 20% > 15% > 10% > 0%, and material loss of those using low MW PVAc with plasticizer was higher than those of high MW PVAc with plasticizer. In cases, the amount of material lost from the matrices polymer might be due to the solubilization of plasticizer and polymeric erosion into the medium (Gutierrez- Rocca and

McGinity, 1993). The results obtained showed that the type of plasticizer did not affect water uptake and the material loss, but different MW PVAc polymer gave different amount of material lost from the matrices polymer. The result of water uptake and weight loss was clearly observed that the decrease weight of polymer matrices at day 28th was corresponding with an increase of plasticizer from 0, 10, 15to 20% are shown in Figure 4.3.

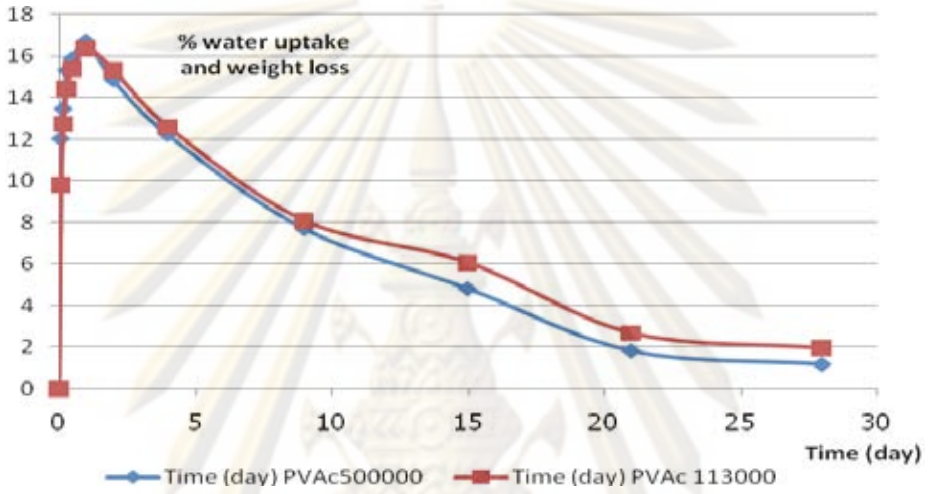
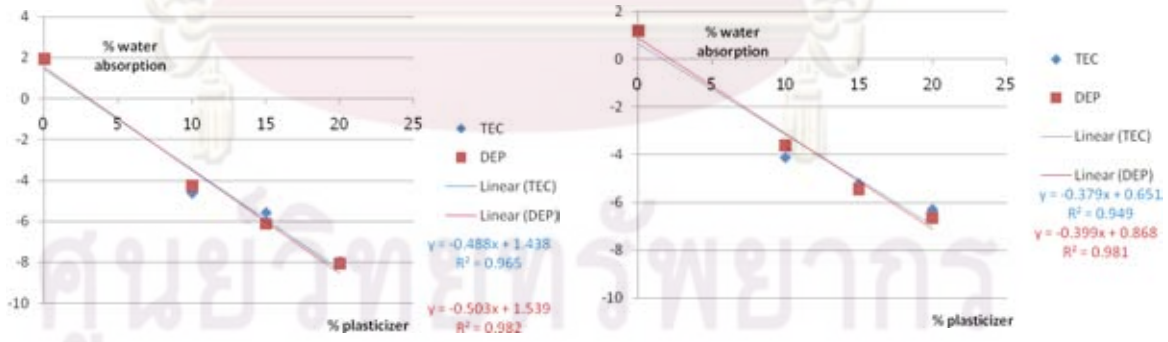


Figure 4.2 Water uptake and erosion of implant using low and high MW of PVAc without plasticizer at various time intervals within 28 days.



(a) (b)

Figure 4.3 Percent water absorption and erosion at day 28th of implant using low (a) or high (b) MW PVAc with 0%, 10%, 15% and 20% of TEC (T) and DEP (D).

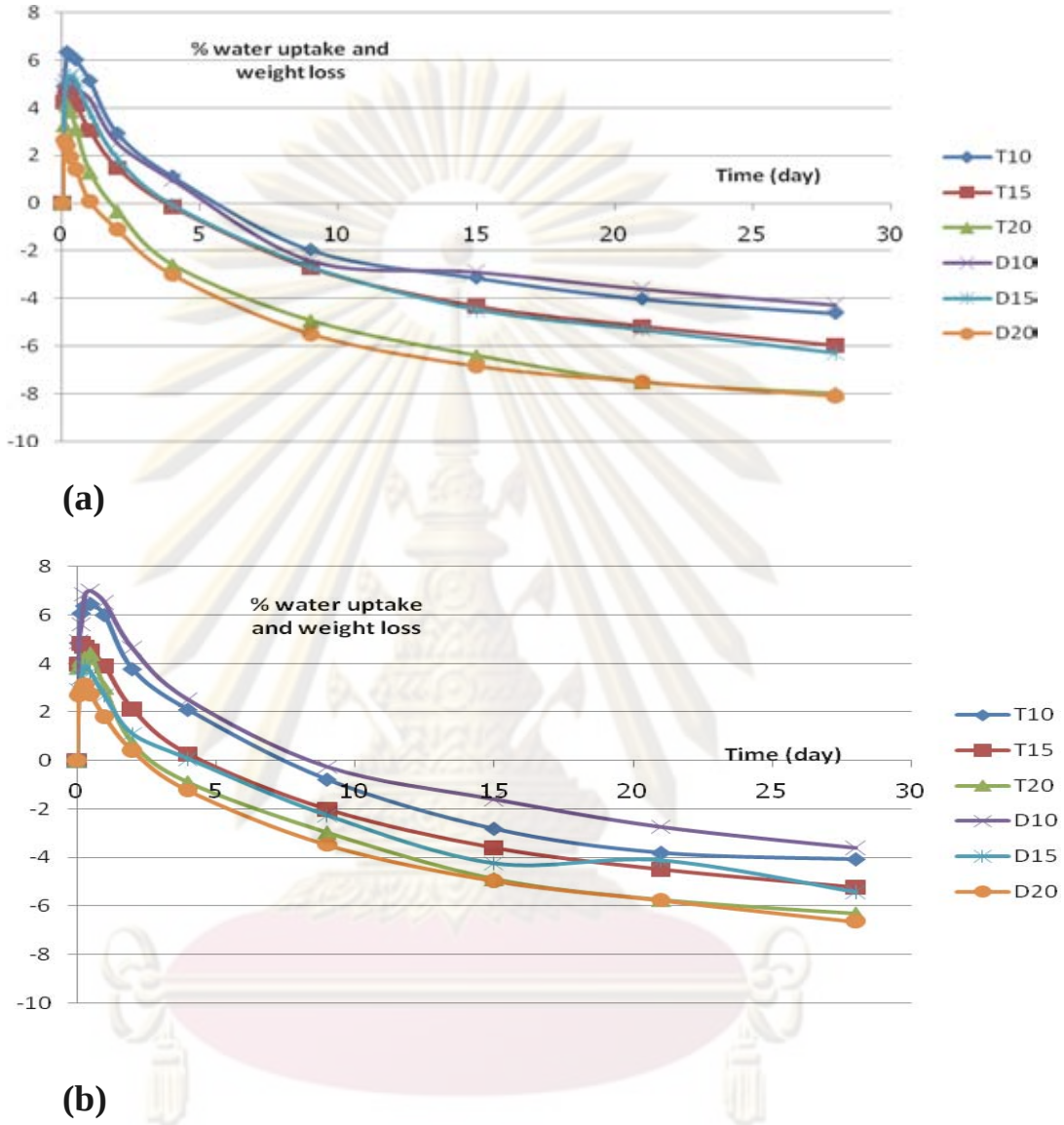


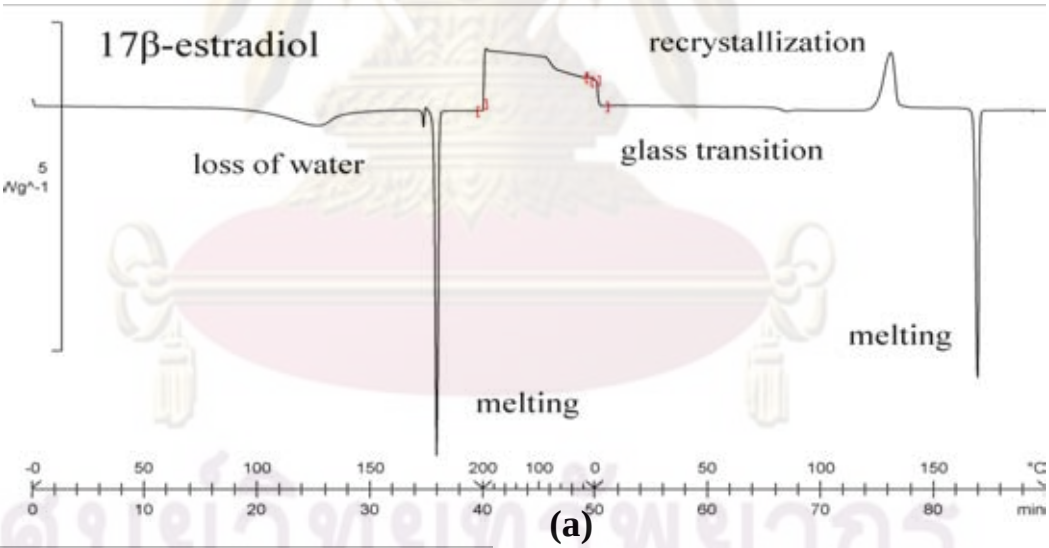
Figure 4.4 Water uptake and erosion of implant using low (a) or high (b) MW PVAc with 10-20% of TEC (T) and DEP (D) at various time intervals within 28 days.

2.2 Thermal Analysis

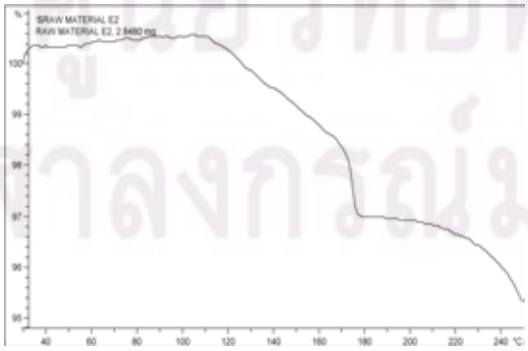
2.2.1 DSC and TGA of E_2

DSC thermograme of E_2 is shown in Figure 4.5. In the first heating run, three endothermic peaks were observed. The first endothermic peaks corresponded to the weight loss around 3.5 % as shown in the thermogravimetrical (TGA) curve of E_2 (Figure 4.5 b). The weight loss around 3.5 % indicated that the

stoichiometry of E_2 should be $C_{18}H_{24}O_2.½H_2O$. The third endothermic peak at 179°C was the melting point of E_2. This corresponed with that of E_2 hemihydrate assigned to EA form. However, it might be contaminated with ED form which has been reported to exhibit endothermic peak at 169°C (Variankaval, Jacob, and Dinh, 2000). The DSC curve during cool down exhibited glass transition of E_2 around 84°C corresponding to Tg of E_2 observed in the second heating run. This indicated that E_2 changed to an amorphous form after heated to 200°C and then rapidly cooled down because of manifesting Tg from its amorphous nature. In the second heating run, the amorphous form of E_2 in the glassy state was changed to rubbery state when the temperature was higher than 80°C. Exothermic peak in the second heating run was observed around 126°C, followed by endothermic peak at around 169°C. This result indicated that the endothermic peak was assigned to ED form of E_2 (Park et. al., 2005) which melted at 170°C, 10°C lower than E_2 hemihydrate.



(a)



(b)

Figure 4.5 DSC thermogram (a) of E_2 scan with heating program: (1) heating up to 200°C at 5°C/min; (2)cooling down to 0°C at 20°C/min; (3) heating up to 200°C at 5°C/min and TGA curve (b) of E_2 scan with heating up to 250°C at 5°C/min

2.2.2 DSC of matrix implants

2.2.2.1 The glass transition temperature of implant using PVAc with and without plasticizer

The glass transition temperature of different molecular weights of PVAc without plasticizer was determined by differential scanning calorimeter with the following heating program: heating up 200°C at 5 °C/min; and cooling down to 0°C at 20°C/min; heating up to 200°C at 5°C/min. The T_g of low and high molecular weight of PVAc were determined to be 46.26°C and 46.93°C, respectively, as shown in Table 4.1 and Figure 1B (Appendix B). The T_g values of low and high MW PVAc were not different.

When 10%, 15%, 20 % of TEC or DEP was incorporated to high MW PVAc implant, the Tg values were determined in the following order: 29.80°C, 24.64°C, 18.48°C for TEC and 32.97°C, 26.72 °C, 21.64°C for DEP, respectively. For low MW PVAc implant, the Tg values were determined in the following order: 29.56°C, 23.81°C, 18.98°C for TEC and 30.64°C, 25.89°C, 20.89°C for DEP, as shown in Table 4.1 and Figures 2B and 3B (Appendix B). The increase in plasticizer from 0% to 20% caused a decrease in T_g values.

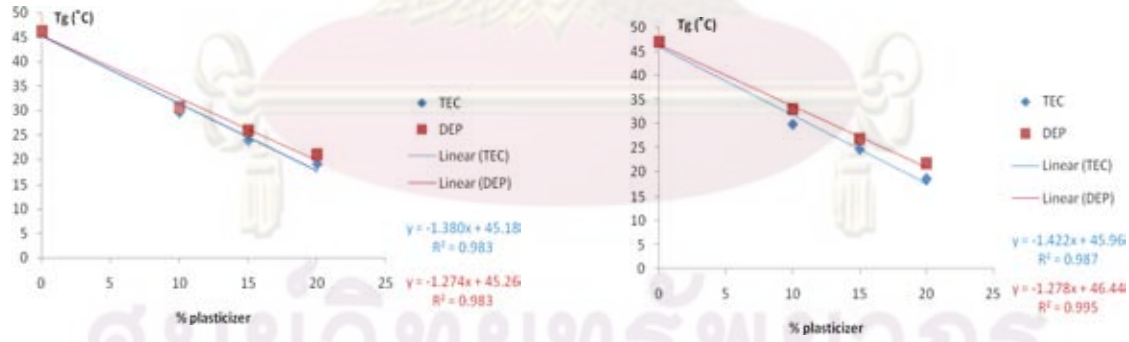
2.2.2.2 The glass transition temperatures of implants using PVAc containing weight percent of PVP and plasticizer

The effect of amount and type of plasticizer on glass transition temperature of PVAc implant containing PVP in the ratio of 90:10, 80:20 and 70:30 are shown in Table 4.1 and Figures 4.7 and 4.8.

Table 4.1 demonstrate that the glass transition temperature of PVAc with 0%, 10%, 15% and 20% TEC or DEP decreased as a function of percentage of TEC or DEP level increased. A linear relationship between the glass transition temperature and percent of TEC or DEP with high correlation was observed. The

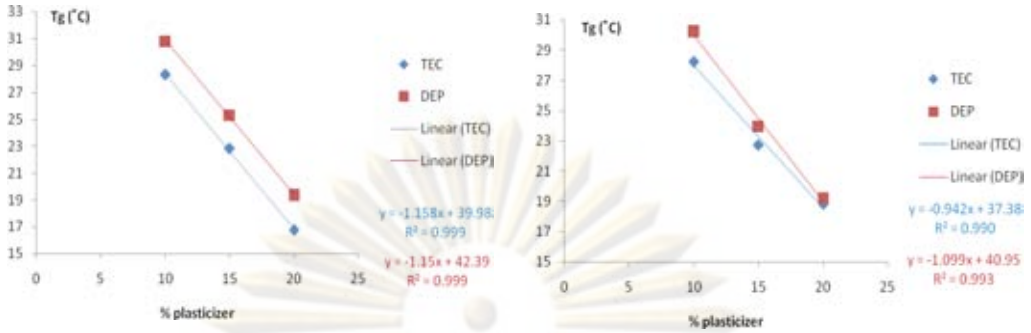
glass transition temperature decreased 1.38°C for each percentage of TEC and 1.27°C of DEP of low MW PVAc (both of R^2 = 0.983). A decreased value of 1.42°C for TEC and 1.28°C for DEP were observed for high MW PVAc as shown in Figure 4.6.

From DSC thermograms and the relationship between the glass transition temperature and percent of plasticizer demonstrated that TEC or DEP could plasticized PVAc in the same efficiency. In all case, the addition of the plasticizers had a significant effect on T_g decrease. These effects on T_g decrease could be related to a flexibility of the structure of polymer molecules and the compatibility of the plasticizers with the polymer and copolymer. The T_g values of implants of low and high MW PVAc with plasticizer after immersion in medium for 28 days were determine as shown in Figure 4.9 and 4.10. In comparison of T_g values obtained from the matrix implants before and after 28 days immersed in medium, in all cases, the T_g values of matrices polymer were increased after immersion in medium for 28 days which might be due to a decreased plasticizers in the matrices and are shown in Table 4.2.

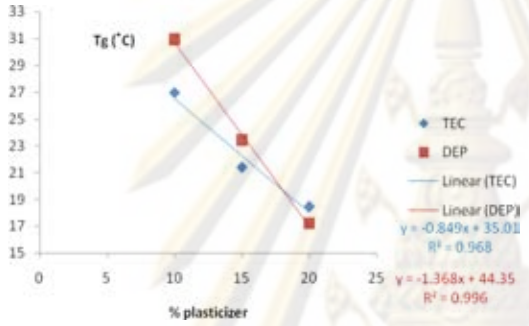


(a) **(b)**

Figure 4.6 Glass transition temperature of low (a) and high (b) MW PVAc with 0%, 10%, 15% and 20% of TEC or DEP

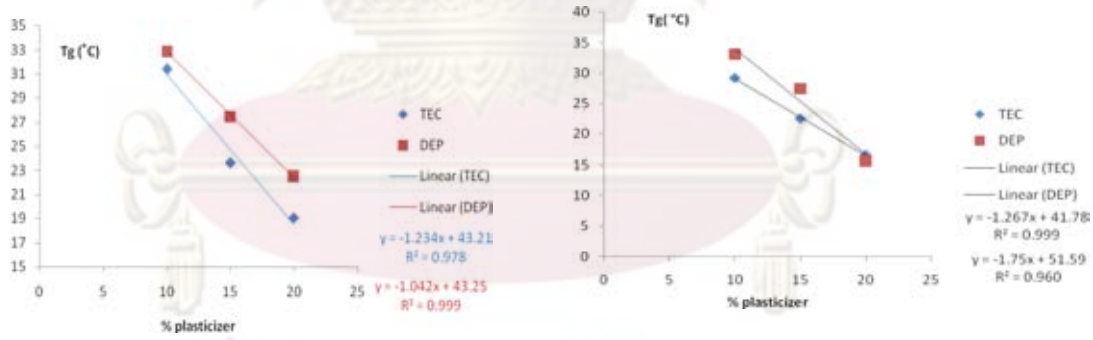


(a) **(b)**

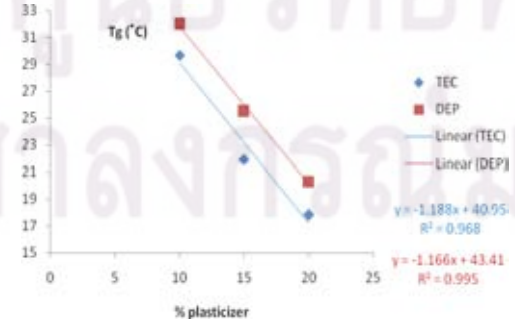


(c)

Figure 4.7 Glass transition temperature of implant using low MW PVAc with PVP in the ratio of 90:10(a), 80:20(b) and 70:30(c) containing 10, 15, 20% TEC and DEP



(a) **(b)**



(c)

Figure 4.8 Glass transition temperature of implant using high MW PVAc with PVP in the ratio of 90:10(a), 80:20(b) and 70:30(c) containing 10, 15, 20% TEC and DEP

Table 4.1 Glass transition temperature of matrix implants

Formulation	Tg(°C)					
	Water-soluble plasticizer (TEC)			Water-insoluble plasticizer (DEP)		
	10%	15%	20%	10%	15%	20%
Low MW PVAc	29.56	23.81	18.98	30.64	25.89	20.89
Low MW PVAc:PVP(90:10)	28.31	22.81	16.73	30.81	25.31	19.31
Low MW PVAc:PVP(80:20)	28.23	22.72	18.81	30.22	23.97	19.23
Low MW PVAc:PVP(70:30)	26.97	21.39	18.48	30.90	23.39	17.22
High MW PVAc	29.80	24.64	18.48	32.97	26.72	21.64
High MW PVAc:PVP(90:10)	31.40	23.64	19.06	32.90	27.49	22.48
High MW PVAc:PVP(80:20)	29.23	22.56	16.56	33.07	27.40	15.57
High MW PVAc:PVP(70:30)	29.69	21.90	17.81	31.98	25.48	20.32

Table 4.2 Glass transition temperature (T_g) of matrices polymer with 0-20% TEC or DEP after immersion in water at 0 and 28 days

Plasticizer	Glass transition temperature(T_g, °C)			
	PVAc MW 113000		PVAc MW 500000	
	Initial	At 28 day	Initial	At 28 day
0 %	46.26	45.72	46.93	44.64
10 % TEC	29.56	37.23	29.80	35.98
15% TEC	23.81	35.31	24.64	31.97
20 % TEC	18.98	33.30	18.48	30.97
10 % DEP	30.64	38.39	32.97	36.72
15% DEP	25.89	35.73	26.72	35.38
20 % DEP	20.89	34.64	21.64	32.47

This confirm the release of plasticizer from implant matrices when immersed in medium causing the weight loss of implant as previously discuss in 2.1

2.3 X-ray Powder Diffractometry

X-ray powder diffraction patterns of E_2, low and high MW PVAc and E_2 in PVAc solid dispersions are shown in the Figure 4.9. The characteristic peaks could not be observed in X-ray powder diffraction patterns of 2 % w/w E_2 in low and high MW PVAc as shown in Figure 4.9. X-ray powder diffraction patterns of these two solid dispersions did not exhibit a distinct X-ray pattern as same as that of PVAc but E_2 solid dispersion exhibited small peak intensity at a diffraction angle of 2θ, at 27.42°. This result indicated an E_2 might be mixture in PVAc solid dispersion but percent of weigh E_2 lower than detection limit characterized by XRPD. Due to an amorphous nature of PVAc, higher weight percent of PVAc corresponding to lower weight percent of E_2 in solid dispersion resulted in less crystalline fraction.

