ไมโครแอนแคปซูเลชันของสารสกัดฟ้าทะลายโจรในรูปออกฤทธ์นานซึ่งเตรียมโดย
เทคนิคการระเหยตัวทำละลาย

นายสาโรช อ่อนละออ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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MICROENCAPSULATION OF *ANDROGRAPHIS PANICULATA* NEES EXTRACT FOR SUSTAINED RELEASE PREPARED BY SOLVENT EVAPORATION TECHNIQUE

Mr.Saroch Onlaor

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By	Mr. Saroch Onlaor
Filed of study	Pharmacy
Thesis Advisor	Associate Professor Suchada Chutimaworapan, Ph.D.
Thesis Co-advisor	Associate Professor Chaiyo Chaichantipyuth, M.Sc.in Pharm.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…….Dean of Faculty of Pharmaceutical Sciences
(Associate Professor Sunibhond Pummangura, Ph.D.)

THESIS COMMITTEE

…….Chairman
(Associate Professor Pranom Pothiyanon, M.Sc.in Pharm.)

…….Thesis Advisor
(Associate Professor Suchada Chutimaworapan, Ph.D.)

…….Thesis Co-advisor
(Associate Professor Chaiyo Chaichantipyuth, M.Sc.in Pharm.)

…….Member
(Associate Professor Phensri Thongnopnua, Ph.D.)

…….Member
(Nontima Vardhanabhuti, Ph.D.)

สาโรช อ่อนละออ : ไมโครเอนแคปซูเลชันของสารสกัดฟ้าทะลายโจรในรูปออกฤทธิ์นานซึ่งเตรียมโดยเทคนิคระเหยตัวทะละลาย. (MICROENCAPULATION OF *ANDROGRAPHIS PANICULATA* NEES EXTRACT FOR SUSTAINED RELEASE BY SOLVENT EVAPORATION TECHNIQUE) อาจารย์ที่ปรึกษา : รศ.ดร.สุชาดา ชุติมาวรพันธ์, อ. ที่ปรึกษาร่วม : รศ.ชัยโย ชัยชาญทิพยุทธ, 187 หน้า. ISBN 974-13-1279-2.

การเตรียมไมโครแคปซูลเพื่อออกฤทธิ์นานของสารสกัดฟ้าทะลายโจร (*Andrographis paniculata* Nees) โดยเทคนิคการระเหยตัวทำละลายชนิดน้ำมันในน้ำมัน ได้ศึกษาผลของปัจจัยของกระบวนการและสูตรตำรับต่อการเกิดไมโครแคปซูลและลักษณะการปลดปล่อยยา การเพิ่มอัตราการกวนจาก 250-1200 รอบต่อนาที พบว่า ขนาดของไมโครแคปซูลลดลง โดยอัตราเร็ว 1000 รอบต่อนาที ให้ผลได้สูงมีปริมาณยาและปริมาณแกนถูกห่อหุ้มสูงที่สุดและให้ค่าคงที่อัตราการปลดปล่อยสูงที่สุดที่พีเอช 6.8 จากความเข้มข้นของสารก่ออิมัลชัน 0 - 2.0% พบว่า สแปน80 0.5% ให้ไมโครแคปซูลขนาดเหมาะสม มีปริมาณยาและปริมาณแกนที่ถูกห่อหุ้มสูงที่สุด ที่พีเอช 1.2 สแปน80 0.5%ให้ค่าคงที่อัตราการปลดปล่อยและประสิทธิภาพการละลายสูงที่สุด อัตราส่วนแกนต่อผนัง เท่ากับ 1:2 ทำให้ปริมาณแกนที่ถูกห่อหุ้มมากที่สุด ส่วนอัตราส่วน 1:1 ให้ผลได้และมีปริมาณยามากที่สุด ขนาดของไมโครแคปซูล ที่เตรียมจาก อัตราส่วน 2 : 3 มีขนาดใหญ่มากเนื่องจากวัฏภาคภายในมีความเข้มข้นสูง ไมโครแคปซูลที่เตรียมจากยูดราจิตอาร์แอล100 จะให้ผลได้สูงกว่าขนาดเล็กกว่า ปริมาณแกนที่ถูกห่อหุ้มมากกว่าและค่าคงที่การปลดปล่อยและประสิทธภาพการละลายสูงกว่ายูดราจิตอาร์เอส100 การเติมพอล็อกซาเมอร์188ความเข้มข้น 20% แม้จะให้ปริมาณแกนที่ถูกห่อหุ้มลดลงเล็กน้อยและอนุภาคเกิดการเกาะกลุ่มแต่ให้ค่าคงที่อัตราการปลดปล่อยและประสิทธิภาพการละลายดีกว่าเมื่อไม่เติมพอล็อกซาเมอร์188 กลไกการปลดปล่อยของแอนโดรกราโฟไลด์จากไมโครแคปซูลสอดคล้องกับโมเดลแบบควบคุมโดยการแพร่

ภาควิชา เภสัชกรรม
สาขาวิชา เภสัชกรรม
ปีการศึกษา 2543

ลายมือชื่อนิสิต .....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEYWORD: *ANDROGRAPHIS PANICULATA* / ANDROGRAPHOLIDE / SUSTAINED RELEASE / EUDRAGIT / MICROCAPSULE / SOLVENT EVAPORATION

SAROCH ONLAOR : MICROENCAPSULATION OF *ANDROGRAPHIS PANICULATA* NEES EXTRACT FOR SUSTAINED RELEASE PREPARED BY SOLVENT EVAPORATION TECHNIQUE

THESIS ADVISOR:ASSOC.PROF.SUCHADA CHUTIMAWORAPAN, Ph.D. THESIS CO-ADVISOR: ASSOC. PROF. CHAIYO CHAICHANTIPYUTH, M.Sc. in Pharm. 187 pp. ISBN 974-13-1279-2.

The present study was designed to develop sustained release microcapsules of *Andrographis paniculata* Nees extract via the oil in oil solvent evaporation technique. Effect of process and formulation factors on microencapsulation and release characteristics were investigated. As the stirring rate increased from 250 to 1200 rpm, the microcapsules size decreased, whereas the rate at 1000 rpm gave the most appropriate yield, highest drug content and core entrapment and highest release rate constant at pH 6.8. Among the emulsifier concentration of 0-2.0%, the 0.5% Span 80 gave appropriate size, highest drug content and core entrapment. At pH 1.2, the 0.5 %Span80 showed the significantly higher release rate constant and dissolution efficiency. The 1:2 core to wall ratio gave the highest core entrapment, whereas the 1:1 ratio showed the highest yield and drug content. The particle size of microcapsules from 2:3 ratio was markedly large due to the high concentration of the internal phase. Eudragit RL100 was superior to Eudragit RS100 as it gave the higher yield, smaller size, higher core entrapment and superior release rate constant and dissolution efficiency. Addition of Poloxamer 188 of 20%, inspite of a slight decrease of core entrapment and particle agglomeration, gave superior release rate constant and dissolution efficiency to those without Poloxamer 188. The release mechanism of andrographolide from microcapsules was demonstrated to follow diffusion- controlled model.

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

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LIST OF ABBREVIATIONS

ANOVA	=	analysis of variance
AG	=	andrographolide
°C	=	degree Celcius
cm	=	centimeter
conc.	=	concentration
CV	=	coefficient of variation
DAG	=	dehydroandrographolide
DE	=	dissolution efficiency
e.g.	=	for example (exampli gratia)
et al	=	and others
ERL100	=	Eudragit RL100
ERS100	=	Eudragit RS100
FTIR	=	Fourier transform infrared
g	=	gram
GI	=	gastrointestinal tract
gr	=	group
h	=	hour
HLB	=	hydrophilic-lipophilic balance
HPLC	=	High performance liquid chromatography
i.e.	=	that is (id est)
k	=	release rate constant
Kg	=	kilogram
k_h	=	Higuchi release rate constant
L	=	liter
mg	=	milligram
min	=	minute
mL	=	milliliter
MW	=	molecular weight
n	=	number of sample
nm	=	nanometer
no.	=	number

o/o	=	oil in oil
o/w	=	oil in water
p	=	probability
P.I.	=	Polydispersibility index
pp.	=	page
PXM	=	Poloxamer188
r	=	correlation coefficient
R^2	=	coefficient of determination
rpm	=	revolution per minute
SD	=	standard deviation
SEM	=	scanning electron microscope
t	=	time
UV	=	ultraviolet
Wt	=	weight
w/o/w	=	water in oil in water
μg	=	microgram
μL	=	microlitter
μm	=	micrometer
λmax	=	wavelength of maximum absorption
%	=	percent
% v/v	=	percent volume by volume
%w/v	=	percent weight by volume
%w/w	=	percent weight by weight

CHAPTER I
INTRODUCTION

There are many successful medications in current use derived from medicinal plants. They offer people an access to safe and effective products to be used in prevention and treatment of illness through self-medication.

Andrographis paniculata (Burm.) Wall. ex Nees or Fa thalaai joan in Thai name is one of the medical plants that has been used as a treatment of inflammation, diarrhea, fever and even an antibacterial. Phytochemical investigations indicated that its leaves contain various diterpenoid lactones such as andrographolide (main diterpenoid lactone), dehydroandrographolide, neoandrographolide and deoxyandrographolide (Preeprame, 1992). These lactones have demonstrated several pharmacological activities (Saxena et al., 1998).

In Thailand, the policy on promotion of the usage of this plant in the community hospitals throughout the country was established. Recently, dry powder of *Andrographis paniculata* leaves has been prepared in as 250 mg-capsules. The daily dosage administration is 1 to 4 g as 4 divided dose. It means that the patient has to take 4 to 16 capsules per day (กระแส วัชรปาน, 1990). This leads to the challenge for the development of the sustained release dosage form of this medicinal plant to improve patient compliance.

Sustained release dosage form can be approached by several techniques including microencapsulation. Many techniques such as coacervation, spray drying, pan coating, air suspension, interfacial polymerization and solvent evaporation are applied (Bakan, 1986). Solvent evaporation is a simple technique carried out in a liquid vehicle. The core material is dissolved or dispersed in the coating polymer solution. With agitation, this solution or dispersion is emulsified in immiscible liquid phases containing an emulsifier to form microdroplets. After evaporation of the solvent, the microdroplets are solidified (Bakan, 1986). Although the solvent evaporation process is conceptually simple, many variables such as stirring rate,

emulsifier concentration, core to wall ratio and type of polymer, influence the final product.

Type of polymer potentially involves drug sustained release. Eudragit RS100 and Eudragit RL100 are copolymers synthesized from acrylic acid and methacrylic acid esters. Both polymers are water insoluble while Eudragit RL100 is more permeable to water than Eudragit RS100 (Wade and Weller, 1994a; Kristmundsdóttir, Gudmundsson and Ingvasdóttir, 1996). Due to the swellability property of these polymers in water, the nonaqueous solvent evaporation techniques has been applied in many investigations.

Since both polymers are water insoluble, drug release from microcapsules is slow and effects on therapeutic activity. The drug release may be enhanced by addition of a water-insoluble polymer. Poloxamer188, a block copolymer surfactant, demonstrated an outstanding dissolution improvement on poorly water soluble drugs such as nifedipine in solid dispersion (Chutimaworapan et al., 2000). Incorporation with insoluble Eudragit copolymers, Poloxamer188 exhibited the marked effect to the controlled release of nifedipine solid dispersion (Chutimaworapan et al., 2001).

The present study was designed to develop sustained release microcapsules of *Andrographis paniculata* extract via the solvent evaporation method. Additionally, effects of process and formulation factors on physicochemical properties of the microcapsules were also investigated.

The purposes of the study were as follows:

1. To prepare *Andrographis paniculata* extract microcapsules for sustained release by nonaqueous solvent evaporation technique.

2. To characterize the physicochemical and pharmaceutical properties of *Andrographis paniculata* extract microcapsules; e.g. yield, morphology, size and size distribution, drug content and drug release.

3. To investigate the effects of stirring rate, emulsifier concentration, core to wall ratio, polymer type and Poloxamer188 concentration on physicochemical properties of *Andrographis paniculata* extract microcapsules.

4. To elucidate the release kinetics of andrographolide from *Andrographis paniculata* extract microcapsules.

CHAPTER II
LITERATURE REVIEW

I. *Andrographis paniculata* (Burm.) Wall. ex Nees

Andrographis paniculata Nees is a well-known small shrub belonging to the Acanthaceae family. The plant can grow in all types of soil and is found throughout tropical area of Asia, known in Thai as Fa thalaai, Fa thalaai joan, Yaa kannguu, in Chinese as Khee-panghee. Its leaves vary much in size, the largest are usually 7.5 cm in length and 2.5 cm in width (Figure 1).

Figure 1 *Andrographis paniculata* (Burm.) Wall.ex Nees.

1. The chemical constituents

The chemical constituents of *Andrographis paniculata* Nees including diterpenoids, flavonoids and glycosides have been isolated from different parts of the plants. Phytochemical investigations indicated that it leaves contain the main constituents as diterpenoid lactones such as andrographolide (main diterpenoid lactone), neoandrographolide, deoxyandrographolide and dehydroandrographolide (Figure2). These lactones have demonstrated several pharmacological activities (Saxena et al., 1998).

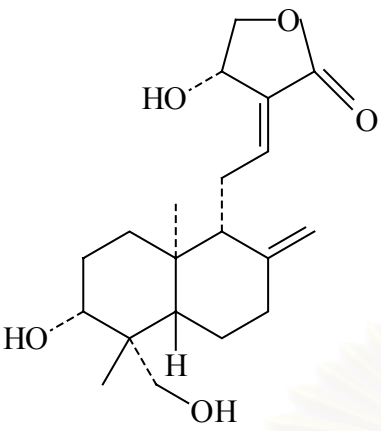
Some physicochemical properties of these diterpenoid lactones were as follows:

1) Andrographolide ($C_{20}H_{30}O_5$) an unsaturated trihydroxy lactone, appears as colorless crystal with bitter taste. It is soluble in methanol, ethanol, acetone, pyridine, acetic acid, slightly soluble in chloroform and water and insoluble in ether. Its melting point is 228-230 °C and UV absorbance at λmax 223 nm.

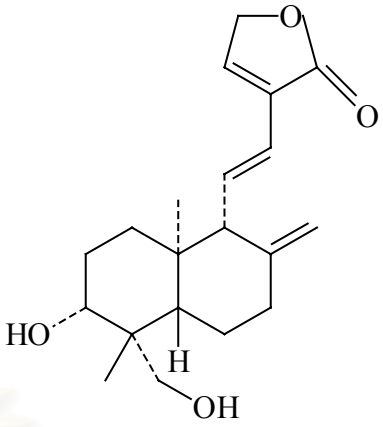
2) Dehydroandrographolide ($C_{20}H_{28}O_4$) an unsaturated dihydroxy lactone, appears as colorless crystal with bitter taste. It is soluble in methanol, ethanol, acetone, pyridine, acetic acid , chloroform and insoluble in water. Its melting point is 203-204 °C and UV absorbance at λmax 248nm.

3) Neoandrographolide ($C_{26}H_{40}O_8$) a diterpene lactone glucoside, appears as colorless crystal with bitter taste. It is soluble in methanol, ethanol, acetone, pyridine, acetic acid , slightly soluble in chloroform and water and insoluble in ether and petroleum ether. Its melting point is 167-168 °C and UV absorbance at λmax 217.4 nm.

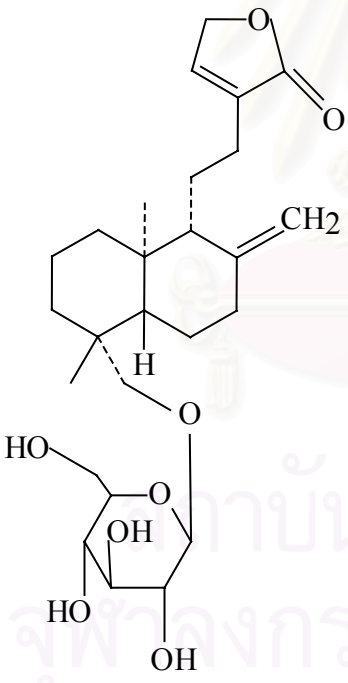
4) Deoxyandrographolide-19β-D-glucoside ($C_{26}H_{39}O_9$) a diterpene lactone glucoside, appears as colorless crystal with bitter taste. It is soluble in methanol, ethanol, acetone, pyridine, acetic acid , slightly soluble in chloroform and water and insoluble in ether. Its melting point is 187-188 °C and UV absorbance at λ max 212 nm.



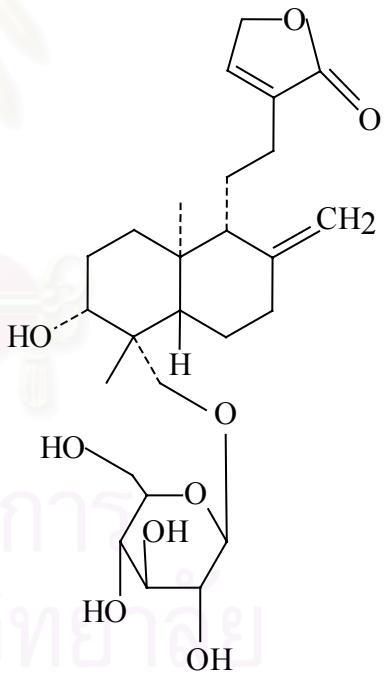
Andrographolide



Dehydroandrographolide



Neoandrographolide



Deoxyandrographolide-19β-D-glucoside

Figure 2 Structure of four major diterpenoid lactones.

2. Pharmacological activity

- *Antimicrobial activity:*

The ethanol extract of *Andrographis paniculata* showed antimicrobial activity against *S.aureus* while the hot water extract showed no effect. However, both extracts were active against *E.coli*. In addition, using the disc diffusion method, the extract and crude lactones were active against *E.coli, B. subtilis, M. luteus, S. aureus* and *β-streptococcus* gr.A. Moreover, dehydroandrographolide at a concentration of 750 μg/disc was active against *M.luteus* while andrographolide and neoandrographolide at equal concentration had no antimicrobial activity against the test organisms. For the antimicrobial activity against oral bacteria, the ethanol extract showed inhibitory activity against all tested bacteria at concentration of 27.5 mg/mL for *Streptococcus mutans* KPSK and GS-5 and 55 mg/mL for *Bacteroides gingivalis* (Farnsworth and Bunyapraphatsara, 1992).

-Antiinflammatory effect:

The antiinflammatory effect of andrographolide, neoandrographolide, dehydroandrographolide and deoxyandrographolide were lower than corticosteroid and nonsteroidal drugs. The pharmacological effect was found highest with dehydroandrographolide followed by deoxyandrographolide , neoandrographolide , and andrographolide (Saxena et al., 1998).

- *Immunostimulant:*

The ethanol extract of *Andrographis paniculata* and purified andrographolide induced significant stimulation of antibody and delated type hypersensitivity response to sheep red blood cells in mice. The plant preparation also stimulated nonspecific immune response of the animal and proliferation of splenic lymphocytes. The stimulation of both antigen specific and nonspecific immune response was, however, of lower order with andrographolide than with ethanol extract, suggesting thereby that substances other than andrographolide present in the extract may also be contributing to immunostimulation (Saxena et al., 1998). Moreover, andrographolide decreased degranulation of mast cells of rats and reduced

the liberation of histamine from the cells when tested *in vitro* at conccentration of 30, 100 and 300 μg/mL (Madav et al., 1998).

- *Antimalarial activity:*

A crude ethanol extract of *Andrographis paniculata* and its fraction studied in four-day suppressive test against *Plasmodium berghei* NK65 in *Mastomys natalensis* could reduce the level of parasitaemia in dose-dependent manner. Four diterpenoids such as andrographolide, neoandrographolide, deoxyandrographolide and andrographiside also reduced the level of parasitaemia but not in dose-dependent manner. Among four diterpenoids, neoandrographolide and deoxyandrographolide were most effective whereas andrographolide and andrographiside presented comparatively less inhibition (Misra et al., 1992).

- *Analgesic activity:*

Andrographolide analgesic activity was tested and compared with narcotic (pethidine) and nonnarcotic (aspirin) drugs. The result presented that andrographolide had a weak peripheral analgesic activity as compared to aspirin. In comparison with pethidine, andrographolide did not show central analgesic activity (Madav et al., 1995).

-Antipyretic activity:

Madav et al. (1995) found that andrographolide produced significant ($p < 0.05$) antipyretic effect after 3 h of 100 and 300 mg/kg oral administration in Brewer's yeast-induce pyrexia in rats.

-Antiulcer activity:

Andrographolide exhibited significant ($p<0.05$) antiulcer activity at 100 and 300 mg/kg dose aspirin-induced ulceration in rats. Moreover, it produced a significant ($P<0.05$) decrease in gastric juice and total acid content in rats (Madav et al., 1995).

-Antidiarrheal activity:

The alcoholic extract of *Andrographis paniculata* showed very good antidiarrheal activity against all types of enterotoxins of *E. coli* in rabbit and guinea pig ileal loop model, at 300 mg ($p < 0.001$) (Gupta, Yadava and Tandon, 1993).

-Effect on platelet activating factor (PAF):

Andrographolide inhibited PAF-induced human blood platelet aggregation in a dose-dependent manner. Mechanism of its action is different from NSAID (Amroyan et al.,1999).

3. Pharmacokinetics

Andrographolide was quickly and almost completely absorbed (91%) into circulation following oral administration of *Andrographis paniculata* extract at a dose of 20 mg/kg body weight in rats. However, its bioavailability decreased four-fold when a 10-times-higher dose was used. Andrographolide has a high affinity for human serum albumin (61.2%) and only a limited amount can enter the cell (40%). The elimination of andrographolide is independent of the dose used. There are less than 10% of andrographolide eliminated via urine after oral administration of *Andrographis paniculata* extract of 20 and 200 mg/kg body weight. Metabolism of andrographolide increased when the dose of andrographolide increased. Thus, the elimination rate of andrographolide increases at higher dose of *Andrographis paniculata* extract ($t_{1/2}$ are 3.1 and 2.5 h for doses of 20 and 200 mg/kg of *Andrographis paniculata* extract in rat, respectively) (Panossian et al., 2000).

II. Sustained release system

Sustained release system is designed to provide prolonged therapeutic action and to maintain a constant level of drug in therapeutic range. The drug must be released from the dosage form that will replace the amount of drug being metabolized and excreted from body.

There are several advantages of sustain released system. The first is to decrease the frequency with which the patient has to take the dosage form to obtain

the desired effect. Also, it reduces inconvenience due to the nighttime dosing, thus it improves patient compliance. In addition, this system reduces fluctuation in drug levels and to obtain more uniform pharmacological response. In the view of adverse effect, it reduces dose related side effect and GI irritation (Welling, 1983).

1. Pharmaceutical technology to achieve oral sustained release

Many pharmaceutical technologies utilized to achieve oral sustained release dosage form were as follows (Ansel, Popovich and Allen , 1995):

1) Coated beads or granules or microencapsulated drug

In this method, a solution of drug in nonaqueous solvent such as a mixture of acetone and alcohol is coated onto small inert seeds or beads made of a combination of sugar and starch. In instance in which the dose of drug is large, the starting granules of material may be composed of drug itself. Then granules were coated, with which some granules receiving a few coats and others many coats. Then the beads or granules of different thickness of coating are blended in the desired proportions to achieve proper blend. The presence of various coating thickness produce the sustained release.

Microencapsulation is a process by which solids, liquids or gases may be encapsulated into microparticle size through the formation of thin coating of wall around the substance being encapsulated.

2) Embedding drug in slowly eroding matrix

In this process, the drug intended to have sustained release is combined with lipid or cellulosic material processed into granules that can be placed into capsules or tablets. The treated granules slowly erode in body fluid. The material type used in preparation of the granules may be varied to achieve different rates of erosion.

3) Embedding drug in inert plastic matrix

The drug in this method is granulated with inert plastic material such as polyethylene, polyvinyl acetate or polymethacrylate and the granulation is

compressed into tablets. The drug is slowly released from the inert plastic matrix by leaching by body fluid.

4) Complex formation

A drug substance is chemically combined with other chemical agents from chemical complexes that slowly dissolves in body fluid, depending upon the pH of environment.

5) Ion-exchange resin

A solution of the cationic drug is passed through a column containing the ion exchange resin, to which it complexes by replacement of hydrogen atoms. The resin-drug complex is washed and may be tableted, encapsulated or suspended in an aqueous vehicle. The release of drug is dependent upon pH and the electrolyte concentration in gastrointestinal tract.

6) Hydrocolloid system

This system is designed to obtain sustained release using hydrodynamically balanced drug delivery system that consists of the matrix so designed for upon contact with gastric fluid. The dosage form demonstrated a bulk density of less than one and remains buoyant.

7) Osmotic pump

This technique is oral osmotic pump composed of a core tablet and semipermeable coating. The semipermeable coating with a hole for drug release is controlled by solvent influx across a semipermeable membrane, which in turn delivers the drug outside through an orifice.

2. Mechanism of sustained release

Mechanism of oral sustained release system can be broadly divided into following:

1) The drug may diffuse out of the carrier by diffusion from the solid matrix. This process is negligibly slow for macroscopic delivery system, but can be

rapid for submicron carriers. The carrier retains its structural integrity in this situation. This mechanism has been designed in such a way that the drug is partitioned largely in the carrier (Washington, 1990). The diffusion of drug from matrix is illustrated in Figure 3. On dilution the drug will diffuse out of the carrier unit until the partition equilibrium is re-established as shown in Figure 4.

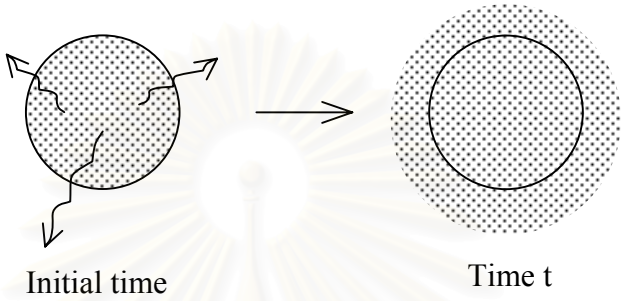


Figure 3 Diffusion of drug from matrix (Kim, 2000a).

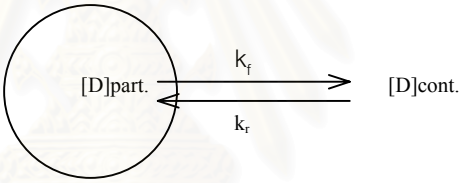


Figure 4 Diffusion equilibrium of drug between particle, $[D]_{part.}$ and continuous phase, $[D]_{cont.}$ (Washington, 1990).

2) The solvent may penetrate into the microparticle and dissolve the drug which then diffuses out in solution. The solvent may gain entry by percolation through pores, or hydration of the particle as shown in Figure 5 (Washington, 1990).

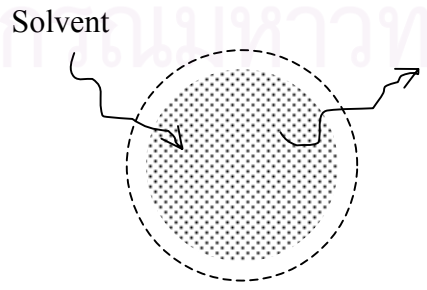


Figure 5 Diagram shows solvent penetration and dissolution of drug (Washington, 1990).

3) The carrier or polymer may be degraded or dissolved by its surrounding, the drug being sufficiently immobile to diffuse from the carrier over the same timescale (Figure 6). In this case, the accumulation of drug in the continuous phase follows the degradation of carrier. (Washington, 1990).

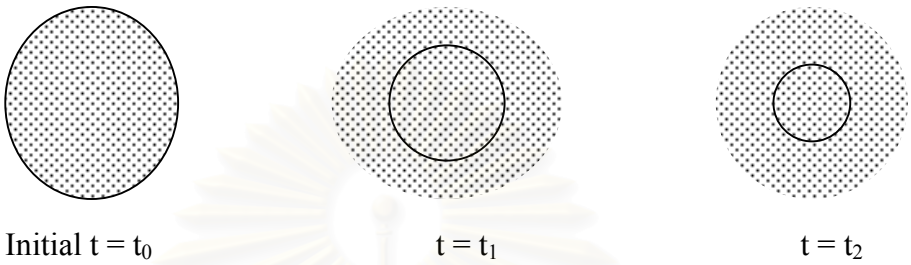


Figure 6 The process of degradation of carrier of microparticles (adapted from Kim, 2000b).