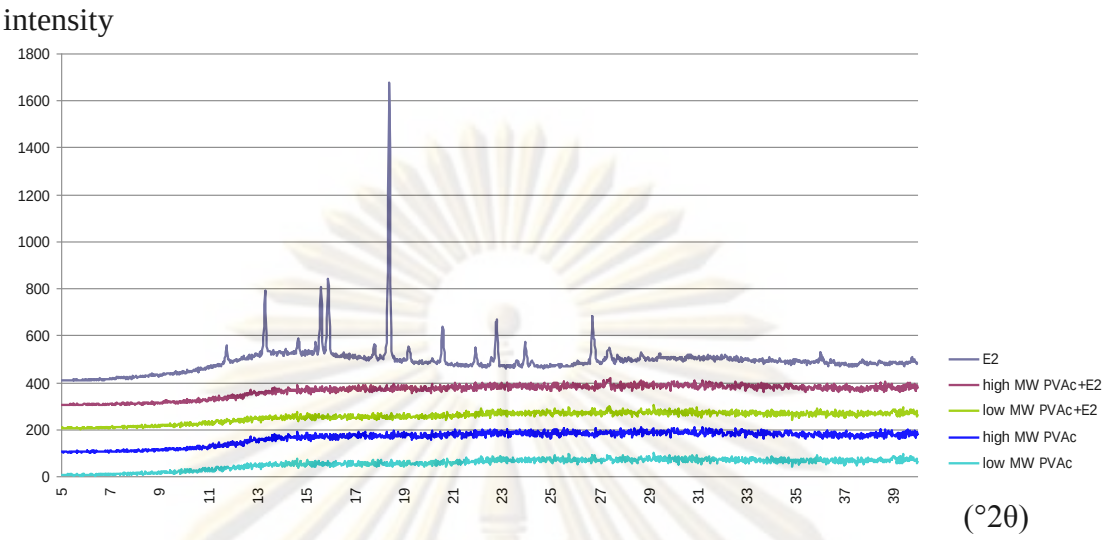


Figure 4.9 X-ray powder diffraction patterns of E2, low and high MW PVAc and 2% w/w E_2 in solid dispersions

2.4 Fourier Transformed Infrared Spectroscopy (FTIR)

FTIR spectroscopy was employed to study the interaction in solid dispersions between E_2 and PVAc, displayed in Figure 4.10. In previous report (Barnett et al, 1995, Chutima, 2004) pure E2 showed broad band centered at 3435.95 and 3232.06 cm^{-1} attributed to O-H strectching of hydroxyl group adjacent to C-17 and C-3 position in E_2 chemical structure, respectively. When adding 2 % w/w of E2 into PVAc, the broad bands of O-H strectching vibration in solid dispersions were not detected. FTIR spectra of low and high MW PVAc displayed peaks at about 1732 cm^{-1} are shown in Figure 4.10. It was seen that the following spectra were appear to be similar peak at about 1732 cm^{-1}: low MW PVAc, high MW PVAc, low MW PVAc+E_2 and high MW PVAc+E_2. This suggested that hydroxyl group adjacent to C-17 and C-3 position of E2 could not engage in inter-associated hydrogen bonding with the ester C=O group in PVAc solid dispersion.

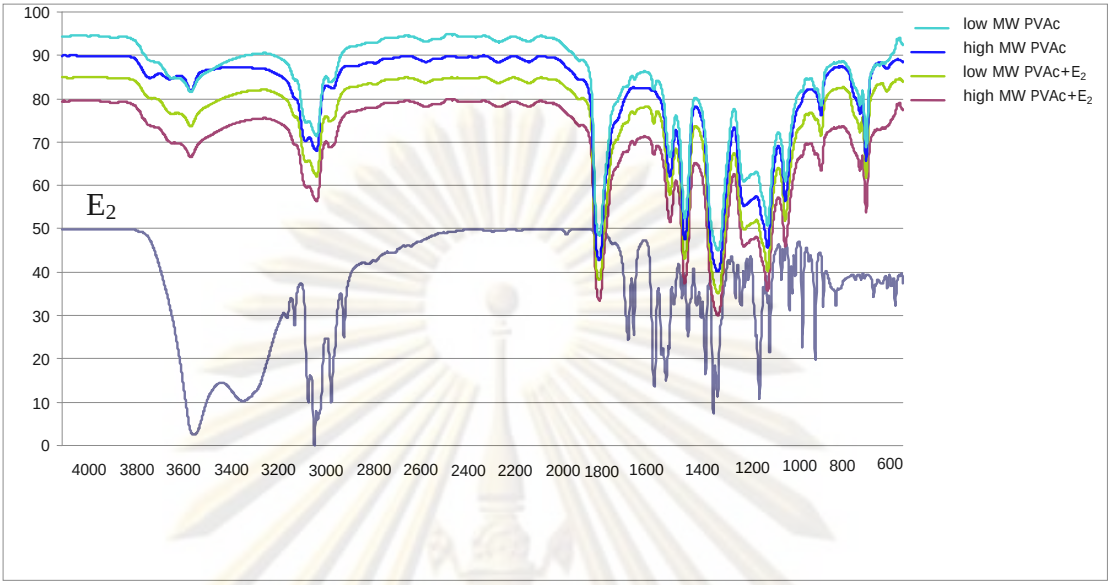


Figure 4.10 FTIR spectra of E2, low and high MW PVAc and 2% w/wE2 in solid dispersions in the range of 4000-450 cm^{-1}

3. Evaluation of Drug Release

3.1 Drug and plasticizer content

3.1.1 E_2 content

Percentages of E_2 content in matrix implants was analyzed by high performance liquid chromatography and the results are shown in the Table 4.3. In all case, E_2 content in matrix implants was well within 95-105%. Moreover, the standard variation of each formulation was very low, confirming homogeneous dispersion of the drug in the matrix.

Table 4.3 The percent drug contents of matrix implants

Formulation	% E_2 content (mean±SD)					
	Water-soluble plasticizer (TEC)			Water-insoluble plasticizer (DEP)		
	10%	15%	20%	10%	15%	20%
Low MW PVAc	97.14(0.65)	100.58(1.04)	102.10(0.64)	99.47(0.90)	98.77(0.30)	98.66(0.86)
Low MW PVAc:PVP(90:10)	98.24(1.41)	101.41(1.15)	102.23(0.85)	97.71(0.76)	99.65(1.80)	101.11(1.46)
Low MW PVAc:PVP(80:20)	100.16(1.52)	99.40(1.82)	99.00(1.09)	99.65(2.26)	99.73(1.08)	97.76(1.90)
Low MW PVAc:PVP(70:30)	98.46(2.23)	98.43(2.93)	98.86(3.22)	99.57(2.65)	99.77(2.09)	97.13(2.89)
High MW PVAc	102.40(0.52)	100.48(0.76)	98.49(0.63)	100.05(0.75)	98.34(0.68)	98.65(0.65)
High MW PVAc:PVP(90:10)	101.85(1.06)	97.84(1.09)	99.99(0.56)	101.75(0.76)	97.70(0.82)	98.16(1.26)
High MW PVAc:PVP(80:20)	103.10(1.33)	98.98(1.42)	98.49(1.35)	97.78(1.21)	99.22(1.24)	97.80(1.46)
High MW PVAc:PVP(70:30)	97.73(2.90)	96.73(2.32)	98.47(3.24)	97.84(3.36)	98.99(3.02)	98.02(3.13)

3.1.2 Plasticizer content

The plasticizer contents in the implant samples before and after immersed dissolution medium at 28 days are shown in Table 4.4. It was found that plasticizer loss from the implant using low and high MW PVAc were about 35-40 % and 25-27%, respectively, which calculated on a percent basis from the amount added to the initial T_g values. This indicated that plasticizer loss from low MW PVAc was higher than from high MW PVAc implant.

Table 4.4 Amount of DEP content remaining in low and high MW PVAc before and after immersed in dissolution medium

	Diethyl Phthalate (DEP)(%)					
	Low MW PVAc			High MW PVAc		
	10	15	20	10	15	20
Before	98.87	92.72	91.47	89.91	92.22	90.21
After	58.94	57.08	54.94	64.48	67.20	63.51
Plasticizer loss	39.63	35.64	36.53	25.43	25.02	26.70

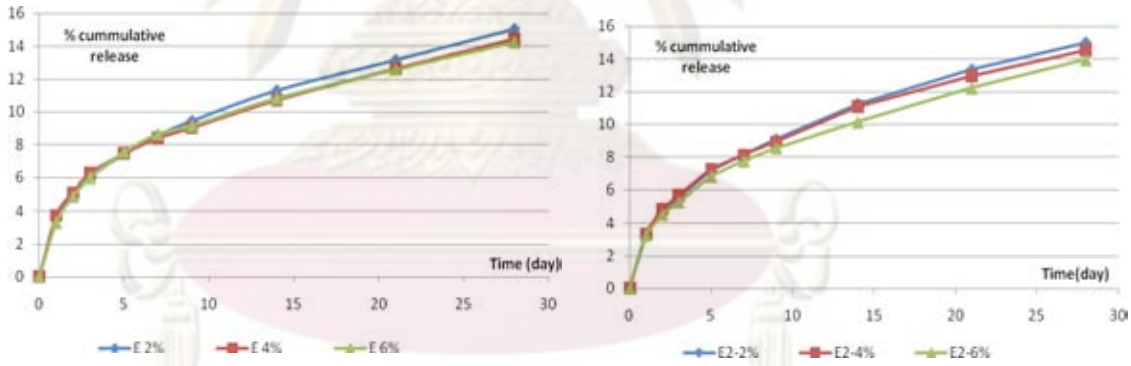
3.2 In vitro E_2 release studies

The dissolution data of all formulations were studied in phosphate buffer pH 7.4 with 3.5% w/v BAC were shown in Table 1C-54C (Appendix C). From these data, the released profile could be plotted between the percentage of amount of drug release against time. Release models generally used to describe drug release phenomena are the zero-order model, the first-order model and the Higuchi model. The release profiles were fitted to those models, it was found that Higuchi model was best described for drug release characteristic from implant (Appendix D).

3.2.1 Effect of Percent Weight of E_2 in low and high MW PVAc Matrix Implants on E2 Release Profile.

The percent cumulative release of E_2 from implant matrices containing 2, 4 and 6% w/w E_2 in low and high MW PVAc polymer (low MW=113,000 and high MW=500,000) are shown in Figure 4.11. E_2 release profiles exhibited about 14 % of E_2 released within 28 days in all cases. The increase in weight

percent of E_2 in PVAc matrix implants did not significantly increase E_2 release rate. In order to compare E_2 release profile obtained from implant matrices containing different weight percent of E_2, the similarity factor (f_2) was use in the assessment. FDA and EMEA have suggested that two dissolution profiles are declared similar if f_2 is between 50 and 100. The f_2 values as a function of weight percent of E_2 in the implant matrices obtain from the in vitro release study are presented in Table 4.5. In all case, f_2 values were higher than 50. Therefore, E_2 release profiles of implant matrices containing E_2 in the range of 2-6 % w/w in PVAc matrix implants were not different. In case of the increase in weight percent of drug in matrices, the porosity upon drug depletion is increased and the tortuosity is reduced, so that rate of drug release should increase. However, rate of E_2 release did not increase when weight percents of E_2 in PVAc matrix implants increased. This suggests that the increase in porosity and decrease in tortuosity in implant matrices cannot elevate E_2 release rate. The porosity and tortuosity might not be the important factors in controlling E_2 release from this system.



(a) **(b)**

Figure 4.11 The release profiles of E_2 from low (a) or high (b) MW PVAc containing various percent weights of E_2 without plasticizer at various time intervals within 28 days

From the previous study by Chutima (2007), it was indicated that intrinsic solubility of poorly water soluble drug affects the duration of drug release from this system. The porosity and the tortuosity did not play the leading role in controlling E_2 release. The solubility (E_2 solubility in 3.5 % w/v BAC in PB 7.4 at

37°C was 891.29 µg/ml) of drug in the release medium predominated in controlling the E_2 release. So the difference of the MW of polymer used as carrier did not significantly changed E2 release profile.

Table 4.5 f_2 values as a function of weight percent of E_2 in low and high MW PVAc obtained from release study

Similarity Factor (f_2)					
Weight percent of E_2 in low MW PVAc			Weight percent of E_2 in high MW PVAc		
(2% vs. 4%)	(2% vs. 6%)	(4% vs. 6%)	(2% vs. 4%)	(2% vs. 6%)	(4% vs. 6%)
98.64	98.38	99.47	99.51	95.88	97.34

3.2.2 Effect of type and amount of plasticizer in low and high MW PVAc matrix implants on E_2 release profile.

3.2.2.1 Low MW PVAc with 10%, 15% and 20% Plasticizer

The dissolution data of E_2 from matrices of low MW PVAc with 10 %, 15% and 20 % TEC or DEP are show in the Tables 27-32 (appendix C). The dissolution profiles were plotted between percent cumulative drug release against time. The dissolution profiles are shown in Figures 4.12.

Increasing the plasticizer 0 to 20 % into polymer resulted in increase in drug released for all formulations. At the 28 day of dissolution time, it was noticed that 20% plasticizer had the fastest drug release, whereas 0% plasticizer had the slowest drug release is shown in Figure 4.11. The results obtained shown that the E_2 release from low MW PVAc of various weight percent of plasticizer increase in the following order: 20% > 15% > 10% > 0%.

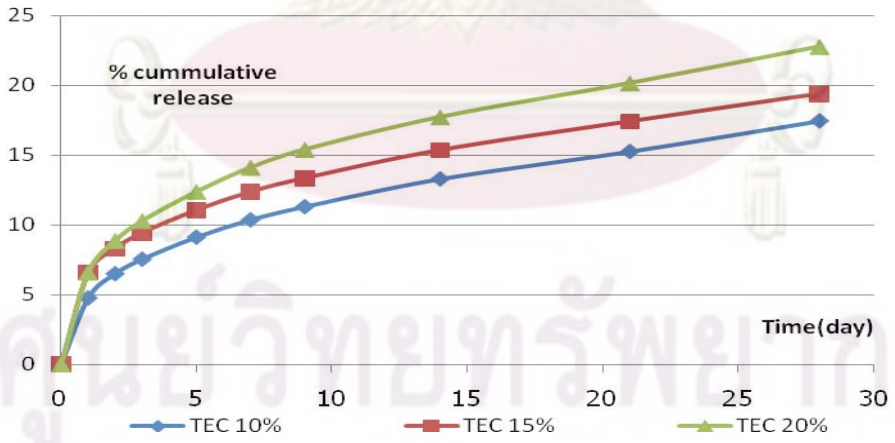
The effect of 10 %, 15% and 20% of TEC on drug release was different from 10%, 15% and 20% of DEP (see Tables 4.6). The Higuchi constant of 10 % of DEP (2.938 % $hr^{-1/2}$) was similar to 10% TEC (2.860 % $hr^{-1/2}$). Whereas the Higuchi constant of 15 % and 20% of DEP (3.527 % $hr^{-1/2}$,4.032 % $hr^{-1/2}$) was slightly faster than 15% and 20% of TEC (2.914 % $hr^{-1/2}$, 3.656 % $hr^{-1/2}$). These results

indicated the influence of different types and various weight percents of plasticizer to drug release rates and an increase of plasticizer causing a decrease of polymer in the formulation might also influence the drug release. For the plasticizer of DEP, the pattern of release profile was faster than TEC at the same percent weight level and the pattern release of plasticizer was faster than the formulation without plasticizer.

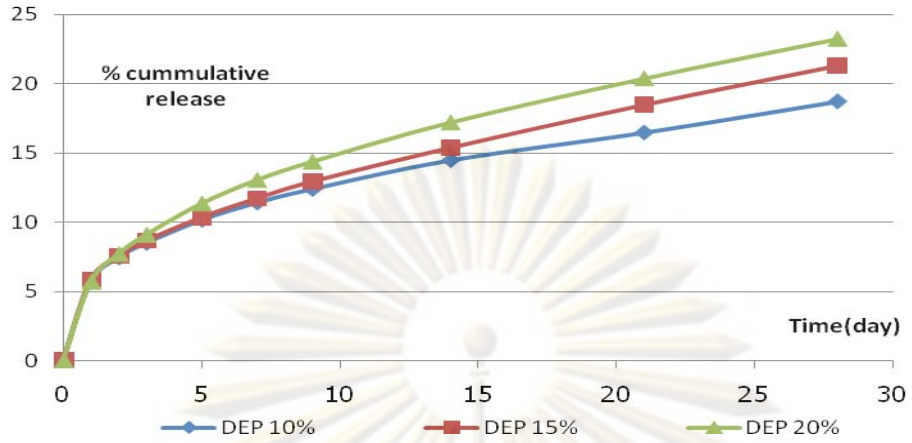
Table 4.6 The effect of 10 %, 15% and 20 % plasticizer on Higuchi constant (k) and coefficient of determination (r^2) of low MW PVAc

	Percent by weight plasticizer						
		TEC			DEP		
	0	10	15	20	10	15	20
k(% $hr^{-1/2}$)	2.723	2.860	2.914	3.656	2.938	3.527	4.032
r^2	0.997	0.995	0.994	0.991	0.995	0.999	0.998

Figure 4.12 The release profiles of E_2 from low MW PVAc with 10 %, 15% and 20 % of TEC (a) and DEP (b) at various time intervals within 28 days.



(a)



(b)

3.2.2.2 High MW PVAc with 10%, 15% and 20% Plasticizer

The dissolution data of E_2 from matrices of high MW PVAc with 10%, 15% and 20 % TEC or DEP are show in the Tables 2-7 (appendixC). The dissolution profiles are shown in Figures 4.13.

Increasing the plasticizer from 0 to 20% into polymer resulted in increased in drug released for all formulations. At the 28 day of dissolution time, it was noticed that 20% plasticizer had the fastest drug release, whereas 0% plasticizer had the slowest drug release. The results obtained shown that the E_2 release from high MW PVAc containing various weight percents of plasticizer increased in the following order: 20% > 15% > 10% > 0%.

The effect of 10 %, 15% and 20% of TEC was different from 10%, 15% and 20% of DEP (see Tables 4.7). The Higuchi constant of all level of TEC (4.169 % $hr^{-1/2}$, 4.953 % $hr^{-1/2}$, 5.049 % $hr^{-1/2}$) was faster than of DEP (3.695 % $hr^{-1/2}$, 3.950 % $hr^{-1/2}$, 4.498 % $hr^{-1/2}$). These results indicated the influence of different type and various weight percent of plasticizer to drug release rates. In the plasticizer of TEC, the pattern of release profile was faster than DEP at the same percent weight level and the pattern release of plasticizer was faster than the formulation without plasticizer.