4) Osmotically controlled release is controlled by solvent influx across a semipermeable membrane, which in turn delivers the drug outside through an orifice. The osmotic and hydrostatic pressure differences on either side of the semipermeable membrane govern fluid transport into the system (Figure 7) (Venkatraman et al., 2000).

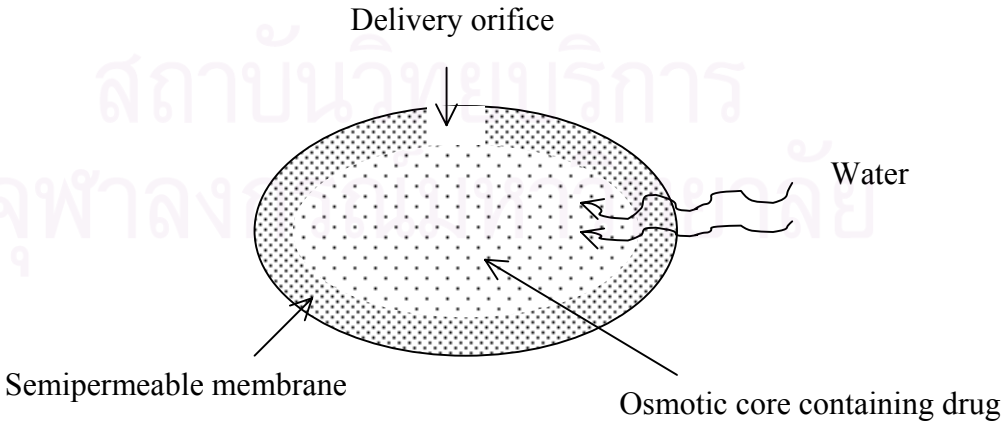


Figure 7 Schematic diagram of elementary osmotic pump (Venkatraman et al., 2000).

5) Ion exchange resin is based on the principle that ionic drug will bind to the functional groups of the resin as drug resinates. The release of drug from drug resinate is dependent upon the reaction with counter ions in gastrointestinal fluid (Kim, 2000). The mechanism of action may explain as follows (Ansel, Popovich and Allen, 1995; Kim, 2000):

In the stomach;

a) Drug resinate + HCl $\rightleftharpoons$ acidic resin + drug

b) Resin salt + HCl $\rightleftharpoons$ resin chloride + acidic drug

In the intestine;

c) Drug resinate + NaCl $\rightleftharpoons$ sodium resinate + drug hydrochloride

b) Resin salt + NaCl $\rightleftharpoons$ resin chloride + sodium salt of drug

The resin are water-insoluble materials containing anionic groups such as amino or quaternary ammonium groups, cationic groups such as carboxylic groups or sulfonic groups in repeating position on the resin chain. A drug-resin complex is formed by prolong exposure of drug to the resin (Venkatraman et al., 2000).

6) Gastroretentive system has been designed to achieve prolonged release by several means, including altering the density of formulation and bioadhesive to the stomach linking (Venkatraman et al., 2000).

III. Mathematical models of drug release

Most mechanisms of drug release have been elucidated by comparing the release data to mathematical models. For example, Donbrow and Benita (1982) used mathematical models in released kinetic study of sparingly soluble drug as salicylamide from ethyl cellulose microcapsules.

Most mathematical models of drug release can be categorized into three types:

1. Zero-order release model
2. First-order release model
3. Square root of time release model or Higuchi model

1. Zero-order release model

An ideal drug release of sustained release dosage form device is one which can deliver the drug at a constant rate until the device is exhausted of active agent. Mathematically, the release rate from this device is given as:

$$\frac{dM_t}{dt} = k \quad (1)$$

Where k is a constant, t is time, and M_t is the mass of active agent released. This model of release is called zero-order release model.

2. First–order release model

The first-order release model is the second common pattern of drug release. This release rate is proportional to the mass of active agent contained within the device. The rate is then given as:

$$\frac{dM_t}{dt} = k\,(M_0 - M_t) \quad (2)$$

Where M_0 is the mass of agent in the device at t = 0. On rearrangement where k_l is first-order release constant, this gives the following:

$$\frac{dMt}{dt} = k\,M_0 e^{-k_1 t} \quad (3)$$

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

On the assumption that the exposed surface area of matrix decreased exponentially with time suggested the drug release from most sustained release matrices could be described by apparent first order kinetic, thus:

$$At = A_0 e^{-k_1 t} \quad (4)$$

Where At is amount of drug remaining in the matrix at time t and A_0 is initial amount of drug

Taking the logarithm of the above equation yielded

$$\log At = \log A_0 - \frac{k_1 t}{2.303} \quad (5)$$

First-order model can be predicted by plotting logarithm of the percentage of drug remaining against time. If the release pattern follows first order model, a linear relationship is obtained. The initial curvature of the plot may be obtained because of the presence of surface drugs and they suggested to be ignored.

3. Square root of time release model or Higuchi model

Square root of time release model or Higuchi model is frequently referred to as Square root of time or $t^{1/2}$ release, providing compound release that is linear with the reciprocal of the square root of time. The release rate is then given as:

$$\frac{dM_t}{dt} = \frac{k}{\sqrt{t}} \quad (6)$$

In contrast to first-order release, the release rate here remained finite as the device approached exhaustion.

The release model of this type can be described by Higuchi equation (Higuchi, 1963).

$$Q = \left[\frac{D\varepsilon(2A-\varepsilon C_s) C_s t}{\tau}\right]^{1/2} \quad (7)$$

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

D is diffusion coefficient of drug in the release medium

ε is porosity of the matrix

τ is tortuosity of matrix

C_s is solubility of drug in release medium

A is concentration of drug in the microcapsules, express as g/mL

The assumptions made deriving from equation (7) are as follows:

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

For purpose of data treatment, equation (7) is simplified

$$Q = k_h t^{1/2} \quad (8)$$

Where k_h is Higuchi constant. Therefore, the plot of amount of drug release from matrix versus the square root of time should be increased linearly if drug release from the matrix is diffusion controlled. Although the above equation was based on the release from a single face, it may be used to desired diffusion-controlled release from all surface matrix. In order to verify further that the release followed the Higuchi model, Higuchi equation was converted into logarithmic form as

$$\log Q = \log k_h + \frac{1}{2} \log t \quad (9)$$

The plot of log Q versus log t must not only yield a straight line but must also have a slope of 0.5.

IV. Microencapsulation technique by solvent evaporation

Microencapsulation is a process which the coating of polymeric materials is deposited around particles of solids or droplets of liquids and dispersions. In the pharmaceutical industries, microencapsulation is recently used for applications such as the conversion of liquid to solid, taste-masking of bitter drugs, prolonged or sustained release, separation of incompatible compounds, reduction of gastric irritation, and environmental protection (Bakan, 1994), isolation from tissues, and detoxification or exchange reaction (Burgess and Hickey, 1994). Examples are shown in Table 1.

Table 1 Pharmaceutical applications of microcapsule products (Burgess and Hickey, 1994).

Apllications	Examples
Taste masking	Fish oil, sulfa drugs, clofibrate, alkaloid and salt
Enteric coating	Aspirin, pancreatolipase, erythromycin
Sustained and controlled release	KCl, ibuprofen, theophylline,
Instability to environment, O_2, H_2O and volatility	Vitamins, aspirin, volatile flavors
Separation of incompatibility	Excipients, buffer, and other drugs
Isolation from tissues	Potassium chloride, aspirin
Administration in solid state and dry handling	Liquids, soft or sticky solids, oils, flavors, vitaminA
Detoxification, exchange reaction	Artificial cells and organs

Many techniques of microencapsulation such as air-suspension, coacervation-phase separation, pan-coating, spray drying, multiorifice-centrifugal, and solvent evaporation, are available to make microcapsules with various types of microcapsule structure. These microcapsule structures are shown in Figure 8 (Deasy, 1984). The most common structure is monospherical.

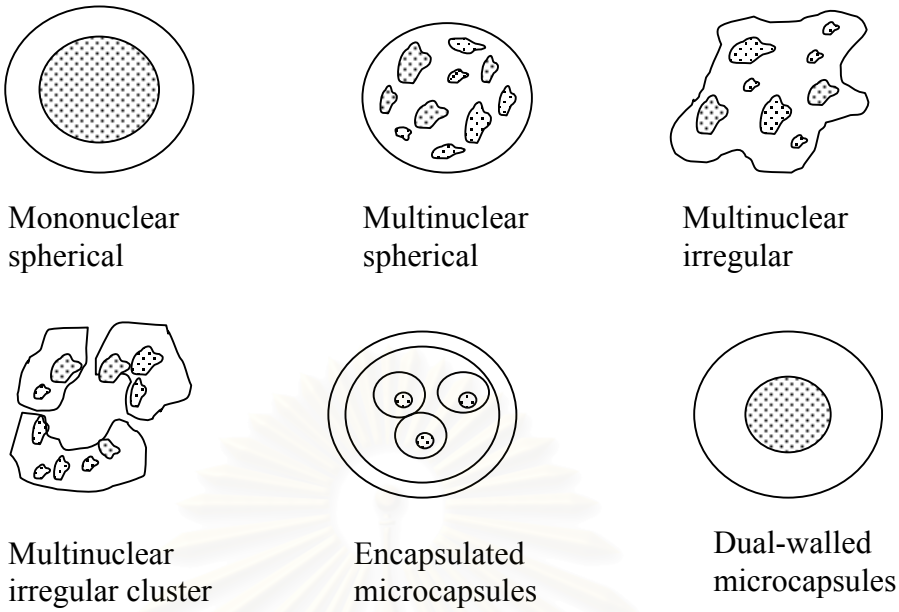


Figure 8 Some typical structures of microcapsules.

Moreover, microcapsules in various size ranges can be manufactured as illustrated in Table 2 (Bakan, 1994).

Table 2 Microencapsulation technique and microcapsule size (Bakan, 1994).

Microencapsulation technique	Microcapsule size (nm)
Air suspension	35-5000
Coacervation-phase separation	1-5000
Multi-orifice-centrifugal	1-5000
Pan- coating	600-5000
Solvent evaporation	1-5000
Spray dry and congealing	5-600

Solvent evaporation is a simple technique to prepare microcapsules.(Bakan, 1986). This technique is carried out in a liquid vehicle. The polymer must be soluble in an organic solvent while the drug may be soluble or dispersible in an organic solvent. The organic solvent containing the polymer and drug is emulsified in immiscible liquid phases containing an emulsifier to form microdroplets. With the

aid of an agitator, the mixture is evaporated to remove solvent from the polymer. The microdroplet solidify and solid microparticles are obtained after complete evaporation. Then the microcapsules are filtrated and dried respectively (Bakan, 1986).

Hincal and Çaliş (2000) concluded that there were two types of solvent evaporation, each having the concept of emulsion and are as follows:

1. Single–emulsion solvent evaporation
2. Multiple–emulsion technique

1. Single–emulsion solvent evaporation

For single-emulsion solvent evaporation, there are two systems to choose: oil in water or water in oil and oil in oil (sometimes referred as water in oil).

1.1 Oil in water emulsion solvent evaporation

Oil in water emulsion is more widely used than w/o emulsion due to the simplicity of the process and easy clean-up requirement for the final product. In this process, both the drug and the polymer should be insoluble in water, while a water-immiscible solvent is required for polymer (Hincal and Çaliş, 2000). The diagram of o/w emulsification-solvent evaporation technique is shown in Figure 9.

Problems to the efficient incorporation of water-soluble active substances into biodegradable polymer microspheres using o/w emulsion solvent evaporation are originating to a great extent from the separation and/or removal of water-soluble material into the aqueous continuous phase. As Bodmeier and McGinity (1988) has found, quinidine sulfate having low solubility in aqueous pH 12 was successfully entrapped, while quinidine sulfate-free microspheres were obtained in external aqueous phase at pH 7.

In this process, the most common solvents used are methylene chloride and chloroform. The solvent used in this process has effect on morphology of microspheres. The diffusion of the water-immiscible organic solvent into the aqueous phase, which causes polymer precipitation, depends on the water solubility

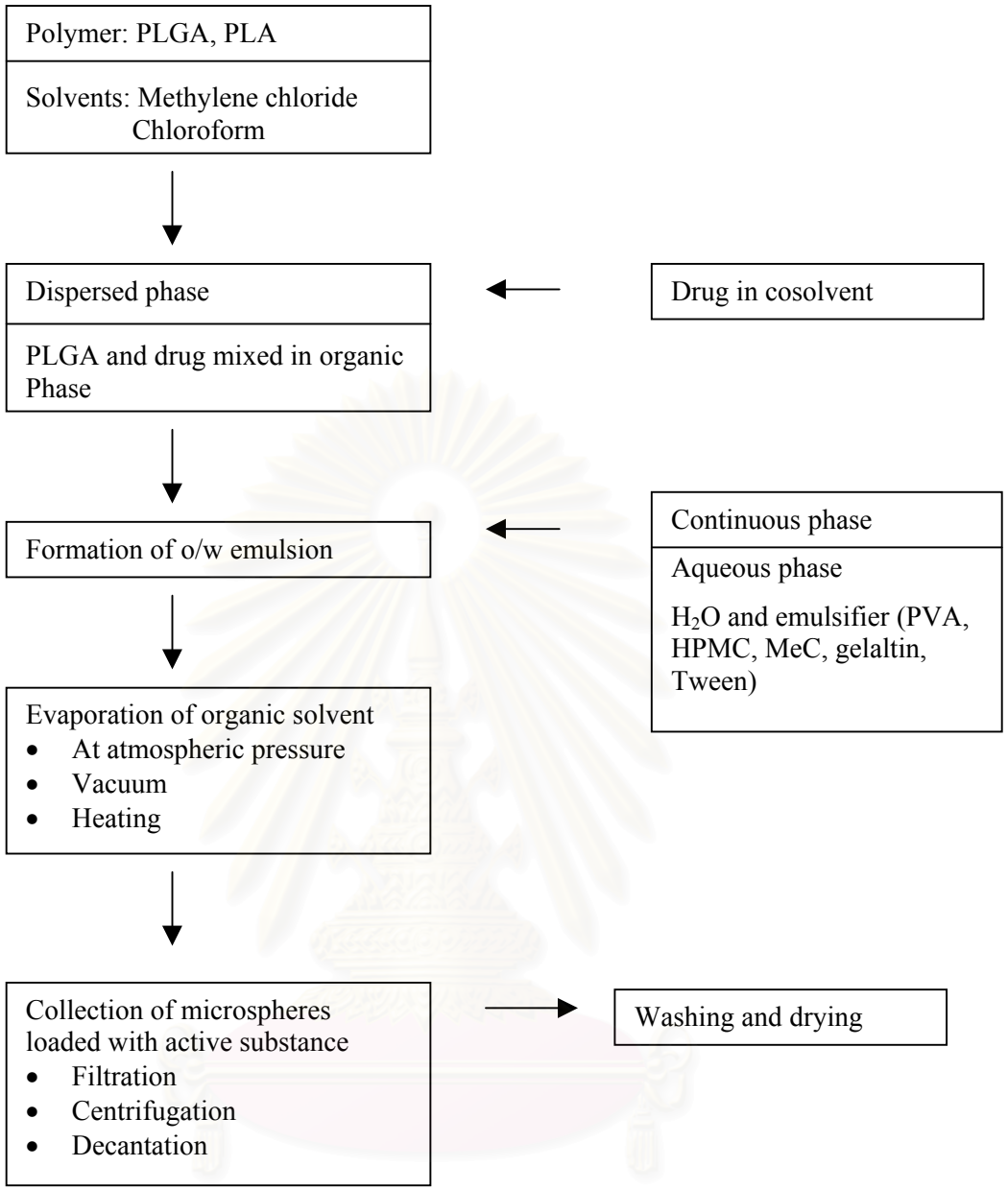


Figure 9 The diagram of o/w emulsification-solvent evaporation technique (Hincal and Çaliş, 2000).

and removal of the organic solvent at the water /air interface (Bodmeier and Chen, 1989). Since methylene chloride has a higher water solubility and a lower heat of evaporation as compared to chloroform, the polymer precipitated prior to the drug while the drug precipitated before the polymer when chloroform is as solvent. Therefore the surface of chloroform-microspheres was irregular and drug crystals were visible as compared to the smooth and crystal-free surface of methylene chloride microspheres.

In general, this method is particularly suitable for microencapsulaton of lipophilic drugs that can be either dispersed or dissolved in the dispersed phase of a solvent. Progesterone (Benita et al., 1984), testosterone (Kobayashi, 1998), dexamethasone (Song et al., 1997) were successfully encapsulated using this technique.

1.2 Oil in oil emulsification-solvent evaporation technique

Oil in oil, sometimes referred as water in oil emulsification process, was developed for the encapsulation of highly water soluble drugs. Due to moderately water soluble and water soluble compounds are low encapsulated in o/w emulsification technique (Hincal and Çaliş, 2000; Jain et al., 1998). Water soluble drugs such as theophylline, caffeine and salicylic acid could not be loaded efficiently using o/w emulsion method, whereas drugs with low water solubility such as diazepam, hydrocortisone, and progesterone were successfully entrapped in microspheres. Also, this system is particularly suitable for drugs sensitive to moisture, such as ascorbic acid (Vanichtanunkul, 1997) and polymer, such as Eudragit RS100, Eudragit RL100 being permeable to water (Wade and Weller, 1994a).

In this technique, polymer and drugs, contained in polar solvent such as acetonitrile, are emulsified into an immiscible lipophilic phase, such as light mineral oil commonly being used, surfactant such as Span. However, an important drawback of using an oil external phase is cleaning up the final product. The oil has to be removed using organic solvent such as n-hexane. The diagram of o/o emulsification-solvent evaporation technique is shown in Figure 10.

2. Multiple-emulsion technique

Multiple-emulsion technique is used for the efficient incorporation of water-soluble peptide, protein, and other macromolecules. In this technique, the polymers are dissolved in an organic solvent and emulsified into an aqueous drug solution to form a water in oil emulsion then reemulsified into an aqueous solution

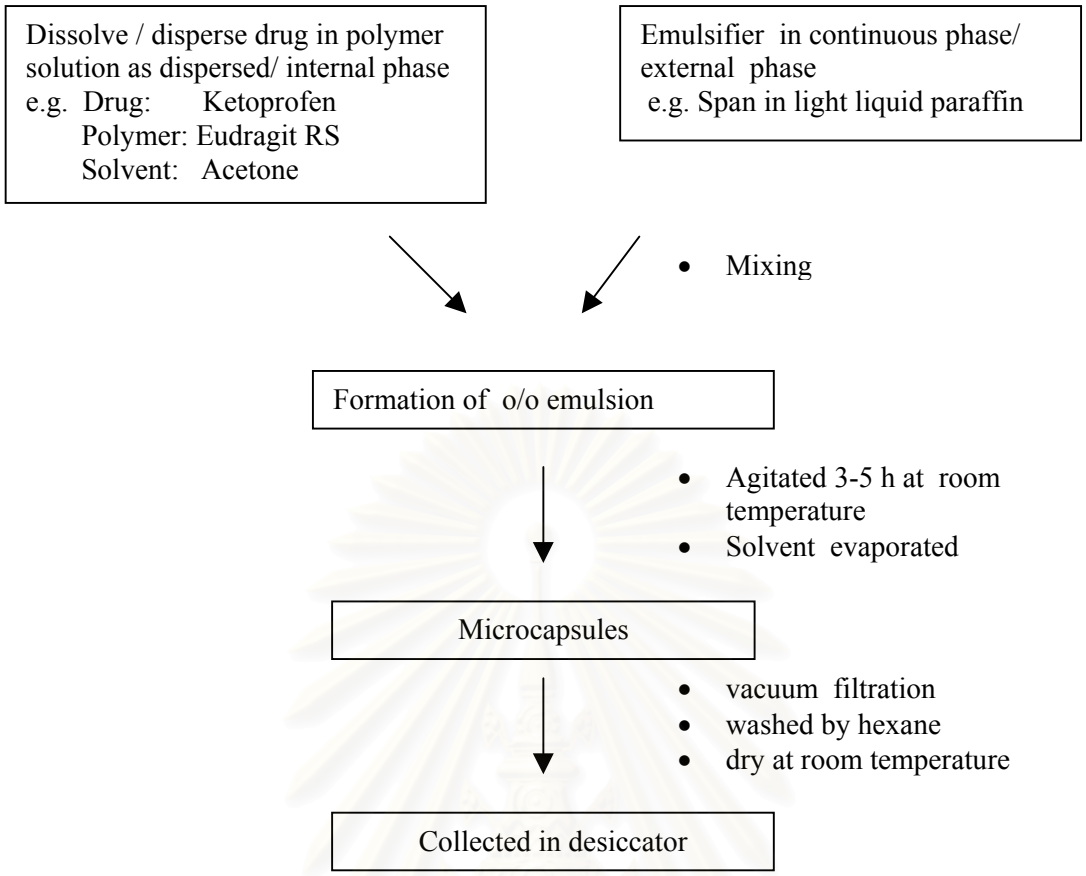


Figure 10 Diagram of o/o emulsification-solvent evaporation technique (Adapted from Kawata et al. , 1986).

containing an emulsifier to produce a multiple w/o/w dispersion. The organic phase acts as a barrier between the two aqueous compartments, preventing the diffusion of the active material toward the external phase.

Hermann and Bodmeier (1998), for example, could not obtain acceptable encapsulation efficiencies to encapsulate the water soluble peptide somato statin acetate by o/w solvent evaporation method. Because of its high water solubility somatostatin acetate diffused into the external phase during microspheres preparation therefore they utilized w/o/w technique. In w/o/w technique, drugs or aqueous solution was dispersed in the organic polymer solution followed by emulsification into the external phase. Partitioning of the drug into the external phase was reported to be prevented.

Various morphologies of microspheres such as porous or nonporous external polymer layers enclosing hollow, macroporous, or microporous internal structure, were discovered in this technique (Crotts and Park, 1995; Crotts, Sah and Park, 1997).

V. Factors affecting on microencapsulation using solvent evaporation

1. Stirring rate

Stirring rate is a parameter of primary importance in emulsification steps. In the forming droplets, the energy and the surface active agent decrease the interfacial tension between the organic droplets and the aqueous phase. The stirring rate providing the energy which is appropriate for the division of the organic phase, so if high energy, small particle and narrow particle size distribution are obtained (Sansdrap and Moës, 1993).

2. Emulsifier

The emulsifier is an important parameter that provides a thin protective layer surrounding the oil droplets and reduces the coalescence and coagulation of microparticles during the solvent evaporation process. Due to a gradual decrease in volume and subsequent increase in viscosity of dispersed oil droplets, these affect the droplet size equilibrium and droplets tend to coalescence and produce agglomerates during the early stages of solvent removal. As the solvent is removed, the emulsifier continues to maintain the spherical shape of the oil droplets and prevents the aggregation, until the microspheres are hardened and isolated as discrete particles (Jain et al.,1998).

The emulsifiers used in this process are the hydrophilic polymeric colloids and anionic or nonionic surfactants. The most commonly emulsifier as PVA is used in o/w emulsion. Others include poly(vinylpyrrolidone), alginate, gelatin, methylcellulose, hydroxyalkyl cellulose, hydroxypropylmethylcellulose, polyoxyethylene derivative of sorbitan fatty esters (Tweens), Cetyltrimethyl ammonium bromide, and fatty acid salts such as sodium oleate. For o/o emulsion, oil soluble emulsifiers such as polyoxyethylene fatty acid ethers (Brijs), Spans, and lecithins have been used (Jain et al., 1998).

Physicochemical properties and concentration of emulsifiers strongly influence size, shape, and encapsulation efficiency. Sandrap and Moës (1993) found that the microsphere size decreased with an increase in emulsifier concentration. Methylcellulose 400 using as emulsifier produced a high viscosity external phase that resulted in distorted, ovoid shaped microparticles (Cavalier, Benoit and Thies, 1986). Also, in the case of porosity, the increase of sodium dodecyl sulfate decreased the porosity of miroparticles (Khawla et al., 1996)

3. Core to wall ratio

As the core to wall ratio decreased, the microcapsule size distribution shifted to the smaller size (Ruiz, Sakr and Sprockel, 1990). The similar result was obtained with preparation of sustained release zidovudine-loaded microspheres (Khawla et al., 1996). Furthermore, Cavalier, Benoit, and Thies (1986) reported the effect of core to wall ratio that a decrease in poly (±-lactide) concentration resulted in a higher drug content in the microspheres.

4. Polymer type

The type of polymers used in solvent evaporation technique was dependent on the purpose of study. Physicochemical properties of polymers such as molecular weight , hydrophilic properties influenced the microcapsules. Polard et al (1996) reported molecular weight (MW) on the characteristics of microparticles, that drug content of polylactide (MW 2000) microparticles was higher than of poly lactide-co-glycolide (MW 9000 and 12000) and polylactide (MW 9000) microparticles due to the rapid rate of polymer precipitation at the droplete surface. In addition, polymer type also effects on the drug release. Kristmundsdóttir, Gudmundsson, and Ingvasdóttir (1996) found that diltiazem released from Eugragit RL microcapsules faster than Eudragit RS microcapsules due to a greater permeability to water of Eudragit RL.

5. Solvent type

For the solvent evaporation, solvent is important for successful microsphere formulation and high drug encapsulation efficiency. Solvent as the dispersed phase should be immiscible or slightly miscible with the continuous phase.

Due to the drug partition, solvent must have a boiling point lower than the continuous phase.

Bodmeier and McGinity (1988) showed that water-miscible solvent such as acetone and dimethyl sulfoxide do not form microspheres in o/w emulisification solvent evaporation technique. In this system, dichloromethane has been used as dispersed phase since it is a good solvent for polymer and its high volatility enables it to be easily removed by evaporation, but the problem is the potential toxicity (Jain et al., 1998).

For the o/o emulsification solvent evaporation technique, the dispersed phase such as acetone (Pradhan, and Vasavada, 1994; Zinutti et al., 1996), ethanol (Zinutti et al., 1996), acetonitrile (Sturesson et al., 1993) have been used and the continuous phase consists of oils such as liquid paraffin (Kawata et al., 1986) light mineral oil (Pradhan and Vasavada, 1994; Zinutti et al., 1996) and sesame oil (Sturesson et al., 1993).

Solvent used in this process effects on morphology and drug content of microspheres. Bodmeier and Chen (1989) found, the diffusion of the water-immiscible organic solvent into the aqueous phase, which causes polymer precipitation, depended on the water solubility and removal of the organic solvent at the water /air interface. Because the organic solvent having a higher water solubility and a lower heat evaporation effects the polymer precipited prior to the drug precipitated, the surface of microspheres were smooth and crystal-free surfaced.

CHAPTER III
MATERIALS AND METHODS

Materials

- Plant material: *Andrographis paniculata* Nees

The leaves of *Andrographis paniculata* Nees were collected in June 2000 from Bangsapannoy Hospital, Bangsapannoy District, Prachuabkerekun Province. They were pulverized into powder using Bticino AEG type AMEB80Fx2 and sieved through a 5-mesh number sieve.

- Eudragit RL100 (Lot no.0860706957, Röhm Gmbh, Germany).
- Eudragit RS100 (Lot no.8370408031, Röhm Gmbh, Germany).
- Acetone (Lot no.K27833314 024, Merck, Germany).
- Light liquid paraffin (Lot no.143605, supplied by S. Tong Chemical Co., LTD. , Thailand).
- Span80 (Lot no.5GD02, supplied by Srichand United Dispensing Co., LTD, Thailand).
- Hexane (Lot no.9309-03, J.T. Baker, USA).
- Poloxamer188 (Lot no.583097, BASF corporation, USA).
- Methanol HPLC grade (Lot no.L919702, BDH Labaratory Suppiles Poole, England).
- Methanol AR grade (Lot no. K28075309 031, Merck, Germany).
- Monobasic potassium phosphate (Lot no.1000 A 986373, Merck, Germany).
- Sodium hydroxide (Lot no.7708 MVKK, Mallinckrodt, Sweden).
- Sodium chloride (Lot no.SHE 49/928, supplied by Srichard united Dispensing Co., LTD., Thailand).
- Hydrochloric acid (Lot no. K25741517, Merck, Germany).
- Chloroform (Lot no. 529 K757545, Merck, Germany).
- Ethanol (Lot no.54467, Excise department, Thailand).
- Deionized water.