Table 4.7 The effect of 10 %, 15% and 20 % plasticizer on Higuchi constant (k) and coefficient of determination (r^2) of high MW PVAc

	Percent by weight plasticizer						
		TEC			DEP		
	0	10	15	20	10	15	20
k(% $hr^{-1/2}$)	2.604	4.169	4.953	5.049	3.695	3.950	4.498
r^2	0.996	0.999	0.998	0.999	0.998	0.997	0.996

Comparison of E_2 release profiles obtained from low MW PVAc implants containing various weight percents of TEC showed that f_2 values were higher than 50 in all cases as shown in Table 4.8.These results indicated that E_2 release profiles obtained from PVAc implants containing 10, 15 and 20% TEC in low MW PVAc were similar, and in case of E_2 release profiles obtained from low MW PVAc implants containing various weight percents of DEP as the same f_2 values of TEC. Furthermore, f_2 values obtained from E_2 release profiles of high MW PVAc implants were the same results low MW PVAc as shown in Table 4.9

Table 4.8 f_2 values as a function of weight percent of E_2 in low MW PVAc obtained from release study

Weight percent of E_2 in low MW PVAc					
10%, 15% and 20% of TEC			10%, 15% and 20% of DEP		
(10%vs.15%)	(10%vs.20%)	(15%vs.20%)	(10%vs.15%)	(10%vs.20%)	(15%vs.20%)
82.91	70.31	82.82	90.84	79.35	89.04

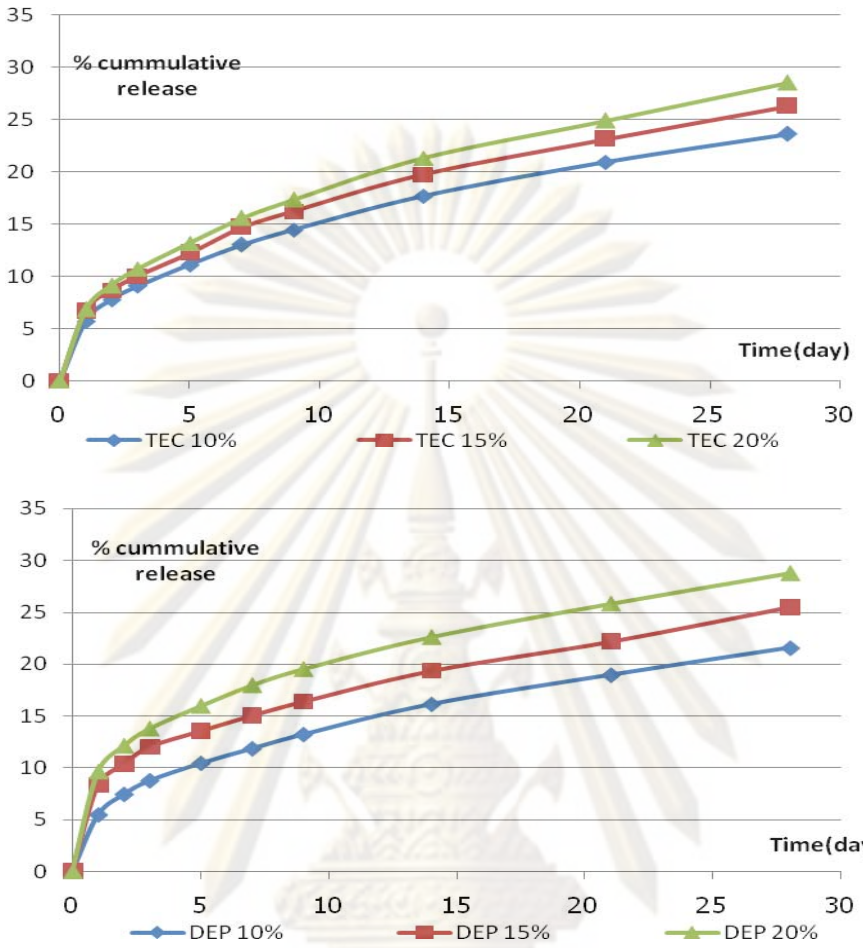


Figure 4.13 The release profiles of E_2 from high MW PVAc with 10 %, 15% and 20 % of TEC (a) and DEP (b) at various time intervals within 28 days

Table 4.9 f_2 values as a function of weight percent of E_2 in high MW PVAc obtained from release study

Weight percent of E_2 in high MW PVAc					
10%, 15% and 20% of TEC			10%, 15% and 20% of DEP		
(10%vs.15%)	(10%vs.20%)	(15%vs.20%)	(10%vs.15%)	(10%vs.20%)	(15%vs.20%)
85.39	75.47	89.69	73.48	61.08	76.85

3.2.3 Effect of percent weight of PVP in low and high MW PVAc polymer with plasticizer on E_2 release profile.

3.2.3.1 Low MW PVAc with 10, 15 and 20% of TEC or DEP containing 10%, 20% and 30% of PVP

The cumulative releases of E_2 from matrix composition of PVP 10%, 20% and 30% with 10%, 15% and 20 % TEC or DEP are shown in Figure 4.14 , 4.15. The effect of 10%, 15% and 20% of TEC or DEP on E_2 release from the matrix was data shown in Table 4.10.

Table 4.10 The effect of 10%, 15% and 20% of TEC or DEP on E_2 release from the high MW PVAc matrix after 28 days.

Ratio of PVAc:PVP	% E_2 release					
	TEC			DEP		
	10	15	20	10	15	20
90:10	24.61	31.44	40.66	22.17	24.68	34.84
80:20	24.71	32.62	41.43	22.44	27.86	36.69
70:30	26.26	33.13	42.60	22.62	30.88	38.28

It was seen that when increased the amount of TEC or DEP from 10, 15 and 20% in PVAc matrices with all PVP levels between 10-30%, the E_2 release from matrices at day 28^{th} increased from 24.61-26.26%, 31.44-33.13% to 41.43-42.60%, respectively, for TEC and 22.17-22.62%, 24.68-30.88% to 34.84-38.28% respectively, for DEP. It was found that increasing PVP into matrices did not significantly alter the drug release rate from the matrices. The E_2 release rate of all formulations were presented in Table 4.13

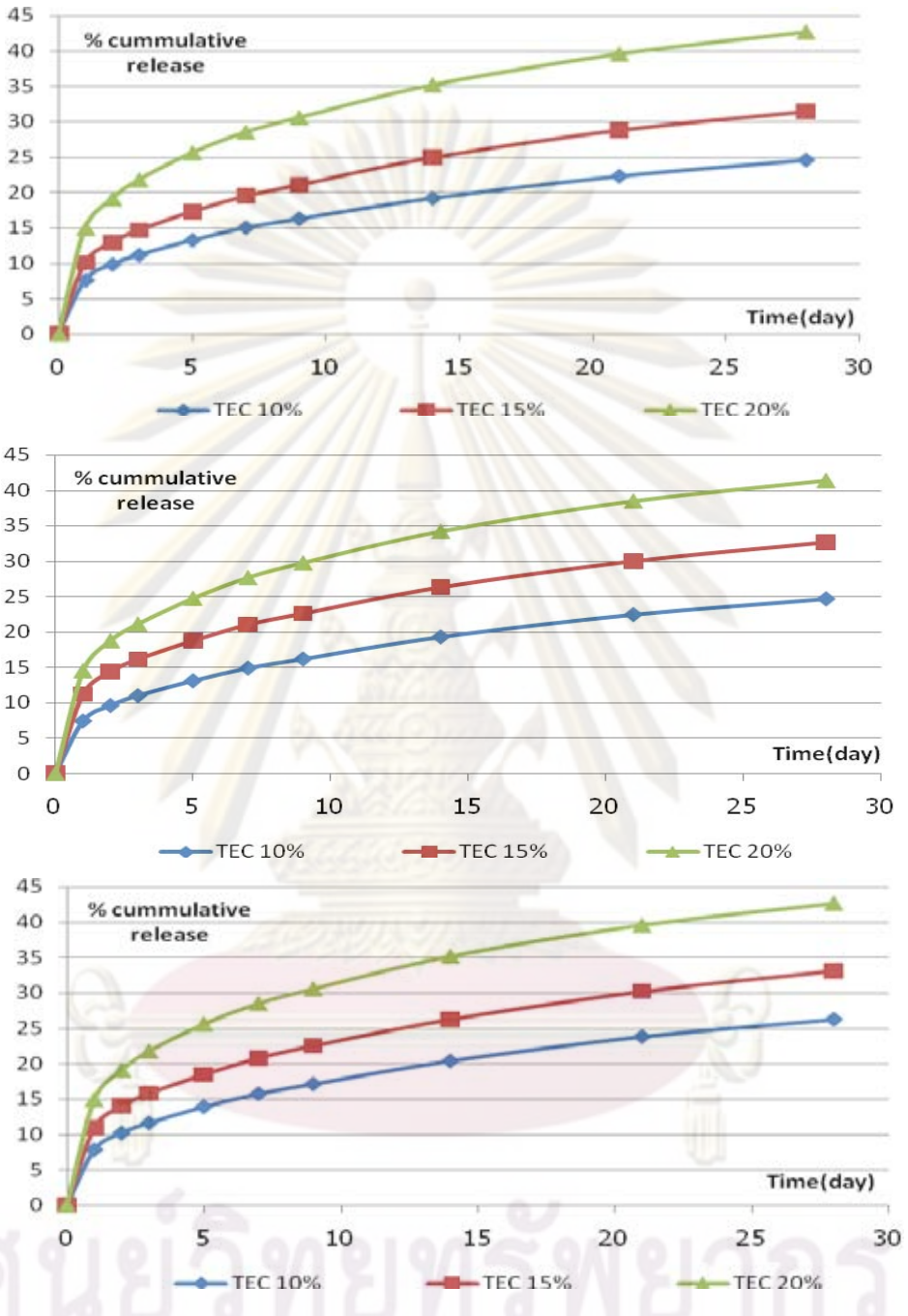


Figure 4.14 The release profiles of E_2 from low MW PVAc containing 10(a), 20(b) and 30% (c) PVP, respectively, with 10%, 15% and 20 % of TEC at various time intervals within 28 days.

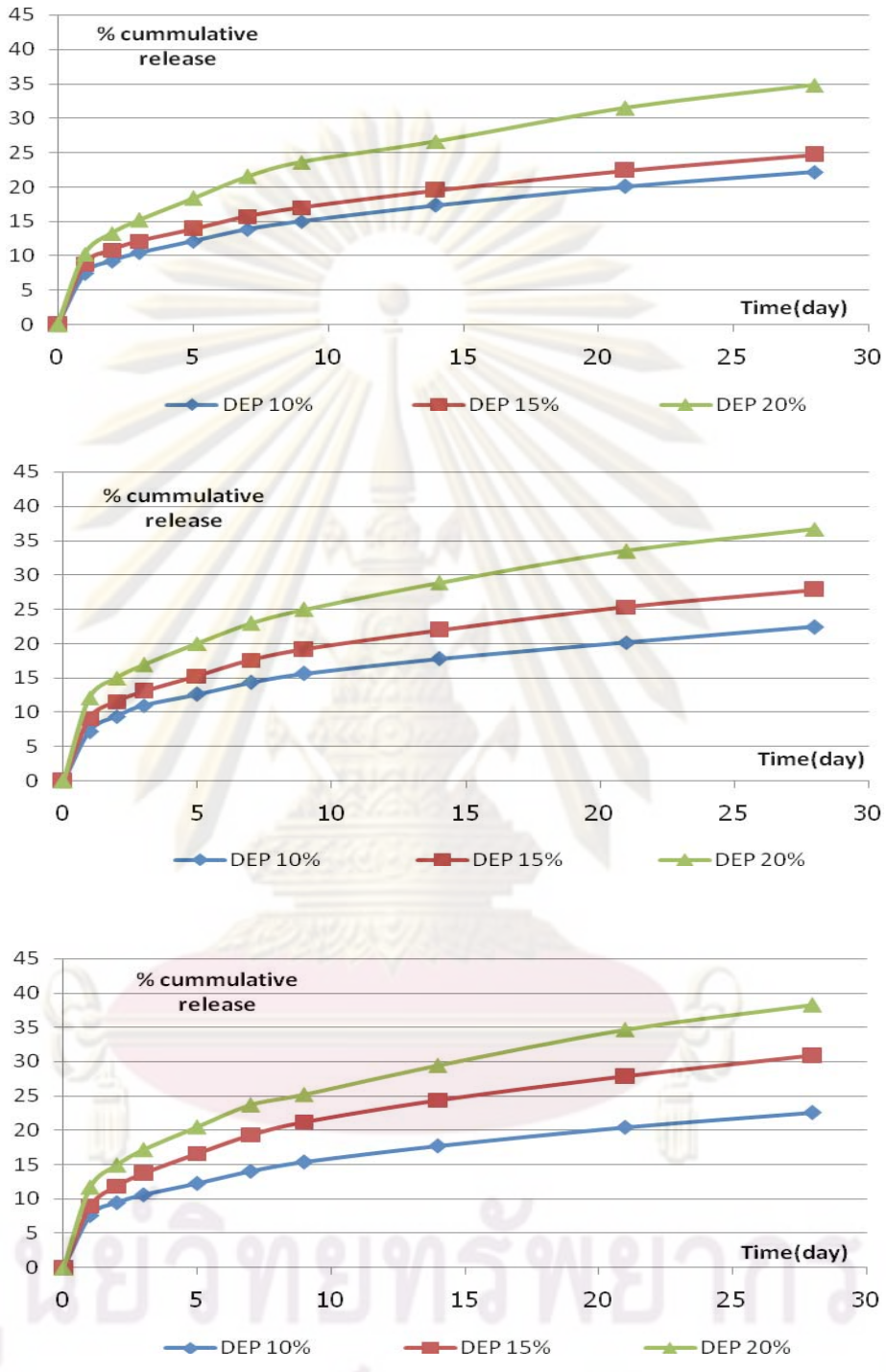


Figure 4.15 The release profiles of E_2 from low MW PVAc containing 10 (a), 20 (b), 30% (c) PVP, respectively, with 10%, 15% and 20 % of DEP at various time intervals within 28 days.

In addition, calculated f_2 values obtained from release profile of the matrices with different percent TEC or DEP of the same amount PVP closed to 50, which indicated , the release rate of E_2 in all case were different as presented in Table 4.11. In case of the increase in weight percent of plasticizer in the matrix, the glass transition temperature of matrix was decreased and the diffusivity of drug was increased, so that rate of drug release should increase. But the calculated f_2 value of the release profile of the matrices containing 10%, 20%, 30% PVP were higher than 70 in all cases as shown in Table 4.12. These results indicated that E_2 release profiles of the matrices containing different amount of PVP were similar. This indicated more similarity among E_2 release profiles containing various percent weight of PVP than of those compose of various percent weights of plasticizer. This indicated that the porosity of copolymer ratio did not play the leading role in controlling E_2 release. The solubility and the diffusivity of drug in the release medium predominates the in control of E_2 release.

Table 4.11 f_2 values as a function of weight percent of plasticizer in low MW PVAc and PVP obtained from release study

Percent weight of plasticizer	Matrix polymer ratio (high MW PVAc :PVP)					
	TEC			DEP		
	90:10	80:20	70:30	90:10	80:20	70:30
10% vs. 15%	65.50	46.48	57.75	83.08	71.56	63.03
10% vs. 20%	43.32	44.53	44.82	54.36	51.07	49.53
15% vs. 20%	52.67	58.45	56.06	59.95	61.15	65.43

Table 4.12 f_2 values as a function of weight percent of PVP in low MW PVAc with plasticizer obtained from release study

Percent weight of PVP	Percent weight of plasticizer					
	TEC			DEP		
	10%	15%	20%	10%	15%	20%
10% vs. 20%	99.73	88.76	91.20	98.26	82.51	84.49
10% vs. 30%	92.96	89.30	83.60	99.05	70.07	79.39
20% vs. 30%	92.83	99.20	94.71	99.20	83.91	94.86

Table 4.13 The effect of 10 %, 20% and 30 % of PVP on Higuchi constant (k) and coefficient of determination (r^2) of low MW PVAc with 10, 15 and 20% of TEC and DEP

Formulation	k (% $h^{-1/2}$)(SD)		r^2	
	TEC	DEP	TEC	DEP
10 % plasticizer-10% PVP	3.931	3.404	0.996	0.997
10 % plasticizer-20% PVP	4.026	3.439	0.997	0.989
10 % plasticizer-30% PVP	4.275	3.474	0.997	0.997
15 % plasticizer-10% PVP	4.963	3.655	0.995	0.997
15 % plasticizer-20% PVP	4.932	4.338	0.993	0.994
15 % plasticizer-30% PVP	5.116	5.055	0.995	0.990
20 % plasticizer-10% PVP	6.156	5.691	0.992	0.993
20 % plasticizer-20% PVP	6.195	5.752	0.985	0.996
20 % plasticizer-30% PVP	6.371	6.297	0.985	0.996

3.2.3.2 High MW PVAc with 10, 15 and 20% of TEC or DEP containing 10%, 20% and 30% of PVP

The cumulative releases of E_2 from matrix composition of PVP 10%, 20% and 30% with 10%, 15% and 20 % TEC or DEP are shown in Figure 4.16, 4.17. The effect of 10%, 15% and 20% of TEC or DEP on E_2 release from the matrix was data shown in Table 4.14.

Table 4.14 The effect of 10%, 15% and 20% of TEC or DEP on E_2 release from the high PVAc matrix after 28 days.

Ratio of PVAc:PVP	% E_2 release					
	TEC			DEP		
	10	15	20	10	15	20
90:10	31.64	36.24	44.80	35.96	41.22	45.43
80:20	33.40	41.35	45.39	37.72	41.76	47.73
70:30	36.50	41.18	45.57	37.37	42.36	48.13

It was seen that when increased the amount of TEC or DEP from 10, 15 and 20% in PVAc matrices with all PVP levels between 10-30%, the E2 release from matrices at day 28th increased significantly from 31.64-36.50%, 36.24-41.18% to 44.80-4.57%, respectively, for TEC and 35.96-37.37%, 41.22-42.36% to 45.43-48.13% respectively, for DEP . It was found that increasing PVP into matrices did not significantly alter the drug release rate from the matrices. The E_2 release rate of all formulations were presented in Table 4.17

Figure 4.16 The release profiles of E_2 from high MW PVAc containing 10(a), 20(b) and 30% (c) PVP, respectively, with 10%, 15% and 20 % of TEC at various time intervals within 28 days

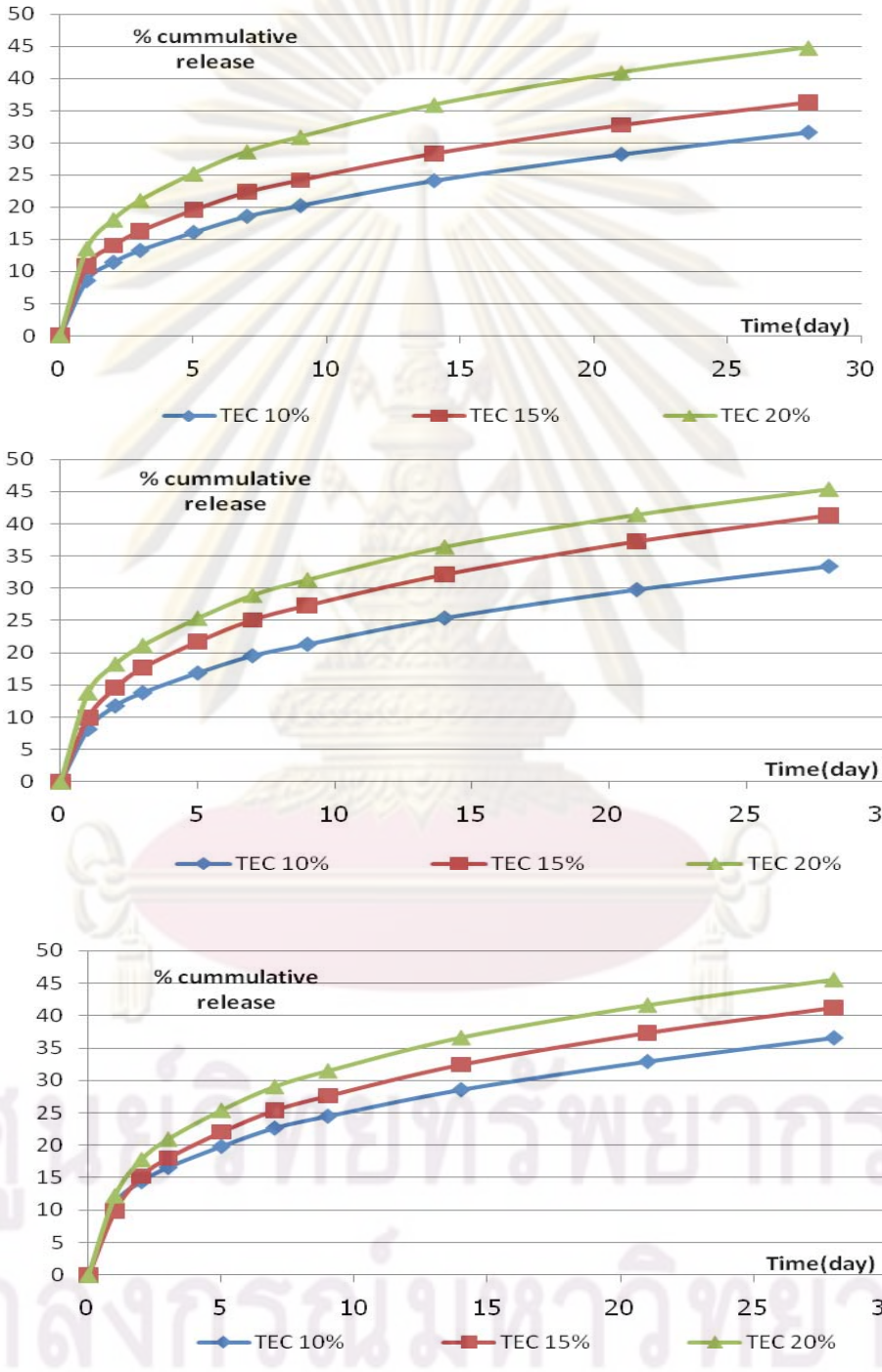
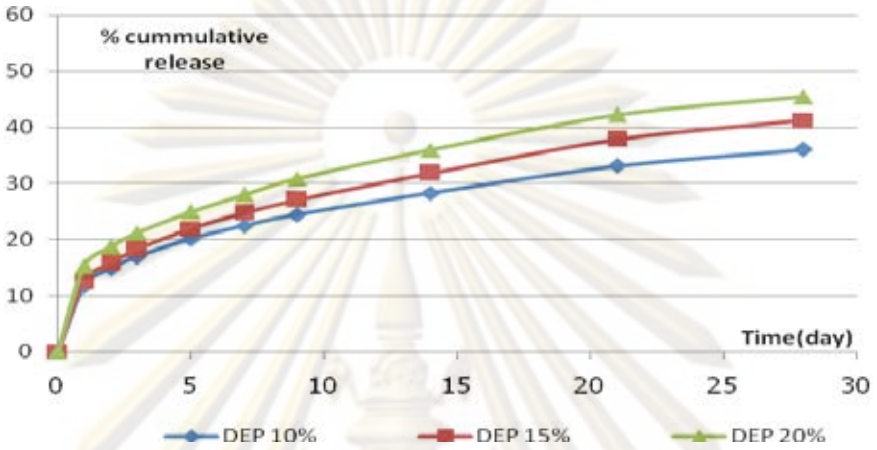
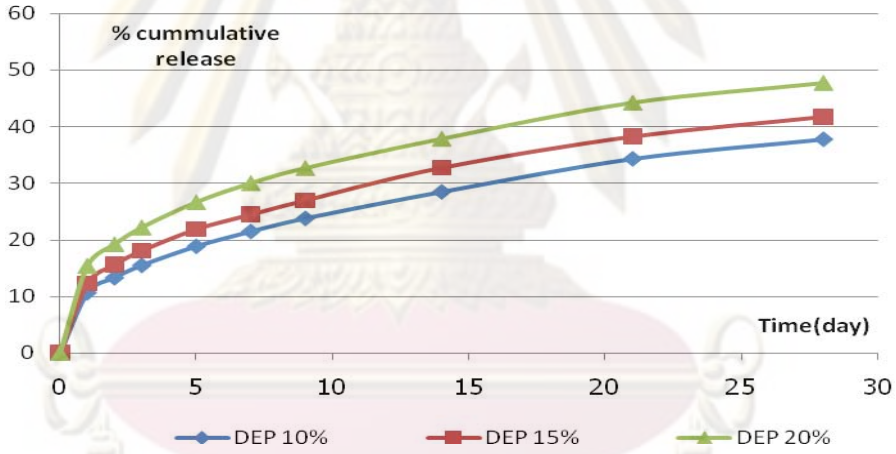


Figure 4.17 the release profiles of E_2 from high MW PVAc containing 10, 20, 30% PVP, respectively, with 10%, 15% and 20 % of DEP at various time intervals within 28 days



(a)



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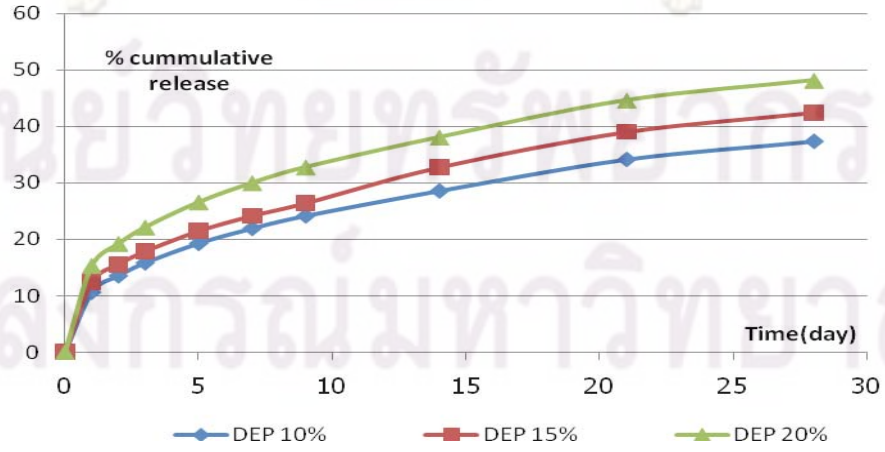


(c)

In addition, f_2 values obtained from the comparison between different percent TEC or DEP and PVP found that f_2 value for comparison between different percent TEC or DEP has value including nearly 50. The lower f_2 value, the release rate of E_2 in all case has different dissolution profiles are obtained are present in Table 4.15 In case of the increase in weight percent of plasticizer in the matrix, the glass transition temperature of matrix is decreased and the diffusivity of drug is increased, so that rate of drug release should increase. Comparison between 10%, 20% and 30% of PVP showed that f_2 value were higher than 70 in all cases as shown in Table 4.16 These results indicated that E_2 release profiles were similar. Furthermore, f_2 values obtained from various percent weight of PVP comparison were higher than that of various percent weight of plasticizer comparison. This indicated more similarity among E_2 release profiles containing various percent weight of PVP than various percent weight of plasticizer.

Table 4.15 f_2 values as a function of weight percent of plasticizer in high MW PVAc and PVP obtained from release study

Percent weight of plasticizer	Matrix polymer ratio (high MW PVAc :PVP)					
	TEC			DEP		
	90:10	80:20	70:30	90:10	80:20	70:30
10% vs. 15%	71.12	62.25	74.77	74.89	73.50	74.04
10% vs. 20%	49.88	51.29	59.09	59.31	54.13	54.25
15% vs. 20%	59.54	69.81	71.32	71.80	64.67	64.09

Table 4.16 f_2 values as a function of weight percent of percent of PVP in high MW PVAc with plasticizer obtained from release study

Percent weight of PVP	Percent weight of plasticizer					
	TEC			DEP		
	10%	15%	20%	10%	15%	20%
10% vs. 20%	91.53	74.07	98.45	90.42	98.27	85.41
10% vs. 30%	70.62	73.30	96.08	93.11	95.49	84.30
20% vs. 30%	75.25	99.04	97.10	98.94	98.17	99.57

Table 4.17 The effect of 10 %, 20% and 30 % of PVP on Higuchi constant (k) and coefficient of determination (r^2) of high MW PVAc with 10%, 15% and 20% of TEC

Formulation	k (% $h^{-1/2}$)(SD)		r^2	
	TEC	DEP	TEC	DEP
10 % plasticizer-10% PVP	5.325	5.649	0.997	0.995
10 % plasticizer-20% PVP	5.750	6.417	0.995	0.999
10 % plasticizer-30% PVP	5.824	6.304	0.994	0.997
15 % plasticizer-10% PVP	5.875	6.744	0.995	0.997
15 % plasticizer-20% PVP	7.146	6.972	0.988	0.997
15 % plasticizer-30% PVP	7.047	7.150	0.985	0.997
20 % plasticizer-10% PVP	7.102	7.169	0.990	0.996
20 % plasticizer-20% PVP	7.094	7.608	0.983	0.993
20 % plasticizer-30% PVP	7.539	7.738	0.981	0.994

CHAPTER V
CONCLUSIONS

This work attempted to apply PVAc as release controlling agent in implantable controlled release drug delivery system. The E_2 implants using PVAc alone released approximately 14 % of E_2 within 28 days. The difference of MW PVAc as release controlling agent and increase weight percent of E_2 in matrices did not change E_2 release. The effect of triethyl citrate (TEC) or dietyl phthalate (DEP) level and percent weight of PVP on E_2 release from matrices implant were investigated. Based on the finding of this study, the following conclusion could be drawn.

1. TEC and DEP provided the same plasticization efficiency when incorporation in low and high MW PVAc.

2. The effect of plasticizer on drug release rates was dependent on the type and amount of plasticizer. When TEC or DEP levels increased, the E_2 release from low and high MW PVAc implants increased for all formulations

3. It was found that implant using high MW PVAc giving higher drug release than of low MW PVAc implant was due to lower leaching of plasticizer

4. An increased the proportion of PVP in matrices resulted increasing drug release rate because of its more water soluble than PVAc. But plasticizer gave more effect on drug release than PVP.

5. The physicochemical study using DSC, X-ray diffractometry and FTIR indicated no interaction of E_2 and PVAc in solid dispersion.

6. The drug release data were fitted to the Higuchi equation and the drug release rate constants was calculated and determines to be diffusion-controlled processes for all formulations.

REFERENCES

Anderson, T.L.G., Stehle, B., Davidsson, B., and Höglund, P. Drug concentration effect relationship of estradiol from two matrix transdermal delivery systems: Menorest® and Climara®. Maturitas 35(2000): 245-252

Bodmeier, R. and Paeratakul, O. Evaluation of drug containing polymer films prepared from aqueous latexes. Pharm. Res., 6,(1989) : 725-730

Bodmeier, R. and Paeratakul, O. Propanolol HCl released from acrylic films prepared from aqueous latexes. Int. J. Pharm. 59, (1990): 197-204

Bodmeier, R. and Paeratakul, O. Leaching of water-soluble plasticizers from polymeric films prepared from aqueous colloidal dispersions. Drug Dev. Ind. Pharm, 18(17), (1992): 1865-1882

Bodmeier, et al. Drug release from ethylcellulose coated pellets in McGinity J.W. (Ed.). Aqueous polymeric coating for pharmaceutical dosage forms. Marcel Dekker, New York. (1997) 62-67

Cameron, C.G., McGinity, J.W., and Coff, G.W. Controlled –release theophylline tablet formulations containing acrylic resins. I. Dissolution properties of tablets, Drug Dev. Ind. Pharm., 9(1), (1987): 57-98

Cheong, W-C., Choi, J-S., and Shin, S-C. Controlled release of Pranoprofen from the ethylene-vinyl acetate matrix using plasticizer. Drug Dev. Ind. Pharm., 33, (2007): 747-753

Chien, Y. W. Fundamentals of controlled release drug administration. In J. Swarbrick (ed), Novel drug delivery systems, (1982), pp465-574. New York: Marcel Dekker.

Chien, Y. W., Cabana, B.E., and Mares, S. E. Implantable controlled-release drug delivery systems. In J. Swarbrick (ed), Novel drug delivery systems, (1982), pp.311-412. New York: Marcel Dekker.

Chutima Wiranidchapong. Development of 17β-estradiol and norethindrone implants using acrylate polymers as release controlling agent. Doctoral dissertation, Department of Pharmaceutic, Chulalongkorn University, 1999

Cobby, J., Mayershon, M., and Walker, G. C. Influence of shape factors on kinetics of drug release from matrix tablets: I. Theorytical. J. Pharm. Sci. 63(1974):725-733

Colombo, P., Conte, U., Gazzaniga, A., Maggi, L., Sangalli, M. E., Peppas, N. A., and La Manna, A. Drug release modulation by physical restrictions of matrix swelling. Int. J. Pharm. 63(1990): 43-48

Costa, P., and Lobo, J. M. S. Modeling and comparison of dissolution profiles. Eur. J. Pharm. Sci. 13(2001): 123-133

El-Arini, S. K., and Leuenberger, H. Modelling of drug release from polymer matrices: Effect of drug loading. Int. J. Pharm. 121(1995): 141-148

Engineer, S., Shao, Z.J. and Khagani, N.A. Temperature/humidity sensitivity of sustained-release formulations containing Kollidon® SR, Drug Dev. Ind. Pharm.,30(10)(2004), 1089-1094

Feng, Z., and McGinity, J.W. Properties of hot-melt extruded theophylline tablets containing poly(vinyl acetate). Drug Dev. Ind. Pharm.,26(9), (2000) : 931-942

Ford, J. L., Rubinnstein, M. H., and F.,Hogan, J. E. Dissolution of a poorly water soluble drug, indomethacin, from hydroxypropylmethylcellulose controlled release tablets. J. Pharm. Pharmacol. 37(1985c): 33P.

Ford, J. L., Rubinnstein, M. H., McCaul, F.,Hogan, J. E., and Edgar, P. J. Importance of drug type, tablet shape, and added diluents on drug release kinetics from hydroxypropylmethylcellulose matrix tablets. Int. J. Pharm. 40(1987): 223-234

Frohoff-Hulsmann, M.A., Schmitz, A. and Lippold, B.C. Aqueous ethylcellulose dispersions containing plasticizers of different water solubility and hydroxypropyl methylcellulose as coating material for diffusion pellets., I drug released rate from coated pellets. Int. J. Pharm., 177, (1999) : 69-82

Fjimori, J., Yonemochi, E., Fukuoka, E. and Terada, K. Application of Eudragit RS to thermos-sensitive drug delivery systems. I thermo-sensitive drug release from acetaminophen matrix tablets consisting of Eudragit RS/PEG 400 blend polymers, Chem. Pharm Bull.50 (3), (2002): 408-412

Fussnegger, B.Kollidon® SR: Polyvinyl acetate based excipient for DC-sustained – release oral dosage forms. BASF, Technical information, 1999

Gibaldi, M., and Feldman, S. Establishment of sink conditions in dissolution rate determinations-theoretical considerations and application to nondisintegrating dosage forms. J. Pharm. Sci. 56: 1238-1242

Gutierrez- Rocca, J.C. and McGinity, J.W.Influence of aging on the physical-mechanical properties of acrylic resin films cast from aqueous dispersions and organic solutions. Drug Dev. Ind. Pharm., 19, (1993): 315-332

Gutierrez- Rocca, J.C. and McGinity, J.W. Influence of water soluble and insoluble plasticizers on the physical and mechanical properties of acrylic resins copolymers. Int. J. Pharm., 103, (1997): 293-301

He, Y., Zhu, B., and Inoue, Y.Hydrogen bonds in polymer blends. Prog. Polym. Sci. 29(2004): 1021-1051.

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

Hülsmann, S., Backensfeld, T., and Bodmeier, R. Stability of extruded 17β-estradiol solid dispersions. Pharmaceut. Dev. Tech. 6 (2001):223-229

Hülsmann, S., Backensfeld, T., Keitel, S., and Bodmeier, R. Melt extrusion-an alternative method for enhancing the dissolution rate of 17β-estradiol hemihydrates. Eur. J. Pharm. Biopharm. 49,(2000) :237-242

Hutchinson, F. G., and Furr, B. J. A. Biodegradable polymer systems for the sustained release of polypeptides. J. Control. Release 13(1990): 279-294

Kim, C-J. Effects of drug solubility, drug loading, and polymer molecular weight on drug release from Polyox® tablets. Drug Dev. Ind. Pharm., 24(7), (1998): 645-651

Lin, S.Y., Chen, K.S. and Run-Chu, L., Organic esters of plasticizers affecting the water absorption, adhesion property, glass transition temperature and plasticizer permanence of Eudragit acrylic films. J. Control. Rel., 68, (2000):343-350

Maejima, T. and McGinity, J.W., Influence of film additives on stabilizing drug release rates from pellets coated with acrylic polymers, Pharm. Dev. Technol. 60(2), (2001), 211-221

Mctaggart, C.M., Ganley, J.A., Sickmueller, A. and Walker, S.E., The evaluation of formulation and processing conditions of a melt granulation process. Int. J. Pharm. 19, (1984):139-148

Mittal, G., Sahana, D.K., Bhardwaj, V., and Ravi Kumar, M.N.V., Estradiol loaded PLGA nanoperticles for oral administration: Effect of polymer molecular weight and copolymer composition on release behavior *in vitro* and *in vivo*. J. Control. Rel., 119,(2007), 77-85

Mulye, N.Y. and Turco, S.J., Matrix type tablets formulation for controlled release for highly water-soluble drugs. Drug Dev. Ind. Pharm. 25, (1994), 2633-2643.

Niwa, T., Takeuchi, H., Hino, T., Itoh, A., Kawashima, Y. and Kiuchi, K., Preparation of agglomerated crystal for direct tableting and microencapsulation by spherical crystallization technique with a continuous system. Pharm. Res.11,(1994) : 278-484

Nokhodchi, A., Farid, Dj., Najafi, M. and Adrangui, M. Studies on controlled release formulation of diclofenac. Drug Dev. Ind. Pharm. 23(11)(1997), 1019-1023

Novoa, G.A.G., Heinämäki, J., Mirza, S., Antikainen, O., Colarte, A.I., Paz, A.S., and Yliruusi, J., Physical solid-state properties and dissolution of sustained-release matrices of polyvinylacetate. Eur. J. Pharm. Biopharm. 59, (2005) : 343-350

Okarter, T.U., Singla, K., The effects of plasticizers on the release of metoprolol tartrate from granules coated with a polymethacrylate film, Drug Dev. Ind. Pharm., 26(3), (2000) : 323-329

Paoletti, A.M., Pilia, I., Nannipieri, F., Bigini, C., and Melis, G.B. Comparison of pharmacokinetic profiles of a 17β-estradiol gel 0.6 mg/g (Gelestra) with a transdermal delivery system (Estraderm TTS 50) in postmenopausal women atsteady state. Maturitas 40(2001): 203-209

Park, J-S., Kang, H. W., Park, S. J., and Kim, C-K. 2005. Use of CP/MAS solid-state NMR for the characterization of solvate molecules within estradiol crystal forms. Eur. J. Pharm. Biopharm. 60, (200): 407-412

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

Repka, M.A., Gerding, T.G., Repka, S.L. and McGinity, J.W. Influence of plasticizers and drug on the physical-mechanical properties of hydroxypropylcellulose films prepared by hot melt extrusion. Drug Dev. Ind. Pharm., 25(5), (1999): 625-633

Repka, M.A. and McGinity, J.W. Influence of cholropheniramine maleate on topical hydroxypropylmethylcellulose films produced by hot-melt extrusion. Pharm. Dev. Tech., 6(3), (2001): 297-304

Rey, H., Wagner, K.G., Wehrle, P. and Schmidt, P.C. Development of matrix-based theophylline sustained-release microtablets, Drug Dev. Ind. Pharm., 26(1), (2000) : 21-26.

Riger, P.L. and Peppas, N.A. A simple equation for description of solute release I. Fickain and non-fickian release from non-swellable devices in the form of slabs, sphere, cylinders or disc. J. Control. Rel., 5, (1987): 23-36

Saettoe, M.F., Perini, G., Rijli, P., Rodriguez, L. and Cini, M. Effect of different polymer-plasticizer combinations on in vitro release of theophylline from coated pellets, Int. J. Pharm., 126,(1995) : 83-88

Sears, J.K. and Darby, J.R. The technology of plasticizers, John Willey &Sons, inc., New York , 1982

Schmidt, W.G., Mehnert, W. and Fromming, H., Controlled release from spherical matrices prepared in a laboratory scale roter granulator release mechanism interpretation using individual pellet data, Eur. J. Pharm. Biopharm. 42, (1996): 348-350

Skultety, P.F. and Sims, S.M. Evaluation of the loss of propylene glycol during aqueous film coating, Drug Dev. Ind. Pharm. 13 (12), (1987) : 2209-2219

Tsong, T., Hsu, P., and Langer, R. Polymers for the controlled release of macromolecules: effect of molecular weight of ethylene-vinyl acetate copolymer. J. Bio. Mat. Res. 19 (1985): 445-460

United States Pharmacopiea & National Formulary. 25th Ed. The United States Pharmacopial Convention Inc., 2002

Wang, C.C., Zhang, G., Infeld, M.H., Malick, A.W. and McGinity, J.W., Influence of plasticizers on the mechanical propeties of pellets containing Eudragit RS 30D. Int. J. Pharm. 152, (1997): 153-163

Wangner, J. G. 1969. Interpretation of percent dissolved-time plots derved from in vitro testing of conventional tablets and capsules. J. Pharm. Sci. 58: 1253-1257.

Wheatley, T.A. and Steuernagel, C.R. in McGinity, J.W.(Ed.) Aqueous polymeric coating for pharmaceutical dosage forms. Dekker , New York (1997) 1-54

Wu, C. and McGinity, J.W. Non-traditional plasticization of polymeric films. Int. J. Pharm. 177, (1999) : 15-27

Wu, C. and McGinity, J.W. Influence of ibuprofen as a solid state plasticizer in Eudragit RS 30D on the physico-chemical properties of coated beads, AAPS Pharm. Sci. Technol. 2 (4) 24, (2001)

Wu, C. and McGinity, J.W. Influence of methylparaben as a solid state plasticizer on the physicochemical properties of Eudragit RSPO hot melt extrudates, Eur. J. Pharm. Biopharm. 56, (2003): 95-100

Ye, W-P, and Chien, Y. W. Dual-controlled drug delivery across biodegradable copolymer. II. Delivery kinetics of levonorgrestrel and estradiol from (matrix/matrix) laminate drug delivery system. J. Control. Release 41(1996):259-269

Zhu Y., Shah N.H., Malick, A.W., Infeld, M.H. and McGinity, J.W. influence of thermal processing on the properties of chlorpheniramine maleate tablets containing an acrylic polymer. Pharm. Dev. Technol., 7(4), (2002) : 481-489

Zhu Y., Shah, N.H., Infeld, M.H., Malick, A.W and McGinity, J.W. Solid state plasticization of an acrylic polymer with chlorpheniramine maleate and triethyl citrate, Int. J. Pharm. 241, (2002) : 301- 310

APPENDICES

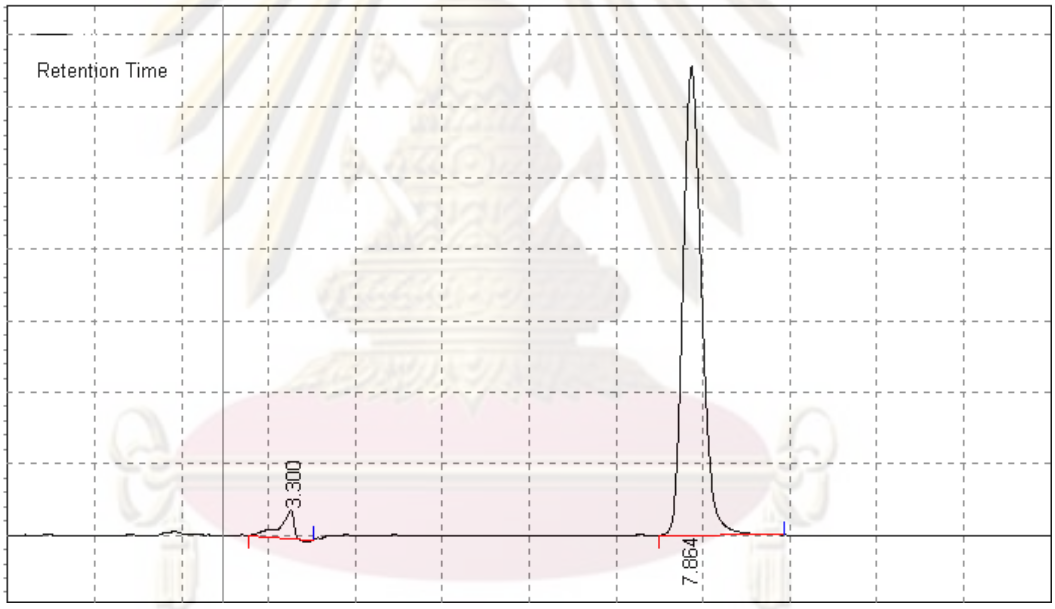
APPENDIX A

Validation of HPLC Method

The HPLC method was used to determine the E_2 and DEP content of PVAc implants. The validation of HPLC methods used was presented as follows:

1. Specificity

The specificity of an analytical method is the ability to measure the analyte accurately and with specificity in the presence of other components in the sample. Figures 1A were shown typical chromatogram of E_2 standard solution, internal standard solution, excipient and DEP matrix solution, respectively. The chromatograms demonstrated that the HPLC condition used in the study had a suitable specificity.