Instruments

- Variable- speed stirring motor fitted with a four-blade stirring shaft (Model R30, GmgH&Co., France).
- Vacuum Pump (DOA-V130-BN, Waters, USA).
- Rotary evaporator (Büchi laboratoriums-technik AG) equipped with
    - Rotator (Type RE-121)
    - Pump (Type Motor Kri TD)
    - Water bath (Type B-461)
- High performance liquid chromatography (HPLC) (Shimadzu, Japan) equipped with
    - LC workstation (Class-LC10 Version 1) (Shimadzu, Japan).
    - Automatic sample injector SIL-10A í (Shimadzu, Japan).
    - Solvent delivery module (LC-10AD; bigradien) (Shimadzu, Japan).
    - Detector (SPD-10A UV-visible detector) (Shimadzu, Japan).
    - Communicator bus module (CBM-10A) (Shimadzu, Japan).
    - Column oven (CTO-10A) (Shimadzu, Japan).
    - Column LiChrospher®100RP-18(5 μm)15 cm with guard column.
- pH meter (Orion model 420A, Orion Research Inc. , USA).
- UV–visible spectrophotometer (UV-160A, Serial No. A113 31034483, Shimadzu, Japan).
- Dissolution apparatus (Sotax AT7, Art no.4100-1, Allschwil, Switzerland) equipped with
    - Suction circulator (Miniplus3, model M312, Gilson, France).
- Optical microscope (BH-2, Olympus, Japan).
- Image Analyzer (KS 400 rel. 2.0, licence 0400526, Kontron Electronik, Germany) equipped with
    - Video camera (Model DXC-930P, serial no. 13113, Sony, Japan).
    - Camera adaptor (Model CMC D2CE no. 13775, Sony, Japan).
- Ultrasonicator (Model 3210E-MTH, Branson, USA).
- Scanning Electron microscope (Model JSM-6400, Japan).
- Fourier transform infrared spectrometer (FT/IR-230, JASCO Corporation, Tokyo, Japan).

Methods

1. Preparation of *Andrographis paniculata* Nees extract

The crude drug powder of *Andrographis paniculata* (4,000 g) was macerated in a soxhlet extractor with 95% ethanol (45 L) at room temperature for 3 days. The extractive was filtered and concentrated under reduced pressure at 65 °C using a rotary vacuum evaporator . The crude extract was then further evaporated on a boiling water bath until andrographolide crystallized. The crystals were collected and further purified by recrystallization with methanol. Both andrographolide crystals and crude extract were dried under vacuum for 48 h. The crystals and dried crude extract were pulverized separately with a laboratory mill and stored in a desiccator for further studies.

Identification of the crystals obtained was performed by Fourier transform infrared (FTIR) spectroscopy. The FTIR spectra were measured by KBr disc method using a Fourier transform infrared spectrometer (FT/IR-230, JASCO) in the wave number range of 650-4000 cm^{-1}. The andrographolide crystals, andrographolide standard and dehydroandrographolide standards were compared.

In order to maintain the presence of some other substances such as dehydroandrographolide and neoandrographolide, the extract used as the core material in the preparation of microcapsules was obtained by mixing 1 part of andrographolide crystal and 1 part of dried crude extract.

2. Preparation of microcapsules of *Andrographis paniculata* extract.

The oil in oil emulsion solvent evaporation technique was applied. Eudragit RL100 was dissolved in 80 mL of acetone and *Andrographis paniculata* extract was dispersed into the polymer solution. The solution was emulsified into light liquid paraffin (220 mL) containing Span80 and maintained under mechanical stirring at room temperature to completely remove acetone. The microcapsules were separated by filtration, and washed twice with 100 mL of hexane to remove light liquid paraffin

from the surface of microcapsules. The microcapsules were air dried at room temperature for 48 h and stored in a desiccator for further studies.

2.1 Effect of stirring rate

The microcapsules containing *Andrographis paniculata* extract were prepared using the procedure described above. From preliminary study, the completely microencapsulation could be obtained by Eudragit RL100 with 1:2 core to wall ratio and 1% Span 80. Thus, the parameters involved were shown in Table 3.

Table 3 The parameters used in the preparation of microcapsules of *Andrographis paniculata* extract.

Parameter	Value
Stirring rate (rpm)	**250, 500, 800, 1000 and 1200**
Span 80 (%w/v)	1
Core to wall ratio	1:2*
Coating polymer	Eudragit RL100
Poloxamer188 (%w/w)	0

*Core = 5.00 g, wall = 10.00 g

The percentage yield, percentage andrographolide content, percentage dehydroandrographolide content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterized. The appropriate stirring speed was considered from these characteristics by ranking score from 1-5 and chosen to apply in the further studies.

2.2 Effect of emulsifier concentration

The microcapsules containing *Andrographis paniculata* extract were prepared using the parameters as shown in Table 4.

Table 4 The parameters used in the preparation of microcapsules of *Andrographis paniculata* extract.

Parameter	Value
Stirring rate (rpm)	From the result of 2.1
Span 80 (%w/v)	**0, 0.5, 1.0, 1.5 and 2.0**
Core to wall ratio	1:2*
Coating polymer	Eudragit RL100
Poloxamer 188 (%w/w)	0

*Core = 5.00 g, wall = 10.00 g

The percentage yield, percentage andrographolide content, percentage dehydroandrographolide content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterized. The appropriate emulsifier concentration was considered from these characteristics by ranking score from 1-5 and chosen to apply in the further studies.

2.3 Effect of core to wall ratio

The microcapsules containing *Andrographis paniculata* extract were prepared using the parameters as shown in Table 5.

Table 5 The parameters used in the preparation of microcapsules of *Andrographis paniculata* extract.

Parameter	Value
Stirring rate (rpm)	From the result of 2.1
Span 80 (%w/v)	From the result of 2.2
Core to wall ratio	**1:1, 1:2, 1:3 and 2:3***
Coating polymer	Eudragit RL100
Poloxamer 188 (%w/w)	0

*Core = 5.00 g, wall = 5.00 g ; core = 5.00 g, wall = 10.00 g ; core = 5.00 g, wall = 15.00 g and core = 10.00 g, wall = 15.00 g .

The percentage yield, percentage andrographolide content, percentage dehydroandrographolide content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterized. The appropriate core to

wall was considered from these characteristics by ranking score from 1-5 and chosen to apply in the further studies.

2.4 Effect of type of polymer

The microcapsules containing *Andrographis paniculata* extract were prepared using the parameters as shown in Table 6.

Table 6 The parameters used in the preparation of microcapsules of *Andrographis paniculata* extract.

Parameter	Value
Stirring rate (rpm)	From the result of 2.1
Span 80 (%w/v)	From the result of 2.2
Core to wall ratio	From the result of 2.3
Coating polymer	**Eudragit RL100 and Eudragit RS100**
Poloxamer 188 (%w/w)	0

The percentage yield, percentage andrographolide content, percentage dehydroandrographolide content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterized. These characteristics were compared between the two polymers and applied in the further studies.

2.5 Effect of Poloxamer188 concentration

The microcapsules containing *Andrographis paniculata* extract were prepared using the parameters as shown in Table 7.

Table 7 The parameters used in the preparation of microcapsules of *Andrographis paniculata* extract.

Parameter	Value
Stirring rate (rpm)	From the result of 2.1
Span 80 (%w/v)	From the result of 2.2
Core to wall ratio	From the result of 2.3
Coating polymer	Eudragit RL100 and Eudragit RS100
Poloxamer 188 (%w/w)	**0, 10 and 20**

The percentage yield, percentage andrographolide content, percentage dehydroandrographolide content, core entrapment, morphology, particle size and the release characteristics of microcapsules were characterized. These characteristics were compared among the Poloxamer 188 concentrations.

3. Physicochemical characterization of microcapsules of *Andrographis paniculata* extract

3.1 Morphology and particle size

The morphology of the microcapsules was observed by scanning electron microscopy (SEM). The sample was coated with gold by ion sputtering under a high vacuum and high voltage. The coated samples were then examined under SEM.

The particle size of the microcapsules was measured using an image analyzer. The image analyzer consists of a computer system linked to a video camera and a stereomicroscope. The microcapsules were randomly sampled and dispersed with light liquid paraffin on a glass slide . The longest diameter of each microcapsule was measured and recorded until the measurements of 600 particles were obtained. The mean particle size was determined from the average of 600 particles. The polydispersibility index was determined from standard deviation divided by the mean.

3.2 The percentage yield of microcapsules of Andrographis paniculata extract

The percentage yield of *Andrographis paniculata* extract was determined from equation 10.

$$\% \text{ Yield} = \frac{\text{Wt. of dried microcapsules (g) X 100}}{\text{Theoretical Wt. of microcapsules (g)}} \qquad (10)$$

Where,

-Theoretical wt. of microcapsules (g) = Wt. of core (g) + Wt. of polymer (g)

-Wt. of core (g) = Wt. of crude extract powder (g) + Wt. of andrographolide crystals (g)

3.3 The percentage content of andrographolide and dehydroandrographolide

3.3.1 HPLC method

The analyses of the major constituent of dry powder crude extract, androghapholide, and other constituent, dehydroandrographolide contents in microcapsules were determined using the modified reverse phase HPLC assayed with UV detection.

Chromatographic conditions for determination of andrographolide developed from a method described by Mahaverawat (1990) were as follows:

Column:	LiChrospher®100RP-18 (5 μm) 15 cm with guard column
Mobile phase:	methanol:water (50:50 v/v)
Flow rate:	1.5 mL/min
Detector:	UV –Vis detector by D2
Detector wave length:	255 nm
Sensitivity:	1.0 Absorbance Units Full Scale (AUFS)
Injection volume:	10 μL
Pump:	LC-10AD; Bigradient
Technique of analysis:	External standard technique

3.3.2 Calibration curve of andrographolide

Six appropriate dilutions of andrographolide made with the same vehicle were prepared to contain 16, 32, 48, 80, 120, and 160 μg/mL in methanol. Retention time, peak height and peak area for each chromatogram were recorded. The calibration curve was plotted between peak area against the concentration (μg/mL) of standard solutions and the linear regression analysis was applied.

3.3.3 Calibration curve for dehydroandrographolide

Six appropriate dilutions of dehydroandrographolide made with the same vehicle were prepared to contain 8, 16, 32, 48, 80 and 120 μg/mL in methanol. Retention time, peak height and peak area for each chromatogram were recorded.

The calibration curve was plotted between peak area against the concentration (μg/mL) of standard solutions and the linear regression analysis was applied.

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3.3.4 Specificity

The specificity of the HPLC method used to determine andrographolide and dehydroandrographolide contents in microcapsules was evaluated by comparing the chromatograms of standard solution and samples, andrographolide and dehydroandrographolide. The peak area of andrographolide and dehydroandrographolide must not be interfered by the other constituent.

3.3.5 Linearity

3.3.5.1 Linearity of calibration curve of andrographolide

The linearity was determined by plotting the standard curve between the peak area of andrographolide and the concentration of andrographolide (μg/mL). The linearity was determined from the coefficient of determination (R^2) and the equation of linear regression was calculated.

3.3.5.2 Linearity of calibration curve of dehydroandrographolide

The linearity was determined by plotting the standard curve between the peak area of dehydroandrographolide and the concentration of dehydroandrographolide (μg/mL). The linearity was determined from the coefficient of determination (R^2) and the equation of linear regression was calculated.

3.3.6 Accuracy

3.3.6.1 Accuracy of andrographolide determination

The determination of accuracy of andrographolide assayed by HPLC method was done by analyzing the percent recoveries of 6 injections of andrographolide solution. The percent recovery of each injection was calculated by dividing the concentration fitted from a calibration curve by the known concentration. The mean, standard deviation and percent coefficient of variation (%CV) were determined.

3.3.6.2 Accuracy of dehydroandrographolide determination

The determination of accuracy of dehydroandrographolide assayed by HPLC method was done by analyzing the percent recoveries of 6 injections of dehydroandrographolide solution. The percent recovery of each injection was calculated by dividing the concentration fitted from a calibration curve by the known concentration. The mean, standard deviation and percent coefficient of variation (%CV) were determined.

3.3.7 Precision

3.3.7.1 Within–run precision

- Within–run precision of andrographolide determination

The within–run precision was evaluated by analyzing peak area of andrographolide of three injections of each concentration injected within the same day. The mean, standard deviation (SD) and percent coefficient of variation (%CV) of each concentration were determined.

- Within–run precision of dehydroandrographolide determination

The within–run precision was evaluated by analyzing peak area of dehydroandrographolide of three injections of each concentration injected within the same day. The mean, standard deviation (SD) and percent coefficient of variation (%CV) of each concentration were determined.

3.3.7.2 Between- run- precision

- Between-run precision of andrographolide determination

The between–run precision was evaluated by analyzing peak area of andrographolide of three sets of calibration curve injected on different days. The mean, standard deviation (SD) and percent coefficient of variation (%CV) of each concentration were determined.

- Between-run precision of dehydroandrographolide determination

The between–run precision was evaluated by analyzing peak area of dehydroandrographolide of three sets of calibration curve injected on different days. The mean, standard deviation (SD) and percent coefficient of variation (%CV) of each concentration were determined.

3.3.8 Calculation of the percentage contents of andrographolide and dehydroandrographolide

The percentage contents and entrapment of andrographolide and dehydroandrographolide were determined using the HPLC method. Triplicate samples of microcapsules of approximately 100 mg were accurately weighed and completely dissolved with methanol in 50 mL volumetric flask. This solution was diluted and assayed by HPLC. The amounts of andrographolide and dehydroandrographolide were determined from the standard curves.