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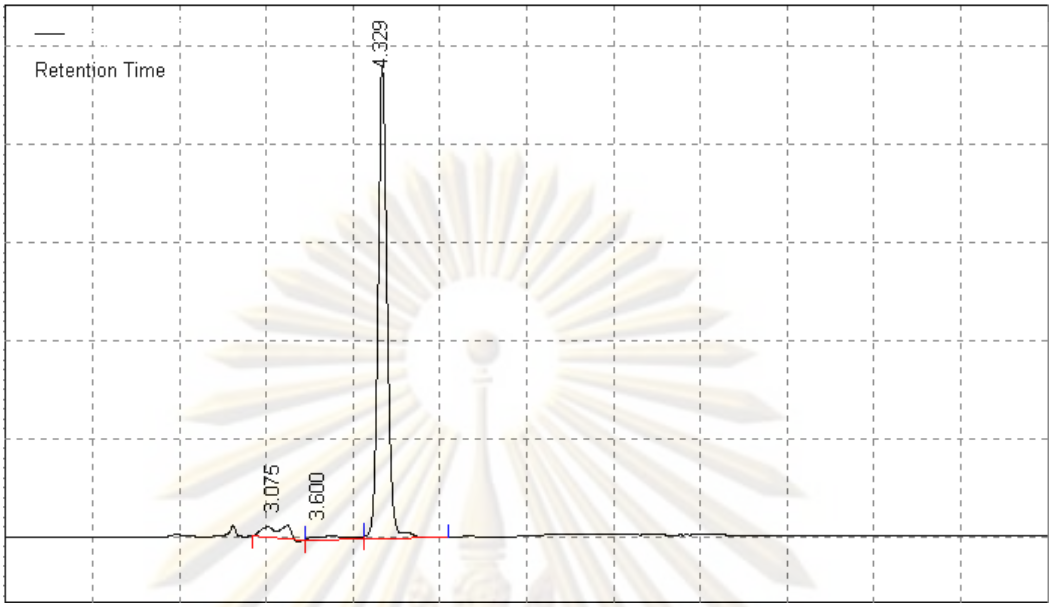

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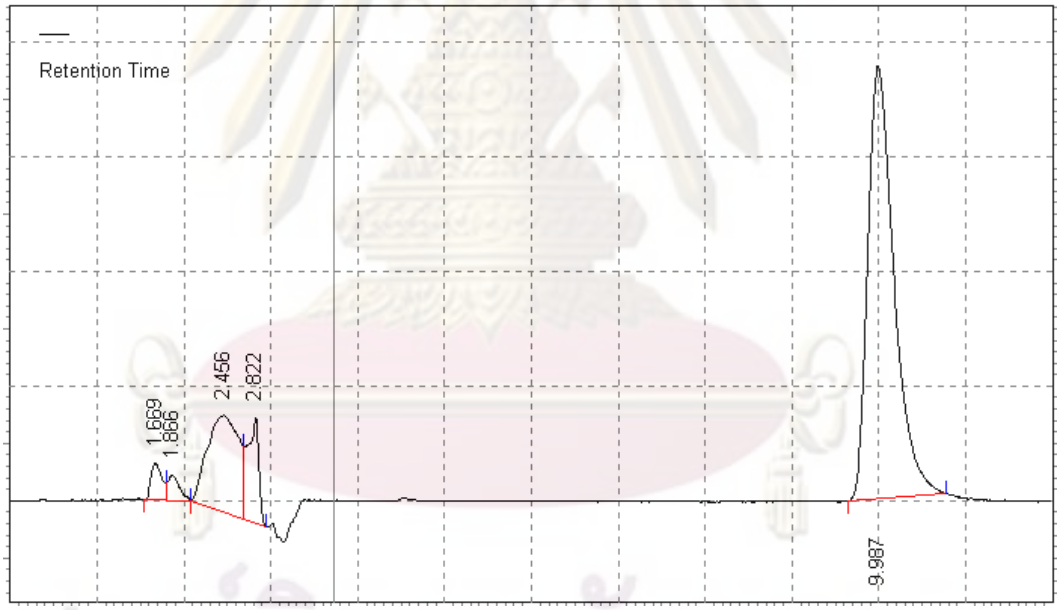


(c)

Figure 1A HPLC chromatograms of mobile phase

(a) 17β-estradiol standard solution

(b) Prednisolone solution

(c) Excipient and DEP matrix formulation

Table 1A Data for calibration curve of E_2 by HPLC method

Concentration (µg/ml)	Peak height ratio			Mean	SD	%RSD
	Set1	Set2	Set3			
2	0.3981	0.3954	0.3942	0.3960	0.0020	0.50
4	0.8193	0.8134	0.7946	0.8090	0.0129	1.59
6	1.1632	1.1549	1.1683	1.1621	0.0068	0.58
8	1.6532	1.6893	1.6324	1.6583	0.0288	1.74
20	4.0174	4.0174	3.9500	3.9608	0.0521	1.34
40	8.0317	7.8242	7.8946	7.9506	0.1055	1.33
R^2	**0.9999**	**0.9997**	**0.9997**	**0.9998**	-	-

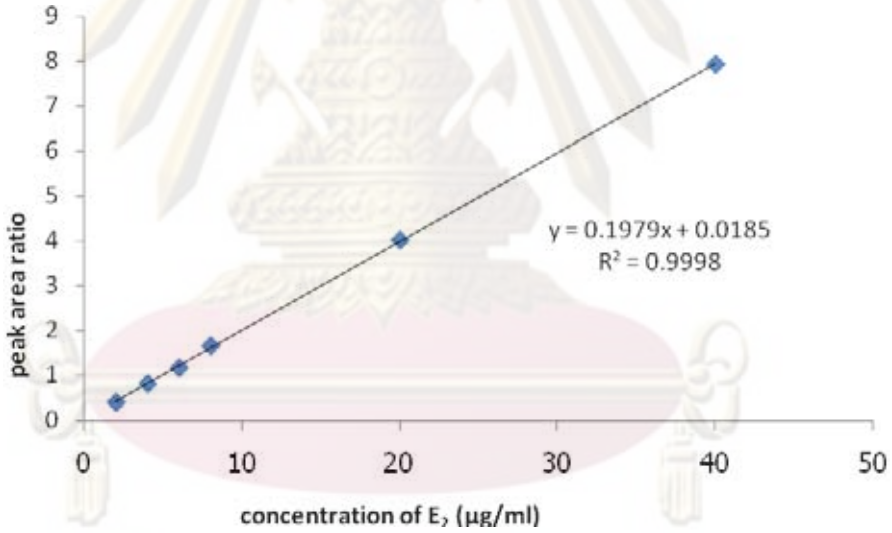


Figure 2A Calibration curve of E_2 by HPLC method

Table 2A Data for calibration curve of DEP by HPLC method

Concentration (µg/ml)	Peak height ratio			Mean	SD	%RSD
	Set1	Set2	Set3			
2	21.613	20.899	21.372	21.29	0.36	1.71
4	54.927	53.919	53.303	54.15	0.82	1.51
6	109.567	108.729	106.759	109.37	1.44	1.32
8	236.873	232.389	233.270	234.18	2.38	1.01
20	530.772	527.325	512.299	522.80	9.82	1.88
R^2	**0.9996**	**0.9996**	**0.9995**	**0.9997**	-	-

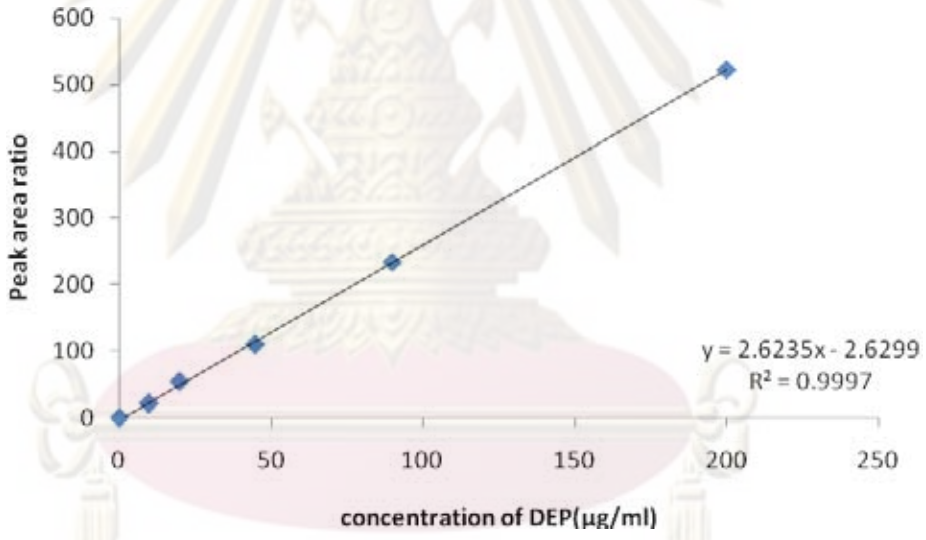


Figure 3A Calibration curve of DEP by HPLC method

2. Accuracy

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value. Accuracy is calculated as percent recovery by the assay of known added amount of analyses. The percentages of analytical recovery of E_2 were shown in Table 3A. The percentages analytical recovery of E_2 was in the range of 95-105%, which indicated that this method could be used for analysis in all concentrations studied with a high accuracy.

Table 3A The percentages of analytical recovery of E_2 solution by HPLC method

Concentration (µg/ml)	%Analytical recovery					Mean ± SD
	1	2	3	4	5	
3	100.78	101.96	99.65	100.27	101.55	100.84±0.93
20	100.11	100.38	101.50	101.75	100.50	100.85±0.73
35	99.48	98.13	100.74	101.66	99.70	99.94±1.33

3. Precision

The precision of E_2 analyzed by HPLC method were determined both within run precision and between run precision as illustrated in Tables 4 and 5. All coefficients of variation values were small, as 1.08-1.37% and 0.93-1.26% respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the HPLC method was precise for quantitative analysis of E_2 in the range studied.

Table 4A Data of within run precision by HPLC method

Concentration (µg/ml)	Peak height ratio					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
3	2.96	2.97	2.98	3.05	2.97	2.99	0.04	1.22
20	19.69	20.29	19.59	19.97	19.91	19.89	0.27	1.37
35	35.15	35.89	35.76	35.06	35.73	35.52	0.38	1.08

Table 5A Data of between run precision by HPLC method

Concentration (µg/ml)	Peak height ratio					Mean	SD	%CV
	Set1	Set2	Set3	Set4	Set5			
3	2.95	2.89	2.98	2.97	2.92	2.94	0.04	1.26
20	20.15	20.19	19.59	19.97	19.85	19.95	0.24	1.22
35	35.05	35.62	35.26	35.06	34.73	35.14	0.33	0.93

APPENDIX B

DSC Thermograms

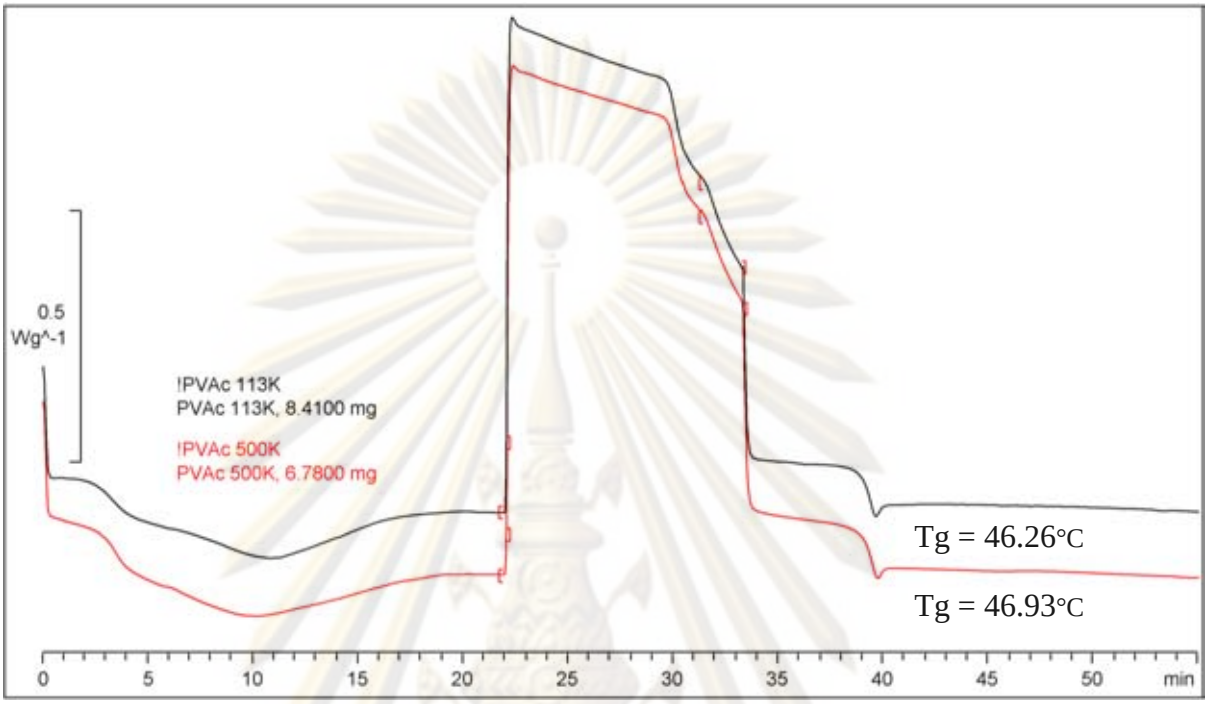
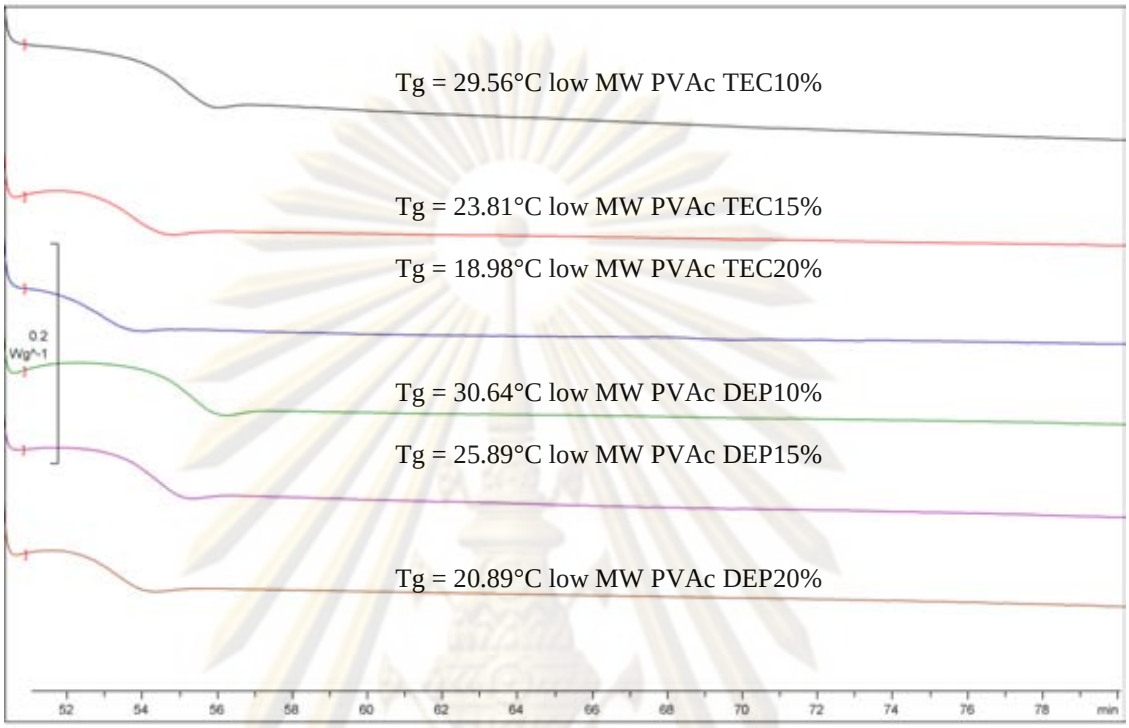
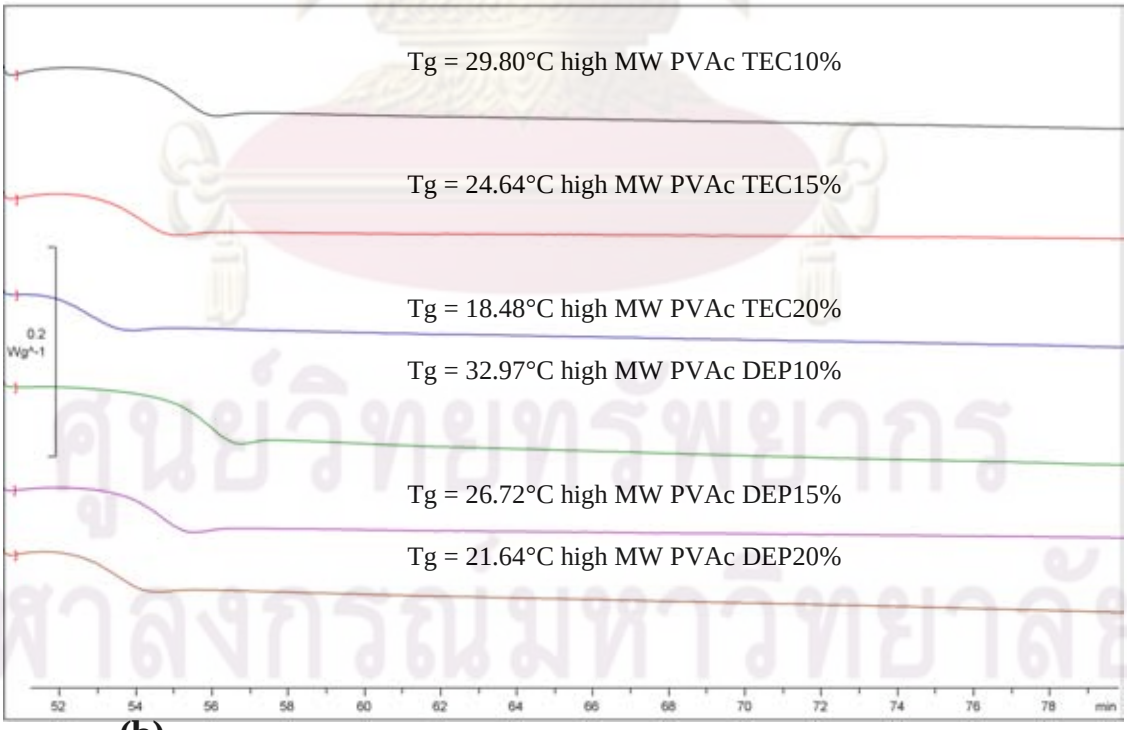


Figure 1B Tg of matrices implant low and high MW PVAc

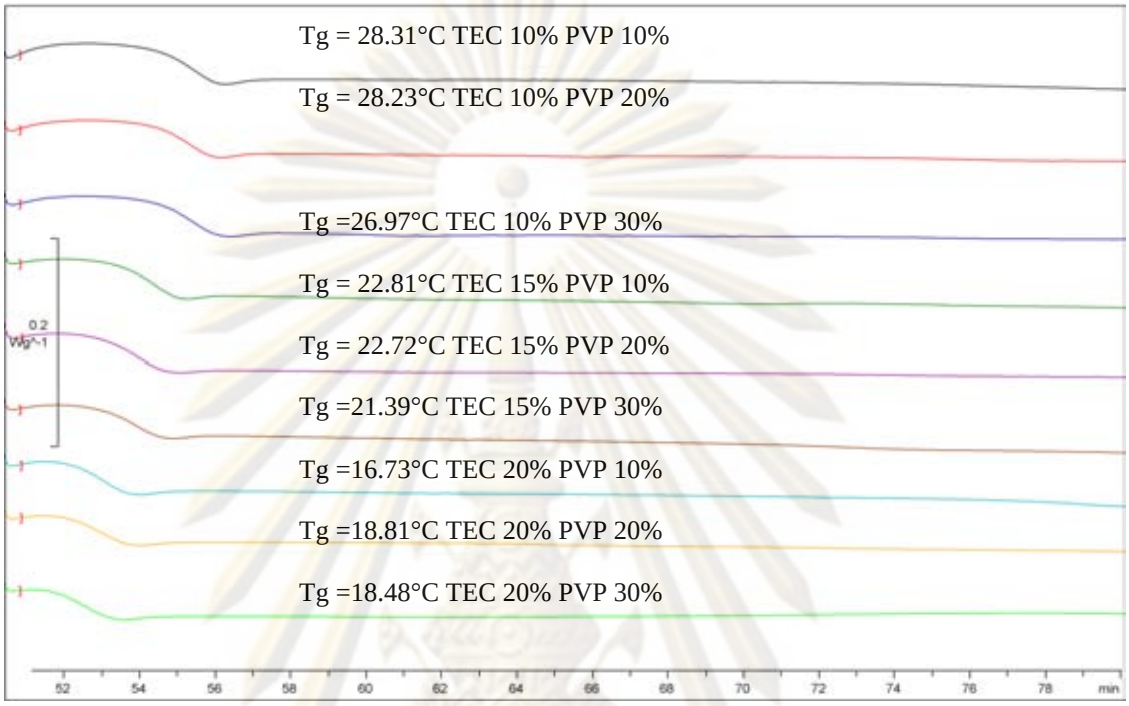


(a)



(b)

Figure 2B Tg of matrices implant low (a) and high (b) MW PVAc with 10, 15and 20% of TEC or DEP



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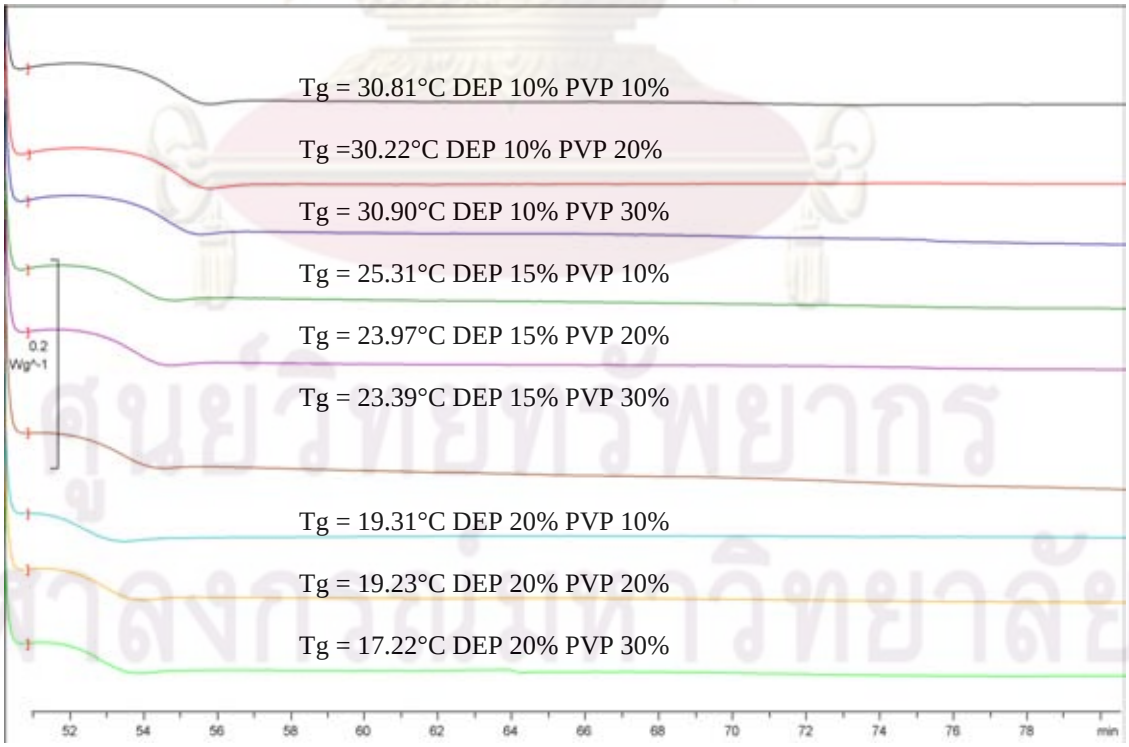
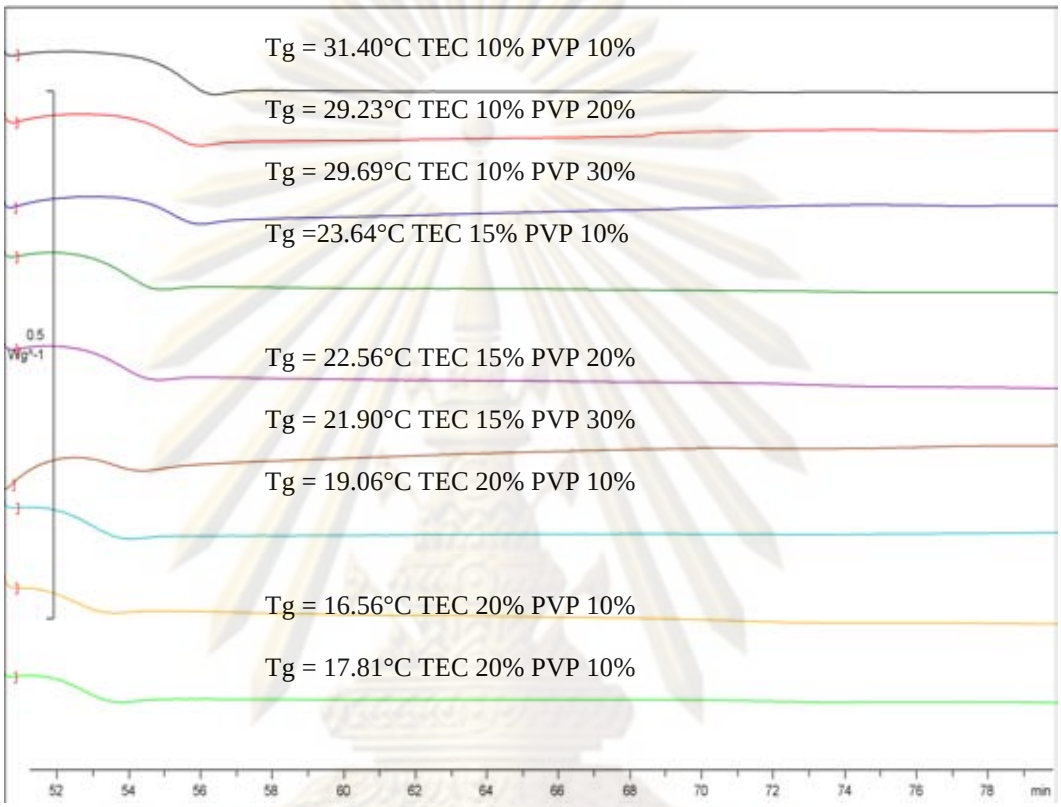
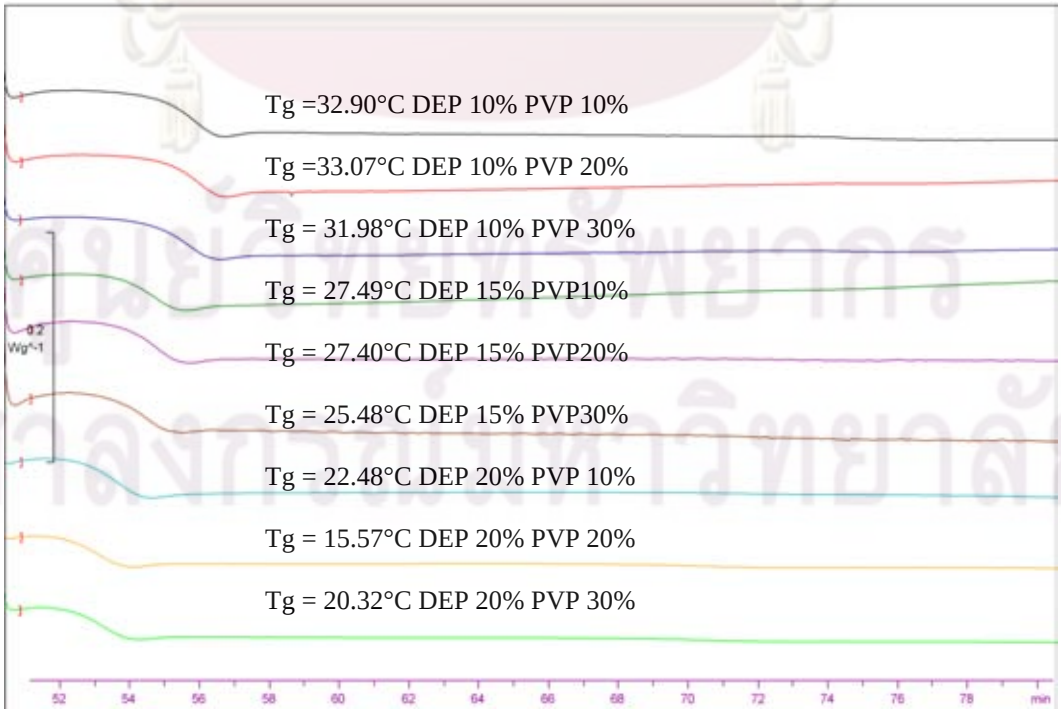


(b)

Figure 3B Tg of matrices implant low MW PVAc containing 10, 20 and 30% PVP with 10, 15and 20% of TEC (a) or DEP (b)

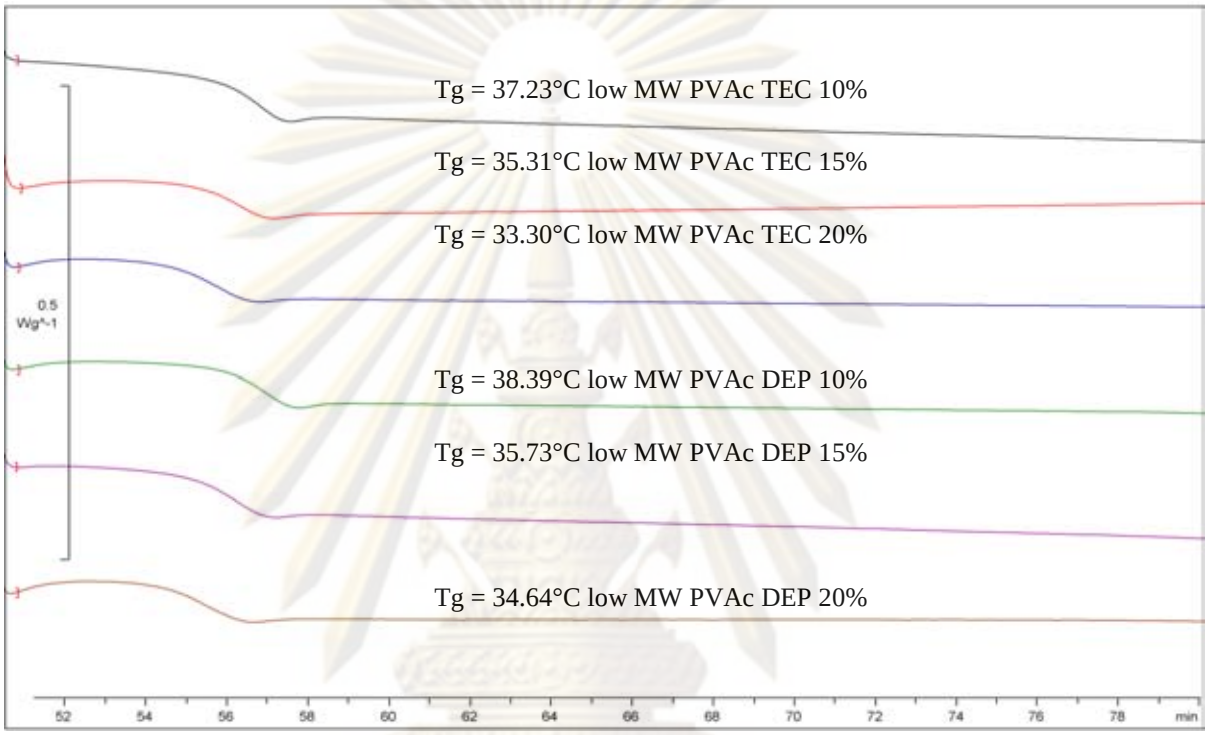


(a)

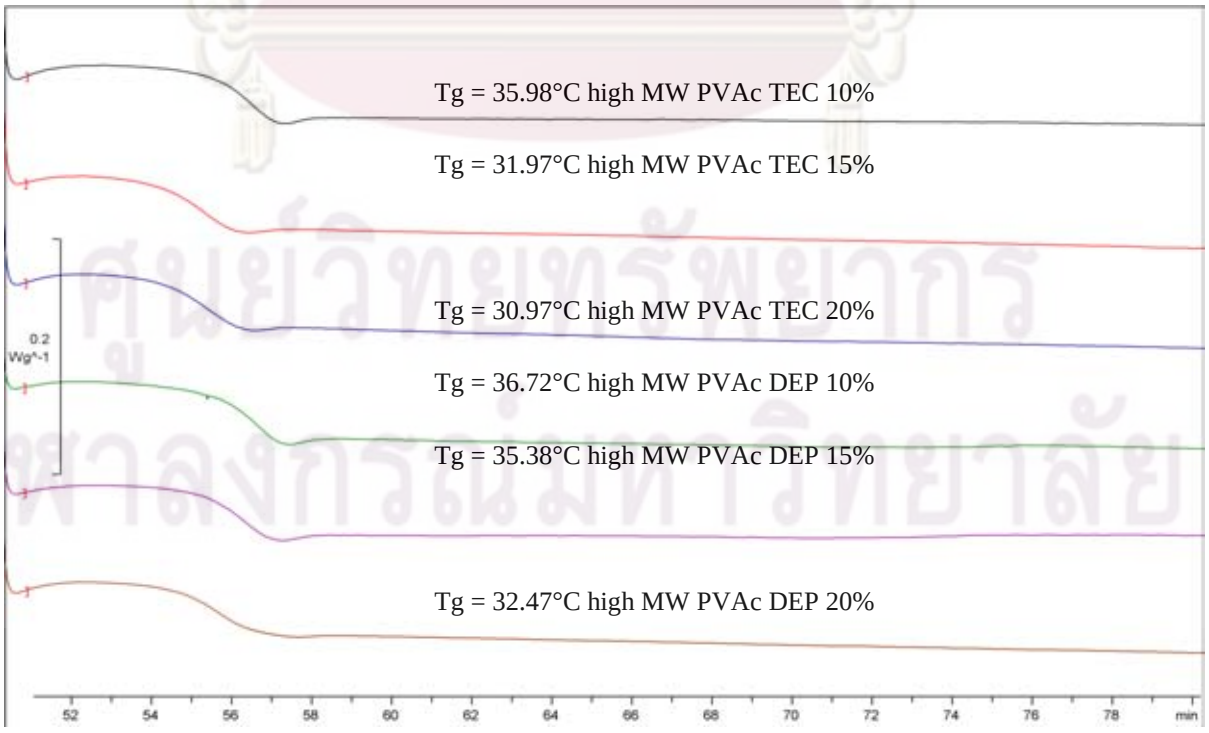


(b)

Figure 4B Tg of matrices implant high MW PVAc containing 10, 20 and 30% PVP with 10, 15and 20% of TEC (a) or DEP (b)



(a)



(b)

Figure 5B Tg of matrices implant low (a) and high (b) MW PVAc with 10, 15and 20% of TEC or DEP after immersion in medium at 28 days

APPENDIX C

Percentage Amount of Drug Release

Table 1C Percentage amounts of 2% w/w 17β-estradiol from matrices containing low MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.69	3.44	3.67	3.60	0.14	4.57
2	1.414	5.05	4.91	5.23	5.06	0.16	4.55
3	1.732	5.98	5.88	6.24	6.03	0.19	4.54
5	2.236	7.41	7.29	7.63	7.44	0.17	4.53
7	2.646	8.49	8.46	8.74	8.56	0.15	4.52
9	3	9.41	9.34	9.58	9.44	0.12	4.51
14	3.742	11.34	11.20	11.34	11.29	0.08	4.49
21	4.583	13.26	13.08	13.13	13.15	0.09	4.46
28	5.292	15.22	14.93	14.96	15.04	0.16	4.44

Table 2C Percentage amounts of 4% w/w 17β-estradiol from matrices containing low MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.68	3.63	3.96	3.76	0.18	4.57
2	1.414	5.22	4.94	5.21	5.12	0.16	4.55
3	1.732	6.44	6.00	6.35	6.26	0.23	4.54
5	2.236	7.84	7.09	7.47	7.47	0.38	4.53
7	2.646	8.91	7.96	8.35	8.41	0.48	4.52
9	3	9.55	8.60	8.95	9.03	0.48	4.51
14	3.742	11.32	10.26	10.59	10.72	0.54	4.49
21	4.583	13.38	12.12	12.42	12.64	0.66	4.47
28	5.292	15.36	13.84	14.15	14.45	0.80	4.45

Table 3C Percentage amounts of 6% w/w 17β-estradiol from matrices containing low MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.26	3.28	3.39	3.31	0.07	4.57
2	1.414	4.76	4.90	5.05	4.90	0.15	4.55
3	1.732	5.90	5.95	6.16	6.01	0.14	4.54
5	2.236	7.40	7.54	7.68	7.54	0.14	4.53
7	2.646	8.54	8.61	8.76	8.64	0.11	4.51
9	3	9.29	9.33	8.82	9.15	0.28	4.51
14	3.742	11.08	10.98	10.40	10.82	0.37	4.49

21	4.583	12.94	12.72	12.05	12.57	0.46	4.47
28	5.292	14.70	14.37	13.61	14.22	0.56	4.45

Table 4C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 10% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	4.58	4.90	4.85	4.77	0.17	4.556
2	1.414	6.28	6.67	6.58	6.51	0.20	4.538
3	1.732	7.31	7.71	7.62	7.55	0.21	4.527
5	2.236	8.88	9.27	9.24	9.13	0.22	4.509
7	2.646	10.11	10.58	10.41	10.37	0.24	4.496
9	3	11.05	11.58	11.31	11.31	0.27	4.485
14	3.742	13.05	13.66	13.18	13.30	0.32	4.462
21	4.583	14.95	15.72	15.14	15.27	0.40	4.439
28	5.292	17.13	17.99	17.27	17.46	0.46	4.413

Table 5C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 15% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.35	6.68	6.75	6.59	0.21	4.537
2	1.414	8.17	8.40	8.38	8.31	0.13	4.518
3	1.732	9.26	9.63	9.41	9.43	0.19	4.506
5	2.236	10.88	11.30	10.99	11.06	0.22	4.488
7	2.646	12.21	12.63	12.27	12.37	0.23	4.473
9	3	13.20	13.61	13.21	13.34	0.23	4.462
14	3.742	15.20	15.69	15.15	15.35	0.30	4.439
21	4.583	17.29	17.73	17.20	17.41	0.28	4.414
28	5.292	19.36	19.75	19.05	19.39	0.35	4.390

Table 6C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 20% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.30	6.68	6.80	6.59	0.26	4.537
2	1.414	8.57	8.92	9.13	8.87	0.28	4.512
3	1.732	9.97	10.34	10.57	10.29	0.30	4.497
5	2.236	12.12	12.32	12.72	12.39	0.31	4.473
7	2.646	13.88	13.99	14.45	14.11	0.30	4.453
9	3	15.21	15.28	15.72	15.40	0.28	4.438
14	3.742	17.53	17.53	18.13	17.73	0.35	4.410

21	4.583	19.93	20.00	20.56	20.16	0.35	4.380
28	5.292	22.52	22.64	23.17	22.78	0.35	4.347

Table 7C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 10% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	5.67	5.76	5.73	3.20	0.05	4.546
2	1.414	7.41	7.43	7.45	4.77	0.02	4.528
3	1.732	8.49	8.51	8.45	5.55	0.03	4.517
5	2.236	10.11	10.35	9.96	7.19	0.20	4.498
7	2.646	11.38	11.63	11.17	8.16	0.23	4.484
9	3	12.35	12.61	12.14	9.08	0.24	4.473
14	3.742	14.45	14.72	14.17	11.25	0.28	4.449
21	4.583	16.48	16.79	16.08	13.34	0.36	4.425
28	5.292	18.73	19.00	18.39	14.98	0.31	4.398

Table 8C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 15% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.40	5.76	5.33	5.83	0.54	4.545
2	1.414	8.28	7.44	6.84	7.52	0.72	4.527
3	1.732	9.49	8.55	7.84	8.63	0.83	4.515
5	2.236	11.08	10.25	9.63	10.32	0.73	4.496
7	2.646	12.37	11.71	11.00	11.69	0.69	4.481
9	3	13.72	12.86	12.14	12.91	0.79	4.467
14	3.742	16.38	15.32	14.34	15.35	1.02	4.439
21	4.583	19.38	18.53	17.44	18.45	0.97	4.401
28	5.292	22.43	21.27	20.13	21.28	1.15	4.366

Table 9C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 20% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	5.83	5.67	5.51	5.67	0.16	4.547
2	1.414	8.12	7.56	7.37	7.68	0.39	4.525
3	1.732	9.44	9.15	8.63	9.07	0.41	4.510
5	2.236	11.40	11.97	10.60	11.32	0.69	4.485
7	2.646	12.96	13.84	12.24	13.02	0.80	4.466
9	3	14.19	15.29	13.55	14.34	0.88	4.450
14	3.742	16.77	18.30	16.37	17.17	1.02	4.417