The percentage content of andrographolide and dehydroandrographolide were calculated using equation 11 and 12, respectively

% Observed andrographolide (AG) content

$$= \frac{\text{Assayed amount of AG (g) X 100}}{\text{Amount of weighed microcapsules (g)}} \quad (11)$$

% Observed dehydroandrographolide (DAG) content

$$= \frac{\text{Assayed amount of DAG (g) X 100}}{\text{Amount of weighed microcapsules (g)}} \quad (12)$$

3.3.10 Calculation of the percentage entrapment of core

The percentage entrapment of core was calculated using equation (13)

$$\%\ \text{Core entrapment} = \frac{\%\ \text{Observed core content X 100}}{\%\ \text{Theoretical core content}} \quad (13)$$

Where,

$$\text{-\% Observed core content} = \frac{\text{Amount of core in microcapsules (g) X 100}}{\text{Wt. of product (g)}}$$

$$\text{-\% Theoretical core content} = \frac{\text{Wt. of core (g) X 100}}{\text{Wt. of core (g) + Wt. of polymer (g)}}$$

-Amount of core in microcapsules

$$= \frac{\text{Wt. of core(g) X (Assayed amount of AG (g) + Assayed amount of DAG (g)}}{\text{Theoretical amount of AG (g) + Theoretical amount of DAG (g)}}$$

3.4 The release of andrographolide from microcapsules

3.4.1 Calibration curve of andrographolide

3.4.1.1 Calibration curve of andrographolide in simulated gastric fluid without pepsin pH 1.2

Six appropriate dilutions of andrographolide made with the same vehicle were prepared to contain 4, 8, 10, 12, 16 and 20 μg/mL in simulated gastric fluid without pepsin pH 1.2. From the UV spectrum scanned at 200-400 nm using UV/Visible spectrophotometer, the maximum absorption of andrographolide in this medium was found at 223 nm. Thus the absorbances of andrographolide standard solutions were determined at this wavelength. The calibration curve was plotted between andrographolide concentration in μg/mL and absorbance at 223 nm and the linear regression was applied.

For each drug release study, the calibration curve was freshly prepared.

3.4.1.2 Calibration curve of andrographolide in simulated intestinal fluid without pancreatin pH 6.8±0.1

Six appropriate dilutions of andrographolide made with the same vehicle were prepared to contain 4, 8, 10, 12, 16 and 20 μg/mL simulated intestinal fluid without pancreatin pH 6.8±0.1. From the UV spectrum scanned at 200-400 nm using UV/Visible spectrophotometer, the maximum absorption of andrographolide in this medium was found at 223 nm. Thus the absorbances of andrographolide standard solutions were determined at this wavelength. The calibration curve was plotted between andrographolide concentration in μg/mL and absorbance at 223 nm and the linear regression was applied.

For each drug release study, the calibration curve was freshly prepared.

3.4.2 Drug release studies in simulated gastric fluid without pepsin pH 1.2

The release studies of microcapsules of *Andrographis paniculata* extract were performed gastric fluid without pepsin pH 1.2 using the USP 24 dissolution apparatus 2 (paddle method) for 24 h. Approximate weight of 150 mg of samples was accurately weighed and transferred into the dissolution medium 900 mL which maintained at 37 ± 0.1°C and stirred at a constant stirring rate of 100 rpm.

Five milliliter samples were withdrawn at definite time intervals and replaced with fresh dissolution medium. The samples were assayed by UV spectrophotometer at λmax of 223 nm.

The percentage release of andrographolide was plotted against time (min) to obtain the release profile. The drug release profile was plotted according to zero order, first order and Higuchi plots. The coefficient of determination (R^2) and release rate constant (k) were calculated from an appropriate portion of the best fitted equation. Each release determination was performed in triplicate.

3.4.3 Drug release studies in simulated intestinal fluid without pancreatin pH 6.8±0.1

The release studies of microcapsules of *Andrographis paniculata* extract were performed intestinal fluid without pancreatin pH 6.8±0.1 using the USP 24 dissolution apparatus 2 (paddle method) for 24 h. Approximate weight of 150 mg of samples was accurately weighed and transferred into the 900 mL dissolution medium which maintained at 37 ± 0.1°C and stirred at a constant stirring speed of 100 rpm.

Five milliliter samples were withdrawn at definite time intervals and replaced with fresh dissolution medium. The samples were assayed by UV spectrophotometer at λmax of 223 nm.

The percentage release of andrographolide was plotted against time (min) to obtain the release profile. The drug release profile was plotted according to zero order, first order and Higuchi plots. The coefficient of determination (R^2) and release rate constant (k) were calculated from an appropriate portion of the best fitted equation. Each release determination was performed in triplicate.

Consequently, dissolution efficiency (DE) was defined as the area under the dissolution curve up to a certain time, t (Khan, 1975). The dissolution efficiency of each release study was determined from the equation (14).

$$DE = \frac{\int_0^t y.dt}{y_{100}.t} \times 100\% \qquad (14)$$

Where y is the percentage drug dissolved at time t and y_{100} equals to 100%

3.4.3 Statistical analysis of dissolution rate constants and dissolution efficiency values

The statistical significance of dissolution rate constant and dissolution values of each release study were tested using one-way analysis of variance (ANOVA) at significant level $\alpha = 0.05$ using SPSS for windows version 7.5.

CHAPTER IV
RESULTS AND DISCUSSION

I. Preparation and characterization of *Andrographis paniculata* extract

The crude extract of *Andrographis paniculata* was obtained by macerating the crude drug powder in a soxhlet extractor with 95% ethanol and the extractive was further evaporated on a boiling water bath until andrographolide crystallized. The andrographolide crystals were collected after recrystallization with methanol. Finally, the andrographolide crystals and dried crude extract were obtained with the yield of 52.48g (1.31%) and 541.49 g (13.54%). Andrographolide crystals were white crystalline powder whereas dried crude extract was dark green powder.

The andrographolide and dehydroandrographolide contents in dry powder crude extract and crystals were determined by HPLC method which validated as shown in next section. The contents in crude drug, crude extract and crystals are shown in Table 8.

Table 8 Andrographolide and dehydroandrographolide content in crude drug, crude extract and crystals.

	Andrographolide content (%)*	Dehydroandrographolide content (%)*
Crude drug	3.73±0.10	0.27±0.04
Crude extract	2.62±0.341	13.04±0.20
Crystal I[a]	81.32±3.48	0
Crystal II[b]	97.02±0.66	0

* Mean±SD determined from triplicate samples

[a] crystal I used in the study of the effect of stirring rate and the effect of amount emulsifier

[b] crystal II used in the study of the effect of core to wall ratio, effect of polymer type and the effect of Poloxamer 188

The identification of andrographolide crystals by FTIR absorption spectra was shown as compared with andrographolide standard and dehydroandrographolide standard (Figure 11). The FTIR absorption spectra of andrographolide crystals were identical with to that of andrographolide standard.

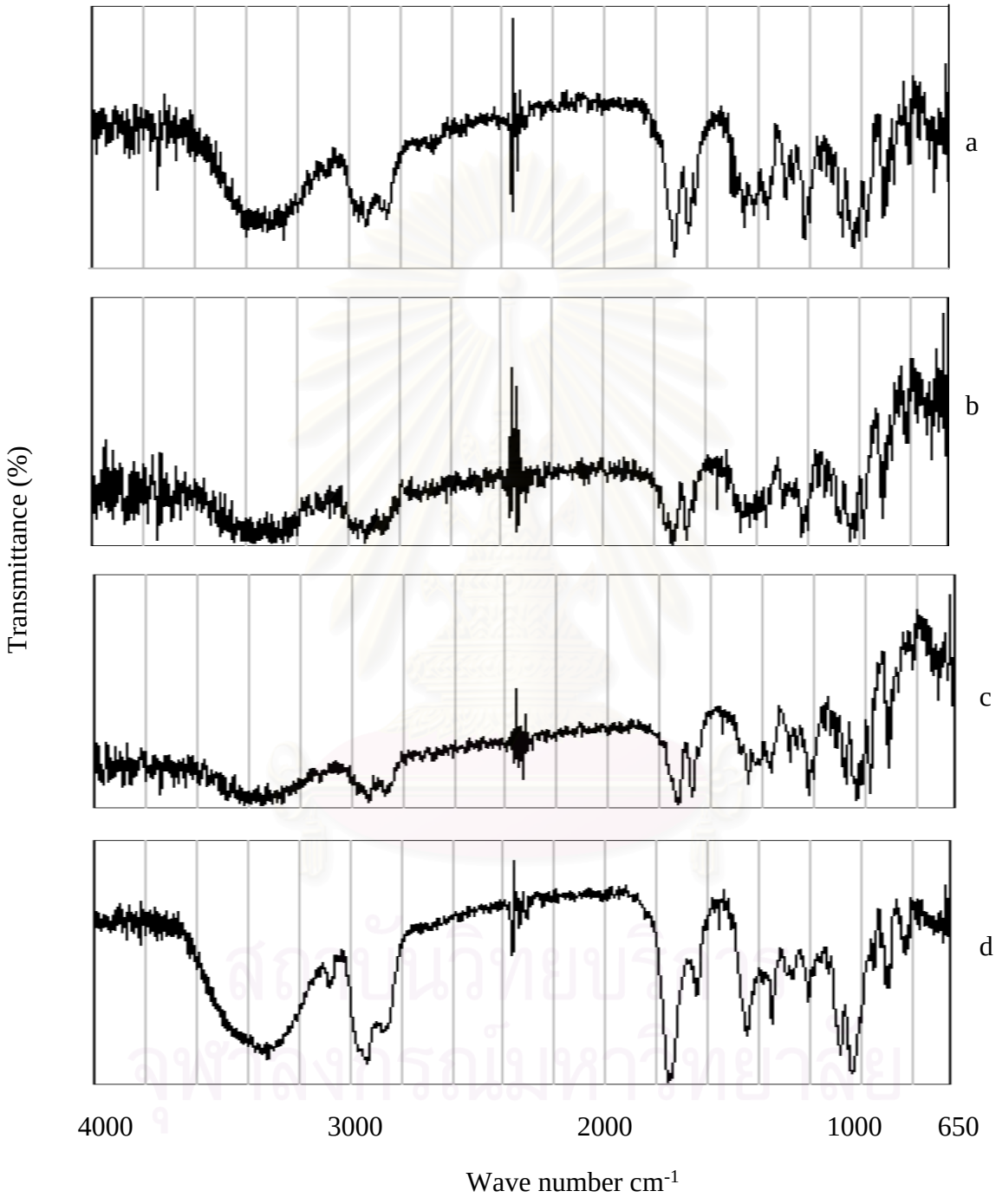


Figure 11 FTIR absorption of andrographolide crystal I (a), andrographolide crystal II (b), andrographolide standard (c), dehydroandrographolide standard (d)

II. Validation of analytical method of andrographolide and dehydroandrographolide by HPLC method

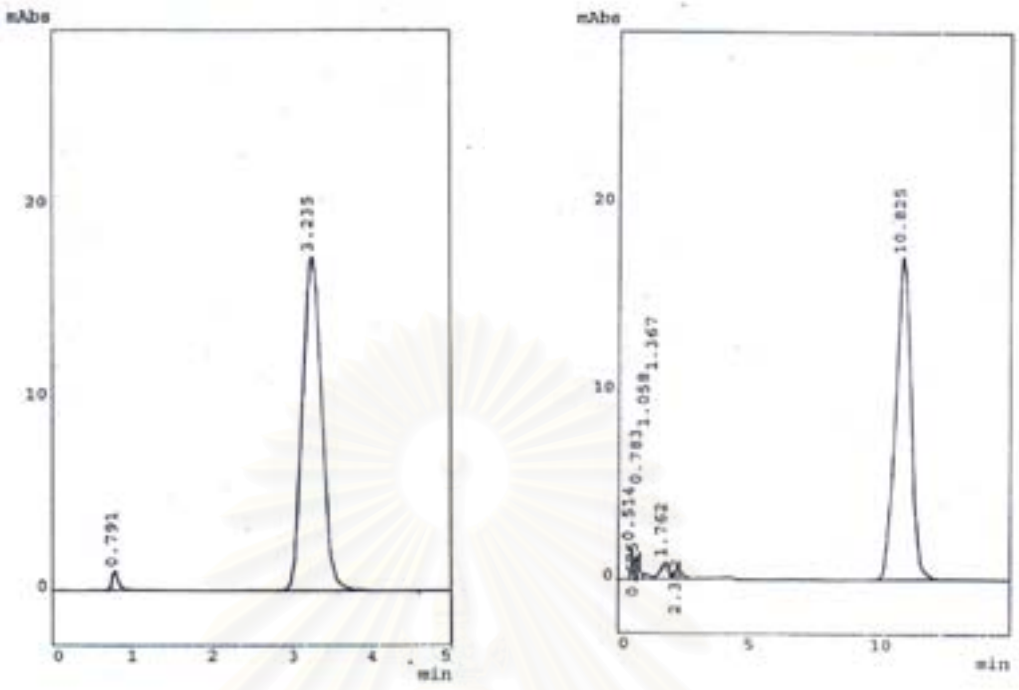
The analyses of the major constituents, andrographolide and dehydroandrographolide, in dry powder crude extract, crystals and microcapsules were determined using the modified reverse phase HPLC with UV detection. Chromatographic condition for determination of andrographolide and dehydroandrographolide was modified from a method described by Mahaverawat (1990). The UV detection wavelength was at 255 nm which was the optimal wavelength given the complete resolution of peak of constituents in the crude extract. The external standard technique was perform by determining the peak area of two working standards. The andrographolide standard and dehydroandrographolide standard were working standards which were supported from the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University. The mobile phase that gave the appropriate separation and sharp peaks of the constituents was methanol-water mixture of 50:50% by volume. The chromatogram of crude extract and Andrographis paniculata extract microcapsules were shown in Figures 1B and 2B, Appendix B.

1. Specificity

The specificity of andrographolide and dehydroandrographolide chromatograms are illustrated in Figure 12. The retention times of andrographolide and dehydroandrographolide were at 3.2 and 10.8 min, respectively. In addition, there was no interference to the peak of both constituents in the chromatogram .

2. Linearity

The calibration curves of andrographolide standard solution and dehydroandrographolide standard solution are shown in Figures 13 and 14, respectively. Both calibration curves were plotted between the peak area and the concentration of andrographolide and dehydroandrographolide in μg/mL. The linear



a b

Figure 12 Chromatograms of andrographolide (retention time = 3.2 min) (a) and dehydroandrographolide (retention time = 10.8 min) (b)

regression was used to test the fitting of data with a straight line. The coefficients of determination (R^2) of andrographolide standard curve and dehydroandrographolide standard curve were 0.99979 and 0.99995, respectively.

3. Accuracy

The determination of accuracy of the analyses of andrographolide and dehydroandrographolide by HPLC method was performed by analyzing the percentage analytical recoveries of 6 injections of both standard solutions (Table 9 and 10). The percentage recoveries obtained ranged from 98.81-101.38 % and 99.36-100.92 % for andrographolide and dehydroandrographolide respectively. It indicated that the HPLC method could be used to determine the drug contents with high accuracy.