21	4.583	19.44	20.84	20.76	20.35	0.79	4.378
28	5.292	22.14	23.75	23.76	23.21	0.93	4.341

Table 10C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 10% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	7.03	8.09	7.73	7.62	0.54	4.526
2	1.414	9.13	10.34	10.06	9.84	0.63	4.502
3	1.732	10.4	11.68	11.43	11.19	0.68	4.486
5	2.236	12.54	13.74	13.55	13.28	0.65	4.463
7	2.646	14.32	15.48	15.35	15.05	0.64	4.442
9	3	15.60	16.74	16.61	16.31	0.62	4.427
14	3.742	18.49	19.67	19.46	19.21	0.63	4.392
21	4.583	13.63	21.50	22.91	22.58	0.74	4.352
28	5.292	15.28	23.75	25.13	24.96	0.75	4.323

Table 11C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 10% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	7.55	7.50	7.21	7.42	0.18	4.528
2	1.414	9.75	9.78	9.31	9.61	0.26	4.504
3	1.732	11.15	11.23	10.66	11.01	0.31	4.489
5	2.236	13.29	13.34	12.71	13.11	0.35	4.465
7	2.646	15.16	15.13	14.45	14.91	0.40	4.444
9	3	16.46	16.48	15.66	16.20	0.47	4.428
14	3.742	19.67	19.65	18.64	19.32	0.59	4.390
21	4.583	22.74	22.93	21.76	22.48	0.63	4.351
28	5.292	24.98	25.26	23.90	24.71	0.72	4.321

Table 12C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 10% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.71	8.23	8.56	7.84	0.99	4.524
2	1.414	9.09	10.69	10.85	10.21	0.97	4.497
3	1.732	10.50	12.09	12.30	11.63	0.98	4.482
5	2.236	12.76	14.40	14.56	13.91	1.00	4.455
7	2.646	14.53	16.31	16.39	15.74	1.05	4.434
9	3	15.92	17.72	17.72	17.12	1.04	4.417
14	3.742	19.14	21.21	20.78	20.38	1.09	4.377
21	4.583	22.55	24.86	24.05	23.82	1.17	4.333
28	5.292	24.82	27.38	26.58	26.26	1.31	4.301

Table 13C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 15% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	10.10	10.36	9.75	10.07	0.31	4.499
2	1.414	12.98	13.24	12.60	12.94	0.32	4.467
3	1.732	14.78	14.98	14.24	14.67	0.38	4.447
5	2.236	17.48	17.59	16.80	17.29	0.43	4.415
7	2.646	19.76	19.78	18.89	19.48	0.51	4.389
9	3	21.40	21.36	20.38	21.05	0.58	4.369
14	3.742	25.22	25.32	24.25	24.93	0.59	4.318
21	4.583	28.75	29.42	28.19	28.79	0.62	4.266
28	5.292	31.16	32.12	31.05	31.44	0.59	4.228

Table 14C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 15% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.36	11.16	11.02	11.18	0.17	4.487
2	1.414	14.38	14.38	14.27	14.34	0.06	4.450
3	1.732	16.09	16.20	16.00	16.10	0.10	4.430
5	2.236	18.65	18.87	18.62	18.72	0.14	4.398
7	2.646	20.97	21.15	20.86	20.99	0.15	4.370
9	3	22.50	22.75	22.44	22.56	0.16	4.350
14	3.742	26.05	26.50	26.24	26.26	0.23	4.301
21	4.583	29.62	30.17	30.05	29.95	0.29	4.249
28	5.292	32.23	32.88	32.75	32.62	0.34	4.210

Table 15C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 15% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.22	10.91	10.80	10.97	0.22	4.489
2	1.414	14.36	13.97	13.72	14.01	0.32	4.454
3	1.732	16.21	15.72	15.41	15.78	0.40	4.433
5	2.236	18.98	18.41	18.00	18.46	0.49	4.401
7	2.646	21.41	20.74	20.22	20.79	0.60	4.372
9	3	23.13	22.51	21.95	22.53	0.59	4.350
14	3.742	26.66	26.20	25.93	26.26	0.37	4.301
21	4.583	30.47	30.25	29.92	30.21	0.28	4.245
28	5.292	33.42	33.08	32.89	33.13	0.27	4.203

Table 16C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 20% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	13.48	13.93	14.76	14.06	0.65	4.454
2	1.414	17.09	17.63	18.71	17.81	0.82	4.409
3	1.732	19.28	19.80	21.16	20.08	0.97	4.381
5	2.236	22.63	23.26	24.70	23.53	1.06	4.337
7	2.646	25.42	25.94	27.62	26.33	1.15	4.300
9	3	27.37	27.92	29.59	28.30	1.16	4.272
14	3.742	31.98	32.51	34.27	32.92	1.20	4.206
21	4.583	36.46	37.08	38.91	37.48	1.27	4.135
28	5.292	39.23	40.44	42.30	40.66	1.55	4.083

Table 17C Percentage amounts of17β-estradiol from matrices containing low MW PVAcwith TEC 20% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	14.40	14.49	14.66	14.52	0.13	4.448
2	1.414	18.63	18.62	19.05	18.77	0.25	4.397
3	1.732	20.88	20.95	21.54	21.12	0.36	4.368
5	2.236	24.51	24.48	25.33	24.77	0.48	4.321
7	2.646	27.42	27.35	28.33	27.70	0.55	4.281
9	3	29.53	29.41	30.44	29.79	0.56	4.251
14	3.742	33.87	33.91	34.92	34.23	0.60	4.186
21	4.583	38.08	38.18	39.33	38.52	0.69	4.119
28	5.292	40.86	41.04	42.40	41.43	0.84	4.070

Table 18C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with TEC 20% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	15.28	15.15	14.35	14.93	0.50	4.443
2	1.414	19.48	19.03	18.69	19.07	0.40	4.394
3	1.732	22.14	21.73	21.36	21.74	0.39	4.360
5	2.236	25.94	25.37	25.41	25.57	0.32	4.310
7	2.646	28.75	28.26	28.44	28.49	0.25	4.270
9	3	30.63	30.32	30.67	30.54	0.19	4.241
14	3.742	35.19	34.91	35.28	35.13	0.19	4.172
21	4.583	39.58	39.30	39.67	39.52	0.19	4.102
28	5.292	42.59	42.40	42.80	42.60	0.20	4.050

Table 19C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 10% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	8.05	7.36	7.01	7.47	0.53	4.528
2	1.414	9.89	9.24	8.81	9.31	0.54	4.507
3	1.732	11.05	10.41	9.96	10.47	0.55	4.495
5	2.236	12.82	12.01	11.66	12.16	0.60	4.476
7	2.646	14.52	13.67	13.38	13.86	0.59	4.456
9	3	14.52	14.83	14.57	15.05	0.17	4.442
14	3.742	15.74	14.83	14.57	17.37	0.61	4.414
21	4.583	20.87	19.82	19.59	20.09	0.68	4.381
28	5.292	22.93	21.89	21.70	22.17	0.66	4.355

Table 20C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 10% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	7.02	7.68	7.14	7.24	0.35	4.530
2	1.414	9.27	9.96	9.15	9.41	0.44	4.506
3	1.732	10.73	11.72	10.78	11.01	0.56	4.488
5	2.236	12.38	13.26	12.41	12.68	0.50	4.475
7	2.646	14.07	14.92	14.18	14.39	0.46	4.455
9	3	15.37	16.20	15.49	15.68	0.45	4.440
14	3.742	17.42	18.38	17.58	17.79	0.51	4.414
21	4.583	19.86	20.88	19.89	20.21	0.58	4.385
28	5.292	22.08	23.18	22.05	22.44	0.64	4.530

Table 21C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 10% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	8.02	7.65	7.29	7.65	0.37	4.526
2	1.414	9.91	9.51	9.11	9.51	0.40	4.505
3	1.732	11.08	10.66	10.25	10.66	0.42	4.492
5	2.236	12.76	12.31	11.87	12.31	0.45	4.474
7	2.646	14.66	14.09	13.53	14.09	0.57	4.453
9	3	16.03	15.43	14.82	15.43	0.61	4.438
14	3.742	18.42	17.76	17.10	17.76	0.66	4.410
21	4.583	21.22	20.47	19.73	20.47	0.75	4.376
28	5.292	23.41	22.62	21.82	22.62	0.80	4.349

Table 22C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 15% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	10.53	8.28	7.79	8.86	1.46	4.512
2	1.414	12.48	10.36	9.72	10.85	1.44	4.490
3	1.732	13.74	11.66	10.99	12.13	1.43	4.476
5	2.236	15.47	13.45	12.81	13.91	1.39	4.455
7	2.646	17.28	15.34	14.64	15.75	1.37	4.434
9	3	18.50	16.67	15.90	17.02	1.34	4.419
14	3.742	20.86	19.26	18.38	19.50	1.26	4.388
21	4.583	23.59	22.36	21.26	22.40	1.17	4.352
28	5.292	25.75	24.6	23.74	24.68	1.01	4.322

Table 23C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 15% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	9.62	8.95	8.83	9.13	0.43	4.509
2	1.414	12.11	11.32	11.21	11.55	0.49	4.482
3	1.732	13.62	12.84	12.71	13.06	0.49	4.465
5	2.236	15.72	15.17	15.09	15.33	0.34	4.439
7	2.646	17.85	17.4	17.52	17.60	0.23	4.412
9	3	19.26	19.12	19.06	19.14	0.10	4.393
14	3.742	22.06	22.13	21.68	21.96	0.24	4.357
21	4.583	25.17	25.52	25.28	25.32	0.18	4.313
28	5.292	27.64	28.16	27.78	27.86	0.27	4.279

Table 24C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 15% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	8.25	9.52	9.17	8.98	0.66	4.511
2	1.414	11.17	12.42	12.05	11.88	0.64	4.479
3	1.732	13.17	14.29	13.80	13.75	0.56	4.457
5	2.236	16.08	17.17	16.55	16.60	0.55	4.424
7	2.646	18.92	19.83	19.15	19.30	0.47	4.391
9	3	20.92	21.72	20.93	21.19	0.46	4.367
14	3.742	23.93	24.99	24.11	24.35	0.57	4.326
21	4.583	27.42	28.55	27.61	27.86	0.61	4.279
28	5.292	30.70	31.88	30.06	30.88	0.92	4.236

Table 25C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 20% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	9.93	10.51	10.34	10.26	0.30	4.497
2	1.414	12.97	13.56	13.34	13.29	0.30	4.463
3	1.732	15.05	15.31	15.28	15.21	0.14	4.440
5	2.236	18.32	18.51	18.27	18.37	0.13	4.402
7	2.646	21.59	21.77	21.33	21.56	0.22	4.362
9	3	23.71	23.89	23.32	23.64	0.29	4.335
14	3.742	26.76	26.92	26.15	26.61	0.41	4.296
21	4.583	31.76	31.83	30.86	31.48	0.54	4.227
28	5.292	35.24	35.16	34.10	34.84	0.64	4.177

Table 26C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 20% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	12.07	11.90	12.43	12.13	0.27	4.476
2	1.414	15.00	14.79	15.33	15.04	0.27	4.442
3	1.732	16.90	16.72	17.18	16.93	0.23	4.420
5	2.236	19.99	19.78	20.35	20.04	0.29	4.382
7	2.646	22.93	22.59	23.34	22.95	0.38	4.344
9	3	24.94	24.64	25.31	24.96	0.34	4.318
14	3.742	28.71	28.63	29.28	28.87	0.35	4.265
21	4.583	33.20	33.21	34.07	33.49	0.50	4.197
28	5.292	36.34	36.45	37.27	36.69	0.51	4.148

Table 27C Percentage amounts of17β-estradiol from matrices containing low MW PVAc with DEP 20% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	10.85	12.03	12.22	11.77	0.74	4.480
2	1.414	14.19	15.08	15.43	14.99	0.64	4.443
3	1.732	16.45	17.30	17.60	17.22	0.60	4.416
5	2.236	19.76	20.61	20.79	20.52	0.55	4.376
7	2.646	22.97	23.78	24.01	23.74	0.55	4.334
9	3	25.14	25.94	26.11	25.23	0.52	4.314
14	3.742	29.50	30.05	30.28	29.47	0.40	4.256
21	4.583	34.71	34.80	35.85	34.68	0.63	4.179
28	5.292	38.43	38.34	39.31	38.28	0.54	4.123

Table 28C Percentage amounts of 2% w/w 17β-estradiol from matrices containing high MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.47	2.98	3.16	3.20	0.25	4.57
2	1.414	5.02	4.57	4.74	4.77	0.23	4.56
3	1.732	5.84	5.32	5.51	5.55	0.26	4.55
5	2.236	7.45	6.95	7.17	7.19	0.24	4.53
7	2.646	8.38	7.94	8.15	8.16	0.21	4.52
9	3	9.29	8.87	9.07	9.08	0.20	4.51
14	3.742	11.50	11	11.23	11.25	0.24	4.49
21	4.583	13.63	13.08	13.29	13.34	0.27	4.46
28	5.292	15.28	14.71	14.96	14.98	0.27	4.44

Table 29C Percentage amounts of 4% w/w17β-estradiol from matrices containing high MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.36	3.41	3.25	3.34	0.08	4.57
2	1.414	4.95	4.93	4.72	4.87	0.13	4.56
3	1.732	5.79	5.74	5.51	5.68	0.15	4.55
5	2.236	7.39	7.35	7.03	7.26	0.19	4.53
7	2.646	8.30	8.22	7.86	8.13	0.23	4.52
9	3	9.14	9.03	8.64	8.94	0.27	4.51
14	3.742	11.33	11.23	10.73	11.10	0.32	4.49
21	4.583	13.60	12.91	12.35	12.96	0.63	4.47
28	5.292	15.59	14.36	13.73	14.56	0.94	4.45

Table 30C Percentage amounts of 6% w/w 17β-estradiol from matrices containing high MW PVAc

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	3.39	3.19	3.25	3.28	0.10	4.57
2	1.414	4.76	4.41	4.45	4.54	0.19	4.56
3	1.732	5.62	5.12	5.16	5.30	0.28	4.55
5	2.236	7.21	6.57	6.61	6.80	0.36	4.53
7	2.646	8.24	7.47	7.51	7.74	0.43	4.52
9	3	9.06	8.27	8.30	8.54	0.45	4.51
14	3.742	10.86	9.76	9.77	10.13	0.63	4.49
21	4.583	13.12	11.77	11.77	12.22	0.78	4.47
28	5.292	15.06	13.41	13.39	13.95	0.96	4.45

Table 31C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with TEC 10% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	5.45	5.58	6.17	5.73	0.38	4.55
2	1.414	7.16	8.04	8.09	7.76	0.52	4.52
3	1.732	8.46	9.40	9.44	9.09	0.55	4.51
5	2.236	10.33	11.46	11.53	11.10	0.67	4.49
7	2.646	12.17	13.45	13.48	13.03	0.75	4.47
9	3	13.48	14.95	14.91	14.44	0.84	4.45
14	3.742	16.58	18.34	18.17	17.69	0.97	4.41
21	4.583	20.35	22.18	21.28	20.94	0.92	4.37
28	5.292	22.96	25.24	24.46	23.62	1.16	4.34

Table 32C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with TEC 15% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.49	6.66	6.88	6.67	0.20	4.54
2	1.414	8.35	8.69	8.92	8.65	0.29	4.51
3	1.732	9.70	10.07	10.35	10.04	0.33	4.50
5	2.236	11.87	12.27	12.50	12.21	0.32	4.47
7	2.646	14.21	14.70	15.17	14.69	0.48	4.45
9	3	15.70	16.22	16.70	16.21	0.50	4.43
14	3.742	19.22	19.75	20.27	19.75	0.53	4.39
21	4.583	22.72	23.31	24.29	23.10	0.79	4.34
28	5.292	26.19	26.55	28.00	26.26	0.96	4.30

Table 33C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 20% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	6.61	6.99	6.86	6.82	0.19	4.53
2	1.414	8.75	9.20	9.43	9.12	0.35	4.51
3	1.732	10.21	10.79	11.10	10.70	0.45	4.49
5	2.236	12.58	13.22	13.70	13.16	0.56	4.46
7	2.646	14.91	15.48	16.33	15.57	0.71	4.44
9	3	16.65	17.16	18.14	17.31	0.76	4.42
14	3.742	20.47	21.04	22.39	21.30	0.99	4.37
21	4.583	24.46	24.88	26.49	24.91	1.07	4.32
28	5.292	27.93	28.74	31.02	28.50	1.60	4.27

Table 34C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with DEP 10% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	5.55	5.52	5.27	5.45	0.15	4.55
2	1.414	7.44	7.61	7.26	7.44	0.18	4.53
3	1.732	8.71	8.94	8.63	8.76	0.16	4.51
5	2.236	10.73	10.53	10.05	10.44	0.35	4.49
7	2.646	11.99	12.03	11.54	11.85	0.27	4.48
9	3	13.29	13.51	12.85	13.22	0.34	4.46
14	3.742	16.18	16.47	15.78	16.14	0.35	4.43
21	4.583	18.97	19.11	18.81	18.96	0.15	4.39
28	5.292	21.48	21.70	21.52	21.57	0.12	4.36

Table 35C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 15% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	5.91	6.03	6.60	8.33	0.37	4.52
2	1.414	7.93	8.11	8.59	10.36	0.34	4.50
3	1.732	9.60	9.76	10.30	12.04	0.37	4.48
5	2.236	11.45	11.60	12.16	13.58	0.37	4.46
7	2.646	13.04	13.18	13.82	15.06	0.42	4.44
9	3	14.48	14.60	15.32	16.41	0.45	4.43
14	3.742	17.65	17.53	18.54	19.37	0.55	4.39
21	4.583	20.32	20.46	21.68	22.20	0.75	4.35
28	5.292	22.75	23.35	24.70	25.53	1.00	4.31

Table 36C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 20% w/w

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	7.08	6.63	7.55	9.63	0.46	450
2	1.414	9.57	9.06	10.14	12.13	0.54	4.48
3	1.732	11.25	10.61	11.87	13.79	0.63	4.46
5	2.236	13.38	12.74	14.19	15.98	0.73	4.43
7	2.646	15.45	14.72	16.15	17.98	0.72	4.41
9	3	16.95	16.27	17.80	19.55	0.77	4.39
14	3.742	20.04	19.31	20.95	22.64	0.82	4.35
21	4.583	22.94	22.17	24.05	25.86	0.95	4.31
28	5.292	25.65	25.15	27.20	28.81	1.07	4.27

Table 37C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with TEC 10% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	8.57	9.46	7.67	8.57	0.90	4.52
2	1.414	11.42	12.02	10.81	11.42	0.61	4.48
3	1.732	13.27	13.86	12.69	13.27	0.59	4.46
5	2.236	16.07	16.43	15.70	16.07	0.37	4.43
7	2.646	18.57	16.43	15.70	18.57	1.49	4.40
9	3	20.24	20.22	20.26	20.24	0.02	4.38
14	3.742	24.12	23.72	24.51	24.12	0.40	7.33
21	4.583	28.22	27.40	29.03	28.22	0.82	4.27
28	5.292	31.64	30.50	32.78	31.64	1.14	4.22

Table 38C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 10% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	9.990	7.122	7.436	8.18	1.57	4.52
2	1.414	13.35	10.39	11.62	11.79	1.49	4.48
3	1.732	15.49	12.24	13.79	13.84	1.63	4.46
5	2.236	18.70	15.08	16.89	16.89	1.81	4.42
7	2.646	18.70	15.08	16.89	19.55	1.81	4.39
9	3	23.32	19.34	21.32	21.33	1.99	4.37
14	3.742	27.64	23.25	25.31	25.40	2.20	4.31
21	4.583	32.27	27.47	29.65	29.79	2.40	4.25
28	5.292	36.06	30.96	33.17	33.40	2.56	4.20

Table 39C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 10% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.535	12.267	9.113	10.97	1.65	4.49
2	1.414	14.95	15.79	12.47	14.40	1.73	4.45
3	1.732	17.15	18.07	14.58	16.60	1.81	4.42
5	2.236	20.33	21.42	17.71	19.82	1.91	4.38
7	2.646	20.33	21.42	17.71	22.64	1.91	4.35
9	3	24.93	26.06	22.11	24.46	2.03	4.33
14	3.742	29.07	30.13	26.09	28.52	2.09	4.27
21	4.583	33.41	34.49	30.42	32.86	2.11	4.21
28	5.292	37.04	38.09	34.10	36.50	2.07	4.15

Table 40C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with TEC 15% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.00	10.49	10.90	10.80	0.27	4.49
2	1.414	14.08	13.72	14.19	14.00	0.25	4.45
3	1.732	16.32	16.04	16.28	16.22	0.15	4.43
5	2.236	19.54	19.33	19.63	19.50	0.15	4.39
7	2.646	19.54	19.33	19.63	22.29	0.15	4.35
9	3	24.10	23.95	24.39	24.15	0.22	4.33
14	3.742	28.16	28.10	28.56	28.28	0.25	4.27
21	4.583	32.51	32.49	33.05	32.69	0.32	4.21
28	5.292	36.00	36.08	36.63	36.24	0.34	4.16

Table 41C Percentage amounts of 17β-estradiol from matrices high MW PVAc with TEC 15% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	9.37	12.52	7.95	9.95	2.34	4.50
2	1.414	12.79	18.00	13.01	14.60	2.95	4.45
3	1.732	16.45	20.93	15.73	17.70	2.82	4.41
5	2.236	20.50	25.17	19.61	21.76	2.99	4.36
7	2.646	20.50	25.17	19.61	25.12	2.99	4.32
9	3	26.11	30.93	25.06	27.37	3.13	4.29
14	3.742	30.83	35.78	29.88	32.16	3.17	4.22
21	4.583	36.05	40.85	34.96	37.29	3.13	4.14
28	5.292	40.20	44.82	39.05	41.35	3.05	4.07

Table 42C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 15% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.31	8.35	10.18	9.95	1.49	4.50
2	1.414	16.77	13.36	15.61	15.25	1.73	4.44
3	1.732	19.58	15.99	18.51	18.03	1.84	4.41
5	2.236	23.52	19.92	22.71	22.05	1.89	4.36
7	2.646	23.52	19.92	22.71	25.38	1.89	4.31
9	3	28.91	25.43	28.45	27.60	1.89	4.28
14	3.742	33.49	30.30	33.44	32.41	1.83	4.21
21	4.583	38.17	35.18	38.54	37.30	1.84	4.14
28	5.292	41.90	39.09	42.52	41.18	1.83	4.07

Table 43C Percentage amounts of17β-estradiol from matrices containing high MW PVAc with TEC 20% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	13.65	13.99	13.00	13.55	0.50	4.45
2	1.414	17.57	18.13	18.45	18.05	0.45	4.40
3	1.732	20.38	20.99	21.74	21.04	0.68	4.36
5	2.236	24.32	25.01	26.31	25.21	1.01	4.30
7	2.646	27.60	28.21	30.08	28.63	1.29	4.27
9	3	29.79	30.34	32.59	30.91	1.48	4.22
14	3.742	34.66	35.21	37.84	35.90	1.70	4.15
21	4.583	39.54	40.19	43.04	40.92	1.86	4.07
28	5.292	43.33	44.04	47.04	44.80	1.97	4.00

Table 44C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 20% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	14.44	14.05	12.98	13.82	0.76	4.47
2	1.414	18.86	18.32	17.66	18.28	0.60	4.42
3	1.732	21.83	21.19	20.52	21.18	0.66	4.38
5	2.236	26.19	25.12	24.85	25.39	0.71	4.33
7	2.646	29.78	28.64	28.46	28.96	0.72	4.26
9	3	32.17	30.98	30.86	31.34	0.72	4.25
14	3.742	37.33	35.97	36.01	36.44	0.77	4.18
21	4.583	42.29	40.87	41.17	41.44	0.75	4.10
28	5.292	46.21	44.72	45.23	45.39	0.76	4.03

Table 45C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with TEC 20% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	12.25	13.04	11.56	12.28	0.74	4.47
2	1.414	18.60	17.24	17.68	17.84	0.69	4.41
3	1.732	21.92	20.11	20.93	20.99	0.91	4.37
5	2.236	26.62	24.30	25.42	25.45	1.16	4.31
7	2.646	30.30	27.93	29.00	29.08	1.19	4.26
9	3	32.76	30.35	31.39	31.50	1.21	4.23
14	3.742	38.01	35.43	36.49	36.64	1.30	4.15
21	4.583	43.07	40.55	41.28	41.63	1.30	4.07
28	5.292	47.02	44.56	45.12	45.57	1.29	4.00

Table 46C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 10% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.62	11.45	11.91	11.66	0.23	4.48
2	1.414	14.82	14.56	14.97	14.78	0.21	4.45
3	1.732	16.91	16.61	17.20	16.91	0.30	4.42
5	2.236	20.05	19.70	20.44	20.06	0.37	4.38
7	2.646	22.43	22.00	22.87	22.43	0.44	4.35
9	3	24.39	23.91	24.88	24.39	0.49	4.33
14	3.742	28.34	27.61	28.76	28.24	0.58	4.27
21	4.583	33.16	32.25	33.91	33.11	0.83	4.20
28	5.292	36.06	35.03	36.78	35.96	0.88	4.48

Table 47C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 10% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E_2remained)
0	0	0	0	0	0	0.00	4.60
1	1	10.62	9.63	11.59	10.61	0.98	4.49
2	1.414	13.33	12.21	14.34	13.29	1.07	4.46
3	1.732	15.56	14.28	16.58	15.47	1.15	4.44
5	2.236	19.00	17.49	20.00	18.83	1.26	4.40
7	2.646	21.74	19.98	22.64	21.45	1.35	4.36
9	3	24.08	22.24	24.95	23.76	1.38	4.33
14	3.742	28.87	26.81	29.65	28.44	1.47	4.27
21	4.583	35.20	32.28	35.32	34.27	1.72	4.19
28	5.292	38.78	35.63	38.75	37.72	0.98	4.13

Table 48C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 10% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	10.81	10.57	10.46	10.61	0.18	4.49
2	1.414	13.73	13.45	13.40	13.53	0.18	4.46
3	1.732	16.04	15.71	15.70	15.82	0.19	4.43
5	2.236	19.51	19.10	19.22	19.28	0.21	4.39
7	2.646	22.12	21.75	21.93	21.93	0.19	4.36
9	3	24.29	23.95	24.20	24.15	0.18	4.33
14	3.742	28.69	28.31	28.75	28.58	0.24	4.27
21	4.583	34.08	33.87	34.54	34.16	0.34	4.19
28	5.292	37.15	37.11	37.84	37.37	0.41	4.27

Table 49C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 15% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	14.75	11.39	11.53	12.56	1.90	4.47
2	1.414	18.19	14.46	14.69	15.78	2.09	4.43
3	1.732	20.70	16.81	17.07	18.19	2.17	4.40
5	2.236	24.44	20.43	20.75	21.87	2.23	4.36
7	2.646	27.29	23.35	23.52	24.72	2.23	4.32
9	3	29.65	25.73	25.87	27.08	2.22	4.29
14	3.742	34.26	30.61	30.55	31.81	2.12	4.22
21	4.583	40.13	36.81	36.55	37.83	2.00	4.13
28	5.292	43.47	40.33	39.85	41.22	1.97	4.07

Table 50C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 15% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	11.93	11.83	13.04	12.27	0.67	4.47
2	1.414	15.19	15.08	16.54	15.60	0.81	4.44
3	1.732	17.69	17.57	18.96	18.07	0.77	4.41
5	2.236	21.72	21.33	22.56	21.87	0.63	4.36
7	2.646	24.13	23.70	25.56	24.46	0.97	4.32
9	3	26.72	26.21	28.01	26.98	0.93	4.29
14	3.742	32.10	32.35	33.77	32.74	0.90	4.21
21	4.583	37.48	37.57	39.75	38.27	1.29	4.12
28	5.292	40.95	41.14	43.20	41.76	1.25	4.06

Table 51C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 15% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	12.06	11.88	13.24	12.39	0.74	4.47
2	1.414	15.10	14.93	16.49	15.51	0.86	4.44
3	1.732	17.39	17.16	18.87	17.81	0.93	4.41
5	2.236	21.26	20.83	22.15	21.41	0.67	4.36
7	2.646	23.90	23.41	24.96	24.09	0.79	4.33
9	3	26.13	25.62	27.28	26.34	0.85	4.30
14	3.742	32.58	32.01	33.40	32.66	0.70	4.21
21	4.583	38.39	38.61	39.80	38.93	0.76	4.11
28	5.292	41.76	41.96	43.36	42.36	0.87	4.05

Table 52C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 20% w/w and copolymer PVP 10%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	15.75	15.40	14.58	15.24	0.60	4.44
2	1.414	19.29	19.02	17.70	18.67	0.85	4.40
3	1.732	21.82	21.62	19.97	21.14	1.02	4.37
5	2.236	25.63	25.49	23.48	24.87	1.20	4.32
7	2.646	28.73	28.49	26.69	27.97	1.11	4.28
9	3	31.74	31.40	29.42	30.85	1.25	4.24
14	3.742	36.99	36.52	34.31	35.94	1.43	4.16
21	4.583	43.31	42.81	40.44	42.19	1.53	4.06
28	5.292	46.54	46.15	43.60	45.43	1.60	4.00

Table 53C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 20% w/w and copolymer PVP 20%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	14.66	15.72	15.78	15.39	0.63	4.44
2	1.414	18.20	19.62	19.82	19.21	0.88	4.39
3	1.732	20.85	22.84	22.88	22.19	1.16	4.35
5	2.236	24.83	27.82	27.36	26.67	1.61	4.29
7	2.646	27.90	31.40	30.80	30.03	1.87	4.25
9	3	30.43	34.10	33.54	32.69	1.98	4.21
14	3.742	35.38	39.34	38.85	37.86	2.16	4.13
21	4.583	41.50	45.98	45.28	44.25	2.41	4.02
28	5.292	44.92	49.55	48.71	47.73	2.47	3.96

Table 54C Percentage amounts of 17β-estradiol from matrices containing high MW PVAc with DEP 20% w/w and copolymer PVP 30%

time(d)	time(√d)	1	2	3	Mean	SD	ln(%E2remained)
0	0	0	0	0	0	0.00	4.60
1	1	15.30	14.46	16.08	15.28	0.81	4.44
2	1.414	19.28	18.34	19.93	19.18	0.80	4.39
3	1.732	22.25	21.25	22.81	22.10	0.79	4.36
5	2.236	26.75	25.68	27.22	26.55	0.79	4.30
7	2.646	30.19	29.09	30.62	29.97	0.79	4.25
9	3	32.98	31.94	33.32	32.75	0.72	4.21
14	3.742	38.39	37.23	38.57	38.06	0.73	4.13
21	4.583	45.06	43.80	44.94	44.60	0.70	4.01
28	5.292	48.58	47.45	48.37	48.13	0.60	3.95

APPENDIX D

Release rate and R^2 of E_2 Released with Higuchi Models

Table 1D Correlation of determination (r^2) of relationships between percentage drug released versus square root of time (Higuchi rate constant), log percentage drug remained versus time (first order rate constant)and drug released versus time(Zero order rate constant) of PVAc MW 113,000 with plasticizer and copolymer PVP

Formulation code	$(Q_t = Q_o + k_o t)$		$(Q_t = Q_o * e^{-k1t})$		$(Q_t = k_H t^{1/2})$	
	k_o	R^2	k_1	R^2	k_H	R^2
E_2-TEC 10%	0.637	0.953	0.007	0.964	4.169	0.999
- PVP 10%	0.809	0.942	0.010	0.960	5.325	0.997
- PVP 20%	0.871	0.933	0.011	0.954	5.750	0.995
- PVP 30%	0.885	0.929	0.011	0.92	5.824	0.994
E_2-TEC 15%	0.702	0.952	0.008	0.965	4.953	0.998
- PVP 10%	0.889	0.931	0.011	0.954	5.875	0.995
- PVP 20%	1.074	0.912	0.014	0.944	7.146	0.988
- PVP 30%	1.057	0.905	0.014	0.938	7.047	0.985
E_2-TEC 20%	0.772	0.952	0.009	0.967	5.049	0.999
- PVP 10%	1.077	0.911	0.015	0.948	7.102	0.990
- PVP 20%	1.093	0.913	0.015	0.935	7.094	0.983
- PVP 30%	1.128	0.897	0.016	0.935	7.539	0.981
E_2-DEP 10%	0.566	0.954	0.006	0.965	3.695	0.998
- PVP 10%	0.856	0.934	0.011	0.955	5.649	0.995
- PVP 20%	0.981	0.953	0.013	0.972	6.417	0.999
- PVP 30%	0.959	0.943	0.012	0.964	6.304	0.997
E_2-DEP 15%	0.606	0.958	0.007	0.969	3.950	0.997
- PVP 10%	1.026	0.943	0.014	0.966	6.744	0.997
- PVP 20%	1.061	0.943	0.014	0.966	6.972	0.997
- PVP 30%	1.094	0.955	0.015	0.975	7.150	0.997
E_2-DEP 20%	0.684	0.938	0.008	0.954	4.498	0.996
- PVP 10%	1.090	0.940	0.016	0.965	7.169	0.996

- PVP 20%	1.150	0.927	0.017	0.958	7.608	0.993
- PVP 30%	1.171	0.930	0.017	0.961	7.738	0.994

Table 2D Correlation of determination (r^2) of relationships between percentage drug released versus square root of time (Higuchi rate constant), log percentage drug remained versus time (first order rate constant)and drug released versus time(Zero order rate constant) of PVAc MW 500,000 with plasticizer and copolymer PVP

Formulation code	$(Q_t = Q_o + k_o t)$		$(Q_t = Q_o * e^{-k1t})$		$(Q_t = k_H t^{1/2})$	
	k_o	R^2	k_1	R^2	k_H	R^2
E_2-TEC 10%	0.434	0.921	0.004	0.946	2.860	0.995
- PVP 10%	0.597	0.939	0.007	0.956	3.931	0.996
- PVP 20%	0.611	0.939	0.007	0.953	4.026	0.997
- PVP 30%	0.650	0.942	0.007	0.952	4.275	0.997
E_2-TEC 15%	0.441	0.930	0.005	0.940	2.914	0.994
- PVP 10%	0.753	0.936	0.009	0.953	4.963	0.995
- PVP 20%	0.745	0.927	0.009	0.946	4.932	0.993
- PVP 30%	0.775	0.934	0.010	0.953	5.116	0.995
E_2-TEC 20%	0.551	0.921	0.006	0.936	3.656	0.991
- PVP 10%	0.929	0.924	0.013	0.949	6.156	0.992
- PVP 20%	0.928	0.905	0.013	0.934	6.195	0.985
- PVP 30%	0.955	0.904	0.013	0.935	6.371	0.985
E_2-DEP 10%	0.446	0.937	0.005	0.947	2.938	0.995
- PVP 10%	0.517	0.941	0.006	0.952	3.404	0.997
- PVP 20%	0.518	0.917	0.006	0.931	3.439	0.989
- PVP 30%	0.528	0.941	0.006	0.952	3.474	0.997
E_2-DEP 15%	0.543	0.967	0.006	0.975	3.527	0.999
- PVP 10%	0.555	0.941	0.006	0.953	3.655	0.997
- PVP 20%	0.656	0.931	0.008	0.947	4.338	0.994
- PVP 30%	0.761	0.918	0.009	0.938	5.055	0.990
E_2-DEP 20%	0.614	0.948	0.007	0.960	4.032	0.998
- PVP 10%	0.861	0.929	0.011	0.951	5.691	0.993

- PVP 20%	0.872	0.937	0.011	0.957	5.752	0.996
- PVP 30%	0.956	0.938	0.013	0.961	6.297	0.996

APPENDIX E

Statistic Analysis

Table 1E One-way ANOVA analysis of E_2 release rate from E_2 implant with varying amount of E_2

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Release rate of E2	.158	18	.200*	.932	18	.210

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

Test of Homogeneity of Variances

Release rate of E_2

Levene Statistic	df1	df2	Sig.
2.578	5	12	.083

ANOVA

Release rate of E_2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.693	5	.539	1.123	.399
Within Groups	5.755	12	.480		
Total	8.447	17			

Multiple Comparisons

Dependent Variable:Release rate of E_2

	(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	PVAc 113K E2 2%	PVAc 113K E2 4%	.58667	.56542	.949	-2.2586	3.4319
		PVAc 113K E2 6%	.81000	.56542	.833	-2.0352	3.6552
		PVAc 500K E2 2%	.05333	.56542	1.000	-2.7919	2.8986
		PVAc 500K E2 4%	.48333	.56542	.977	-2.3619	3.3286
		PVAc 500K E2 6%	1.08667	.56542	.609	-1.7586	3.9319
	PVAc 113K E2 4%	PVAc 113K E2 2%	-.58667	.56542	.949	-3.4319	2.2586
		PVAc 113K E2 6%	.22333	.56542	.999	-2.6219	3.0686
		PVAc 500K E2 2%	-.53333	.56542	.966	-3.3786	2.3119
		PVAc 500K E2 4%	-.10333	.56542	1.000	-2.9486	2.7419
		PVAc 500K E2 6%	.50000	.56542	.974	-2.3452	3.3452
	PVAc 113K E2 6%	PVAc 113K E2 2%	-.81000	.56542	.833	-3.6552	2.0352
		PVAc 113K E2 4%	-.22333	.56542	.999	-3.0686	2.6219
		PVAc 500K E2 2%	-.75667	.56542	.867	-3.6019	2.0886
		PVAc 500K E2 4%	-.32667	.56542	.996	-3.1719	2.5186
		PVAc 500K E2 6%	.27667	.56542	.998	-2.5686	3.1219
	PVAc 500K E2 2%	PVAc 113K E2 2%	-.05333	.56542	1.000	-2.8986	2.7919
		PVAc 113K E2 4%	.53333	.56542	.966	-2.3119	3.3786
		PVAc 113K E2 6%	.75667	.56542	.867	-2.0886	3.6019
		PVAc 500K E2 4%	.43000	.56542	.986	-2.4152	3.2752
		PVAc 500K E2 6%	1.03333	.56542	.655	-1.8119	3.8786
	PVAc 500K E2 4%	PVAc 113K E2 2%	-.48333	.56542	.977	-3.3286	2.3619
		PVAc 113K E2 4%	.10333	.56542	1.000	-2.7419	2.9486
		PVAc 113K E2 6%	.32667	.56542	.996	-2.5186	3.1719

	PVAc 500K E2 2%	-.43000	.56542	.986	-3.2752	2.4152
	PVAc 500K E2 6%	.60333	.56542	.943	-2.2419	3.4486
PVAc 500K E2 6%	PVAc 113K E2 2%	-1.08667	.56542	.609	-3.9319	1.7586
	PVAc 113K E2 4%	-.50000	.56542	.974	-3.3452	2.3452
	PVAc 113K E2 6%	-.27667	.56542	.998	-3.1219	2.5686
	PVAc 500K E2 2%	-1.03333	.56542	.655	-3.8786	1.8119
	PVAc 500K E2 4%	-.60333	.56542	.943	-3.4486	2.2419

*. The mean difference is significant at the 0.01 level.

Table 2E One-way ANOVA analysis of E_2 release rate from E_2 implant with varying amount of plasticizer

Test of Homogeneity of Variances

Release rate of E_2

Levene Statistic	df1	df2	Sig.
2.175	11	24	.054

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Release rate of E2	.068	36	.200*	.979	36	.702

a. Lilliefors Significance Correction

*. This is a lower bound of the true significance.

ANOVA

Release rate of E_2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	396.045	11	36.004	44.437	.000
Within Groups	19.445	24	.810		
Total	415.491	35			

Multiple Comparisons

Dependent Variable:Release rate of E_2

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	99% Confidence Interval	
					Lower Bound	Upper Bound
PVAc113M TEC 10	PVAc113M TEC 20	-5.01000*	.73495	.002	-9.2978	-.7222
	PVAc500M TEC 10	6.75667*	.73495	.000	2.4688	11.0445
	PVAc500M TEC 15	4.83333*	.73495	.002	.5455	9.1212
	PVAc500M DEP 10	5.51333*	.73495	.000	1.2255	9.8012
PVAc113M TEC 15	PVAc113K DEP 10	5.34667*	.73495	.001	1.0588	9.6345
	PVAc500M TEC 10	9.45000*	.73495	.000	5.1622	13.7378
	PVAc500M TEC 15	7.52667*	.73495	.000	3.2388	11.8145
	PVAc500M DEP 10	8.20667*	.73495	.000	3.9188	12.4945
	PVAc500M DEP 15	5.63667*	.73495	.000	1.3488	9.9245
PVAc113M TEC 20	PVAc113K DEP10	7.66333*	.73495	.000	3.3755	11.9512
	PVAc113K DEP 15	5.63000*	.73495	.000	1.3422	9.9178
	PVAc500M TEC 10	11.76667*	.73495	.000	7.4788	16.0545
	PVAc500M TEC 15	9.84333*	.73495	.000	5.5555	14.1312
	PVAc500M TEC 20	6.45333*	.73495	.000	2.1655	10.7412
	PVAc500M DEP 10	10.52333*	.73495	.000	6.2355	14.8112
	PVAc500M DEP 15	7.95333*	.73495	.000	3.6655	12.2412
	PVAc500M DEP 20	6.01333*	.73495	.000	1.7255	10.3012
PVAc113K DEP 10	PVAc113K DEP 20	-4.43333*	.73495	.007	-8.7212	-.1455
PVAc113K DEP 15	PVAc500M TEC 10	6.13667*	.73495	.000	1.8488	10.4245
	PVAc500M DEP 10	4.89333*	.73495	.002	.6055	9.1812
PVAc113K DEP 20	PVAc500M TEC 10	8.53667*	.73495	.000	4.2488	12.8245
	PVAc500M TEC 15	6.61333*	.73495	.000	2.3255	10.9012

	PVAc500M DEP 10	7.29333*	.73495	.000	3.0055	11.5812
	PVAc500M DEP 15	4.72333*	.73495	.003	.4355	9.0112
PVAc500M TEC 10	PVAc500M TEC 20	-5.31333*	.73495	.001	-9.6012	-1.0255
	PVAc500M DEP 20	-5.75333*	.73495	.000	-10.0412	-1.4655
PVAc500M DEP 10	PVAc500M DEP 20	-4.51000*	.73495	.006	-8.7978	-.2222

*. The mean difference is significant at the 0.01 level.

VITA

Mr. Supot Wittayanukulluck was born on August 23, 1979 in Nakornsawan, Thailand. He received the Bachelor of Science in Pharmacy from Faculty of Pharmaceutical Sciences, Chulalongkorn University in 2004.