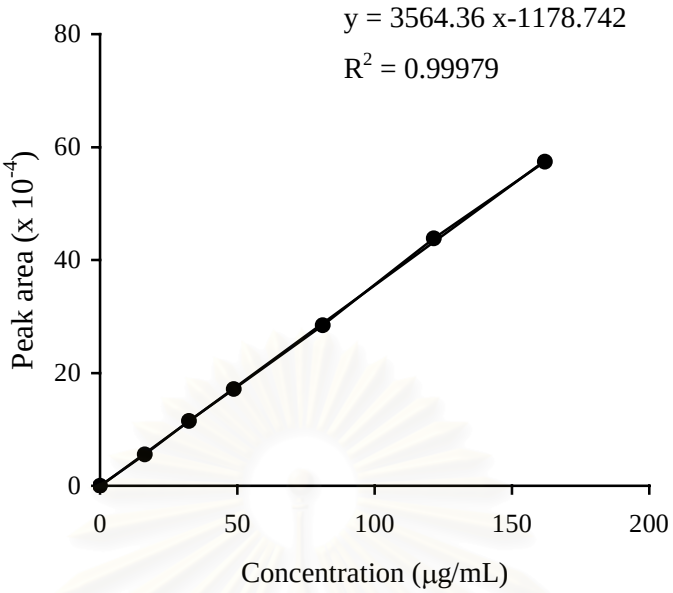


Figure 13 Calibration curve of andrographolide assayed by HPLC method.

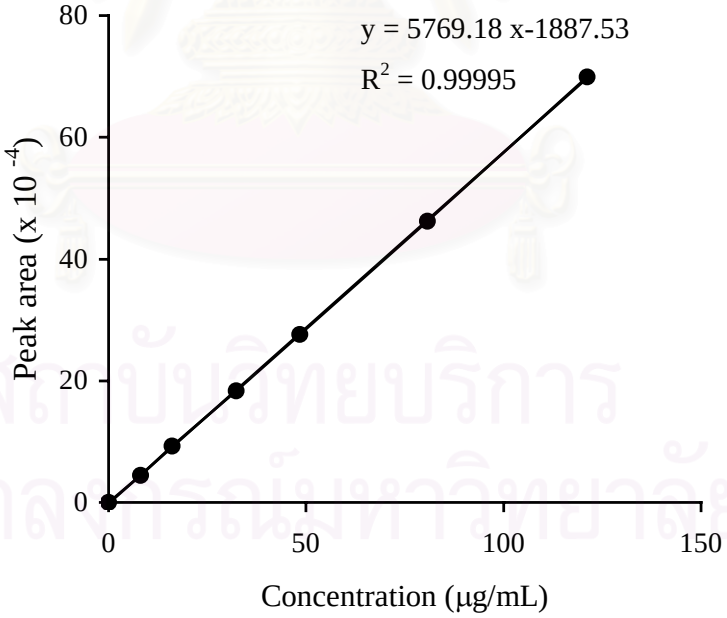


Figure 14 Calibration curve of dehydroandrographolide assayed by HPLC method.

Table 9 The percentage analytical recoveries of the analysis of andrographolide by HPLC method.

Andrographolide conc. (μg/mL)	Fitted conc. (μg/mL)	%Recovery
16.20	16.02	98.87
32.40	32.49	100.28
48.60	48.28	99.35
81.00	80.12	98.91
121.50	123.19	101.39
162.00	161.27	99.55
	mean	99.81
	SD	0.91
	%CV	0.91

Table 10 The percentage analytical recoveries of the analysis of dehydroandrographolide by HPLC method.

Dehydroandrographolide conc.(μg/mL)	Fitted conc. (μg/mL)	%Recovery
8.08	8.04	99.47
16.16	16.34	100.92
32.32	32.18	99.56
48.48	48.17	99.36
80.80	80.42	99.53
121.20	121.60	100.33
	mean	99.93
	SD	0.60
	%CV	0.60

4. Precision

The precision of the analyses of andrographolide and dehydroandrographolide by HPLC method was determined both within run and between-run as illustrated in Tables 11,12, 13 and 14. The percentage coefficients of variation (%CV) of all precision determinations were very low, especially the within run precision of both compounds.

Table 11 The within-run precision of andrographolide by HPLC method.

conc.	Peak area of andrographolide					
(μg/ml)	Inj.1	Inj.2	Inj.3	Mean	SD	%CV
0	0	0	0	0	0	0
16.20	56527	55612	55597	55912.00	532.66	0.95
32.40	114660	114714	114516	114630.00	102.35	0.09
48.60	171418	170509	170857	170928.00	458.64	0.27
81.00	282605	286418	284169	284397.33	1916.73	0.67
121.50	439973	433037	440694	437901.33	4228.03	0.97
162.00	577478	567569	575889	573645.33	5321.90	0.93

Table 12 The between-run precision of andrographolide by HPLC method.

conc.	Peak area of andrographolide					
(μg/ml)	day1	day2	day3	Mean	SD	%CV
0	0	0	0	0	0	0
16.20	55612	51463	59184	55419. 67	3864.09	6.97
32.40	114516	103094	115340	110983.33	6844.77	6.17
48.60	170509	165568	171216	169097.67	3077.15	1.82
81.00	286418	266807	293388	282204.33	13782.37	4.88
121.50	440694	466755	431409	446286.00	18324.51	4.11
162.00	577478	567577	577524	574193.00	5729.67	0.99

Table 13 The within-run precision of dehydroandrographolide by HPLC method.

conc.	Peak area of dehydroandrographolide					
(μg/ml)	Inj.1	Inj.2	Inj.3	Mean	SD	%CV
0	0	0	0	0	0	0
8.08	44379	44134	44932	44481.67	408.79	0.92
16.16	91806	92757	92053	92205.33	493.47	0.54
32.32	183521	183988	183746	183751.67	233.55	0.13
48.48	275605	276800	275621	276008.67	685.36	0.25
80.80	467241	459811	459181	462077.67	4482.66	0.97
121.20	703157	698999	696736	699630.67	3256.77	0.46

Table 14 The between-run precision of dehydroandrographolide by HPLC method.

conc. (μg/ml)	Peak area of dehydroandrographolide					
	day1	day 2	day 3	Mean	SD	%CV
0	0	0	0	0	0	0
8.08	44932	40331	47429	44230.67	3600.60	8.14
16.16	92053	76787	100837	89892.33	12169.72	13.54
32.32	183746	162297	196226	180756.33	17160.94	9.50
48.48	275621	243731	285205	268185.67	21713.74	8.10
80.80	459181	405740	490047	451656.00	42654.27	9.44
121.20	696736	616976	728095	680602.33	57289.44	8.42

In conclusion, the analyses of andrographolide and dehydroandrographolide by HPLC developed in this study showed good specificity, linearity, accuracy and precision. Thus the method was used for the determination of the content in the study.

III. Preparation of *Andrographis paniculata* extract microcapsules by solvent evaporation technique

Andrographis paniculata extract microcapsules were successfully prepared using oil in oil emulsion solvent evaporation method. Acetone and light liquid paraffin were chosen to be internal phase and external phase with the fixed amounts of 80 and 220 mL, respectively. Acetone was used to be the internal organic phase because of the good solubility for both the core and Eudragit polymer.

Additionally, acetone could be easily and completely removed from the system at room temperature. The acetone/liquid paraffin system or oil/oil system was applied in this study to avoid two problems, swelling and fragility, found in the microcapsules prepared by the evaporation process in the water phase (Kawata et al., 1986).

The volumes of internal and external organic phases were kept constant since the variation of the phase volume had a strong influence on the size of the microcapsules (Sansdrap and Moës, 1993). In the preparation process, the geometry

of the manufacturing or preparing systems (e.g. reactor or container, stirrer, etc.) was kept unchanged to prevent any uncontrolled influences on the production of microparticles. The core was comprised of 1 part of andrographolide crystal (81.32% in crystal I or 97.02% in crystal II) and 1 part of crude extract powder (2.62% andrographolide and 13.04 % dehydroandrographolide). Approximately, the core material should contain 41.97-49.82% andrographolide and 6.52% dehydroandrographolide.

Microspheres were completely formed in the external phase . After drying, the core was completely encapsulated as spherical microcapsules as shown in Figure 15. The microcapsules were uniform and free-flowing. There are no aggregates observed.

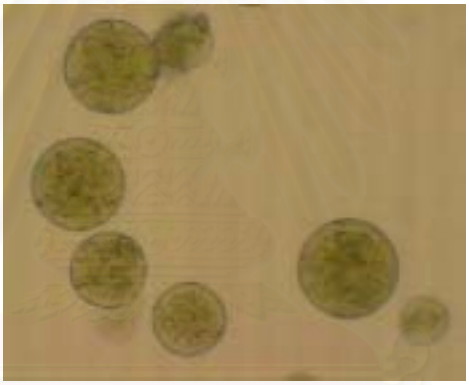


Figure 15 The completely coated microcapsules prepared by oil in oil emulsion solvent evaporation.

IV. Effect of stirring rate

The 1:2 core to wall ratio microcapsules containing *Andrographis paniculata* extract were prepared using varied stirring rate from 250-1200 rpm and other parameters as described in Table 3. The yield, size, and andrographolide and dehydroandrographolide contents of the microcapsules are shown in Table 15. It was found that the percentage yield of microcapsules increased as the stirring rate increased, and the yield was highest at the highest stirring rate of 1200 rpm. The result might be attributable that the stirring rate is the parameter of primary

importance in the emulsification steps. The stirring rate provides the energy for microcapsules formation and reduction of droplet size (Sansdrap and Moës, 1993).

The microcapsules prepared at 1000 rpm had highest drug content and core entrapment (Table 15). The drug content and core entrapment of microcapsules prepared at other stirring rates, i.e. 250, 500, 800 and 1200 rpm, showed no difference. This might suggest that the stirring rate should be appropriate for the maximum entrapment.

Table 15 The percentage yield, mean particle size, polydispersibility index (P.I.) and the percentage andrographolide (AG) and dehydroandrographolide (DAG) contents of *Andrographis paniculata* extract microcapsules prepared at varied stirring rates.

Rate (rpm)	Yield (%)	Size Mean ± SD (μm) [a]	P.I.	AG content Mean ± SD (%) [b]	DAG content Mean ± SD (%) [b]	Core entrapment Mean ± SD (%) [b]
250	95.05	192.16±121.05	0.63	11.68±0.97	1.79±0.09	83.68±5.80
500	99.29	95.67±76.51	0.80	10.71±1.18	1.93±0.07	78.79±7.55
800	97.28	69.88±43.05	0.62	11.98±0.66	1.81±0.04	85.97±4.36
1000	98.38	65.38±34.83	0.53	13.46±1.02	1.81±0.09	93.24±5.74
1200	99.57	53.42±32.39	0.61	12.18±0.97	1.70±0.06	85.40±5.66

a n = 600; b n = 3

The stirring rate exhibited the dramatic influence on the microcapsule size. When the stirring rate was increased, the microparticles became smaller. The cumulative percentage undersize of the microcapsules is displayed in Figure 16. Additionally, the polydispersibility index (P.I.) as shown in Table 15 indicating the size distribution of microcapsules prepared at 1000 rpm was narrowest while the size distribution of microcapsules prepared at 500 rpm was widest.

The result from this study agreed with a previous studies by Benita, Zouai and Benoit (1986), Barkai, Pathak and Benita (1990) and Sandrap and Moës (1993). The stirring rate provided the energy to shearing forces during emulsification step (Sandrap and Moës, 1993) and prevented aggregates of small microcapsules forming (Amperiadou and Georgarakis, 1995).

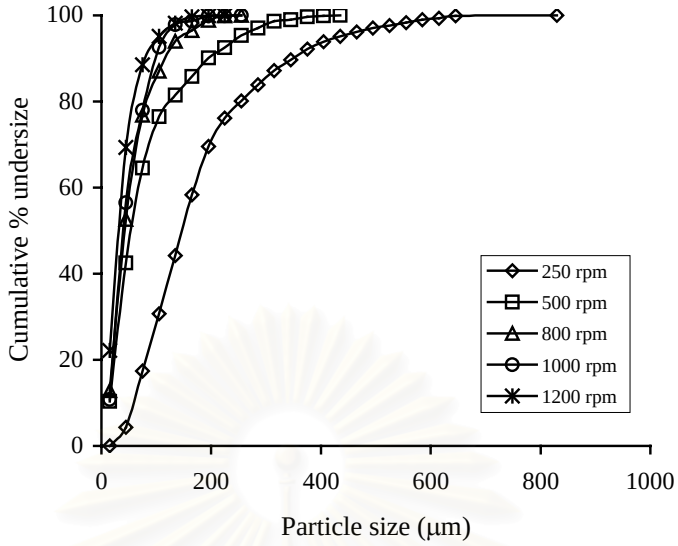


Figure 16 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 250, 500, 800, 1000 and 1200 rpm.

The effect of stirring rate on the *in vitro* drug release is shown in Figures 17 and 18. It could be observed that the drug release profiles obtained from microcapsules prepared using stirring rate at 250 and 500 rpm were lower than microcapsules prepared using stirring rate at 800, 1000 and 1200 rpm. Since the higher stirring rate produced the small particle size thus it resulted in a higher surface area available for drug release. From the drug release profile, the data were analyzed according to different models to obtain the release rate constant (k) and the regression coefficient or coefficient of determination (R^2) was determined to present the linearity as shown in Tables 16 and 17. Among all the models tested, the Higuchi model appeared to provide the best fits for all the investigated formulations. The result presented that the mechanism of drug release would probably be through the dissolution of drug in microcapsules followed by diffusion-controlled release.

From the Higuchi release rate constants (Table16, 17), in the simulated gastric fluid pH 1.2, there was no significant difference among the microcapsules prepared at different stirring rates ($p>0.05$) (Table 1H, Appendix H). In the medium of pH 6.8, the rate constant of the microcapsules prepared at 1200 rpm was significant lowest , whereas that at 1000 rpm was highest ($p<0.05$) (Table 6H, Appendix H).

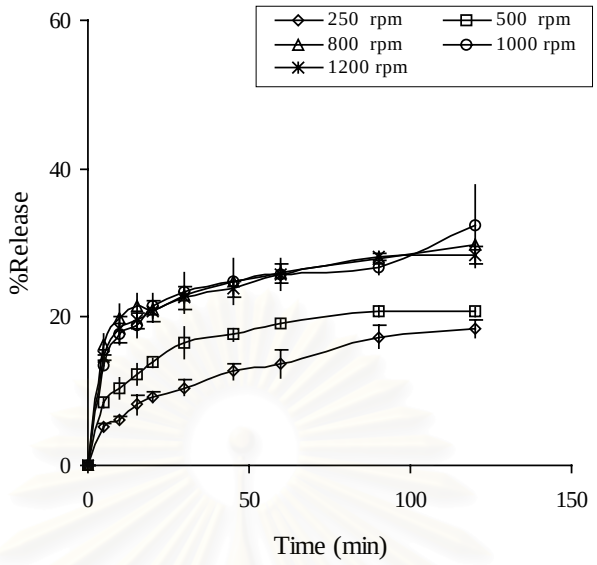


Figure 17 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 250, 500, 800, 1000 and 1200 rpm in simulated gastric fluid without pepsin pH 1.2.

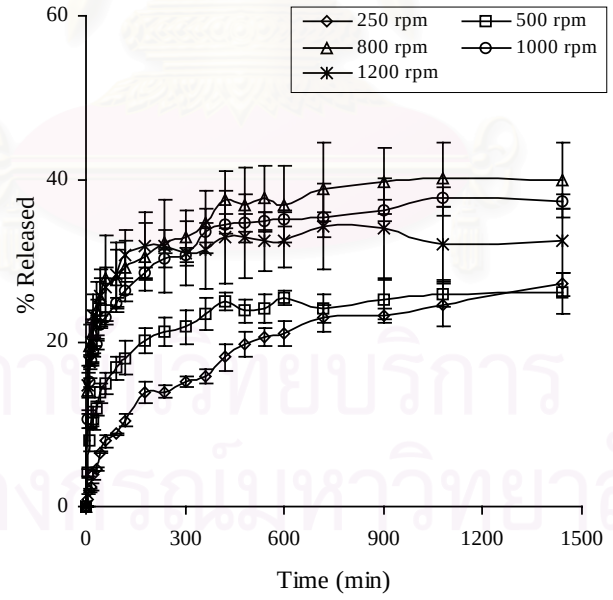


Figure 18 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 250, 500, 800, 1000 and 1200 rpm in simulated intestinal fluid without pancreatin pH 6.8± 0.1.

Table 16 The release rate constants of zero-order (k_0), first-order (k) and Higuchi-model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 at different stirring rates in pH 1.2.

Stirring rate (rpm)	Zero-order		First -order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
250	0.1139 ±0.0124	0.9357 ±0.0256	0.0013 ±0.00002	0.9444 ±0.0223	1.5616 ±0.1889	0.9827 ±0.0066
500	0.1021 ±0.0051	0.7738 ±0.0658	0.0012 ±0.0001	0.7863 ±0.0650	1.4685 ±0.0471	0.8956 ±0.0431
800	0.1008 ±0.0094	0.8477 ±0.0293	0.0013 ±0.0001	0.8647 ±0.0282	1.4051 ±0.1227	0.9228 ±0.0354
1000	0.1312 ±0.0316	0.8220 ±0.0631	0.0017 ±0.0005	0.8373 ±0.0595	1.8289 ±0.3937	0.8990 ±0.0547
1200	0.1073 ±0.0066	0.824 8±0.0625	0.0014 ±0.0001	0.8421 ±0.0594	1.5213 ±0.0698	0.9278 ±0.0412

Table 17 The release rate constants of zero-order (k_0), first-order (k) and Higuchi-model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 at different stirring rates in pH 6.8±0.1.

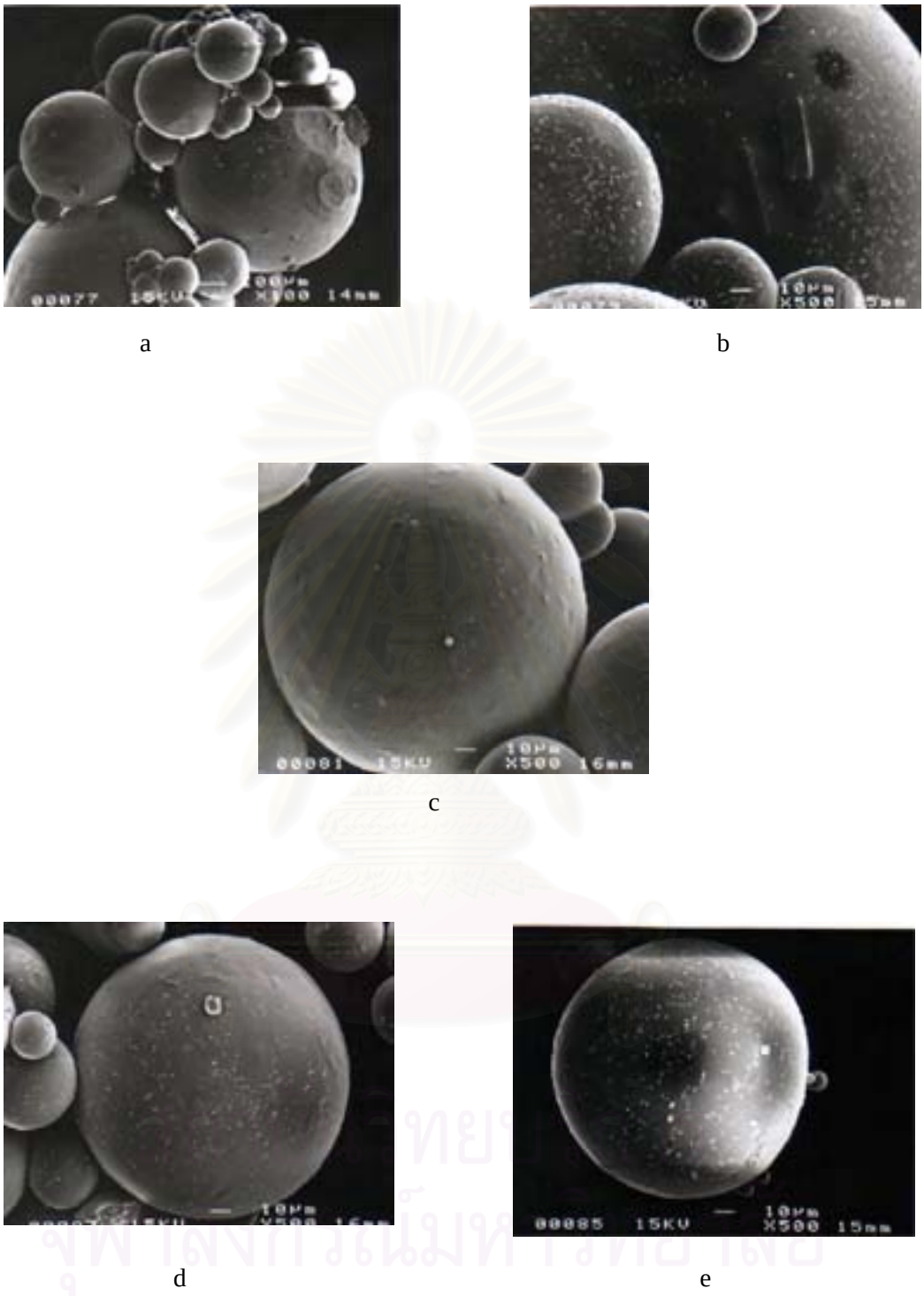
Stirring rate (rpm)	Zero-order		First-order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
250	0.0325 ±0.0028	0.9087 ±0.0117	0.0004 ±0.00003	0.9237 ±0.0113	0.9041 ±0.0777	0.9818 ±0.0068
500	0.0288 ±0.0003	0.7869 ±0.0461	0.0003 ±0.00001	0.8092 ±0.0438	0.8332 ±0.0201	0.9156 ±0.0262
800	0.0312 ±0.0017	0.7870 ±0.0351	0.0004 ±0.00004	0.8150 ±0.0385	0.8962 ±0.0426	0.9019 ±0.0279
1000	0.0339 ±0.0012	0.8139 ±0.0107	0.0005 ±0.00002	0.8447 ±0.0097	0.9738 ±0.0350	0.9330 ±0.0094
1200	0.0220 ±0.0053	0.5703 ±0.0491	0.0003 ±0.0001	0.5859 ±0.0537	0.6687 ±0.1578	0.7376 ±0.0520

In addition, the initial drug release profile exhibited a small burst effect. This observation could be explained by Figure 19. From the scanning electron microphotographs, the microcapsules prepared at 500, 800, 1000 and 1200 rpm had more drug crystals adhering on the microcapsule surfaces than the microcapsules prepared at 250 rpm. Since the stirring rate effected on the evaporation rate of the organic internal phase, the high stirring rate resulted in faster evaporation. In the case where Eudragit polymer precipitated prior to the drug, no crystal was visible on microspheres surface, and when drug crystallized before Eudragit, it resulted in irregular shaped microcapsules and drug crystal adhering on microcapsule surface (Bodmeier and Chen1989).

In this study, the *in vitro* drug release was also evaluated using the dissolution efficiency (DE) which calculated from the drug release from 2 h in medium pH 1.2 and after 24 h in medium pH 6.8. Dissolution efficiency was defined as the area under the dissolution curve up to a certain time, t (Khan, 1975). In Table 18, it was found that the microcapsules prepared at 800, 1000 and 1200 rpm had significant higher DE values than those prepared at 250 rpm and 500 rpm in both pH 1.2 and pH 6.8 ($p<0.05$) (Tables 11H and 16H, Appendix H).

From the results obtained on yield, andrographolide content, dehydroandrographolide content, core entrapment, size, size distribution and rate constant, ranking scores were given from 1 to 5. The highest score of 5 was ranked to the best or most appropriate value of each parameter. In addition, the score of the highest drug release rate constant was given as 5 due to the comparison between the drug release profile of microcapsules with the release profile of the 1:1 mixture of andrographolide crystals and crude extract (core material). The percentage drug release of microcapsules was much less than that shown from the latter (Figures 1G and 2G, Appendix G). Thus, the appreciation of the relatively high drug release rate constant was given with the ranking score of 5.

In the ranking scores, as the stirring rate of 1000 rpm gave the highest score, it was chosen for further studies (Table 1I, Appendix I).



a b c d e

Figure 19 Scanning electron microphotographs of *Andrographis paniculata* extract microcapsules prepared at 250 (a), 500 (b), 800 (c), 1000 (d), and 1200 rpm (e).

Table 18 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 at different stirring rates.

Stirring rate (rpm)	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
250	13.26±1.18	20.35±1.28
500	17.47±0.65	23.65±1.67
800	24.75±0.46	36.63±4.15
1000	24.53±1.86	34.01±0.97
1200	24.21±0.87	32.06±4.33

V. Effect of emulsifier concentration

From the investigation of stirring rate, the rate of 1000 rpm was chosen to prepare microcapsules with varied emulsifier concentrations. Span80 concentration was varied as 0, 0.5, 1.0, 1.5 and 2.0% of the external phase, and other parameters were as described in Table 4. Span80 is a nonionic surfactant with a low HLB value of 4.3. It was incorporated in the external phase (light liquid paraffin) and promoted the emulsification step.

The surface active concentration showed the influence on the yield. As the Span80 concentration increased, the yield increased (Table 19). The surface active agent decreased the interfacial tension between the organic droplets and the external phase.

From the size (Table 19), microcapsules prepared with 1.0, 1.5 and 2.0 % Span80 had larger particle size than those prepared with 0 and 0.5% (Figure 20). This might be suggested that there was an optimal emulsifier concentration for emulsification process. The excess emulsifier presented in the microcapsule with high emulsifier (Span 80) concentration, thus resulted in the unexpected large microcapsule size. The particle size distribution of the microcapsules was evaluated using the polydispersibility index (P.I.). It was found that the size distribution of the microcapsules prepared with 1.0, 1.5 and 2.0 % Span80 was wider than those prepared with 0 and 0.5 %.

Table 19 The percentage yield, mean particle size, polydispersibility index (P.I.) and the percentage andrographolide (AG) and dehydroandrographolide (DAG) contents of *Andrographis paniculata* microcapsules prepared with varied Span80 concentrations.

Conc. (%)	Yield (%)	Size Mean ± SD (μm) [a]	P.I.	AG content Mean ± SD (%)[b]	DAG content Mean ± SD (%)[b]	Core entrapment Mean ± SD (%)[b]
0	95.43	42.42±20.25	0.48	13.64±1.16	1.95±0.03	96.64±7.23
0.5	92.65	46.37±21.63	0.47	15.49±0.80	1.84±0.05	107.38±4.79
1.0	98.38	65.39±34.86	0.53	13.46±1.02	1.81±0.09	93.24±5.74
1.5	98.51	56.58±29.17	0.52	12.92±1.02	1.80±0.06	90.67±6.64
2.0	101.68	60.13±35.72	0.59	14.25±1.66	1.32±0.24	96.04±11.62

a n = 600; b n = 3

The different result was reported by Khawla et al.(1996) that increasing the emulsifier concentration gave smaller particle size. The surface pores were observed to be decreased as Span80 increased (Figure 21). The similar result was also reported by Khawla et al.(1996).

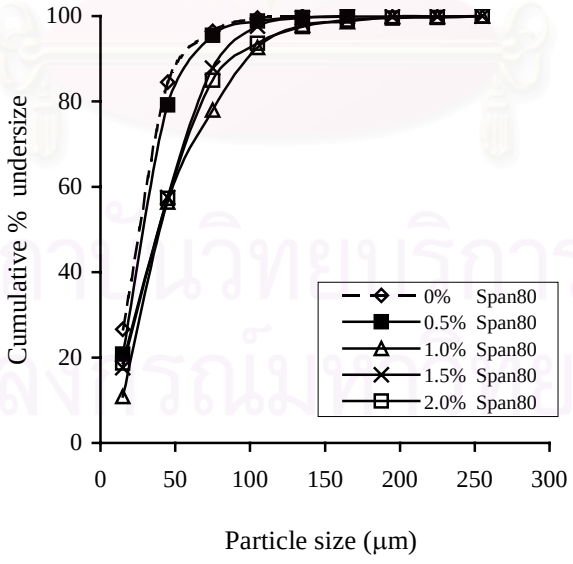


Figure 20 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 0.5, 1.0, 1.5 and 2% Span 80.

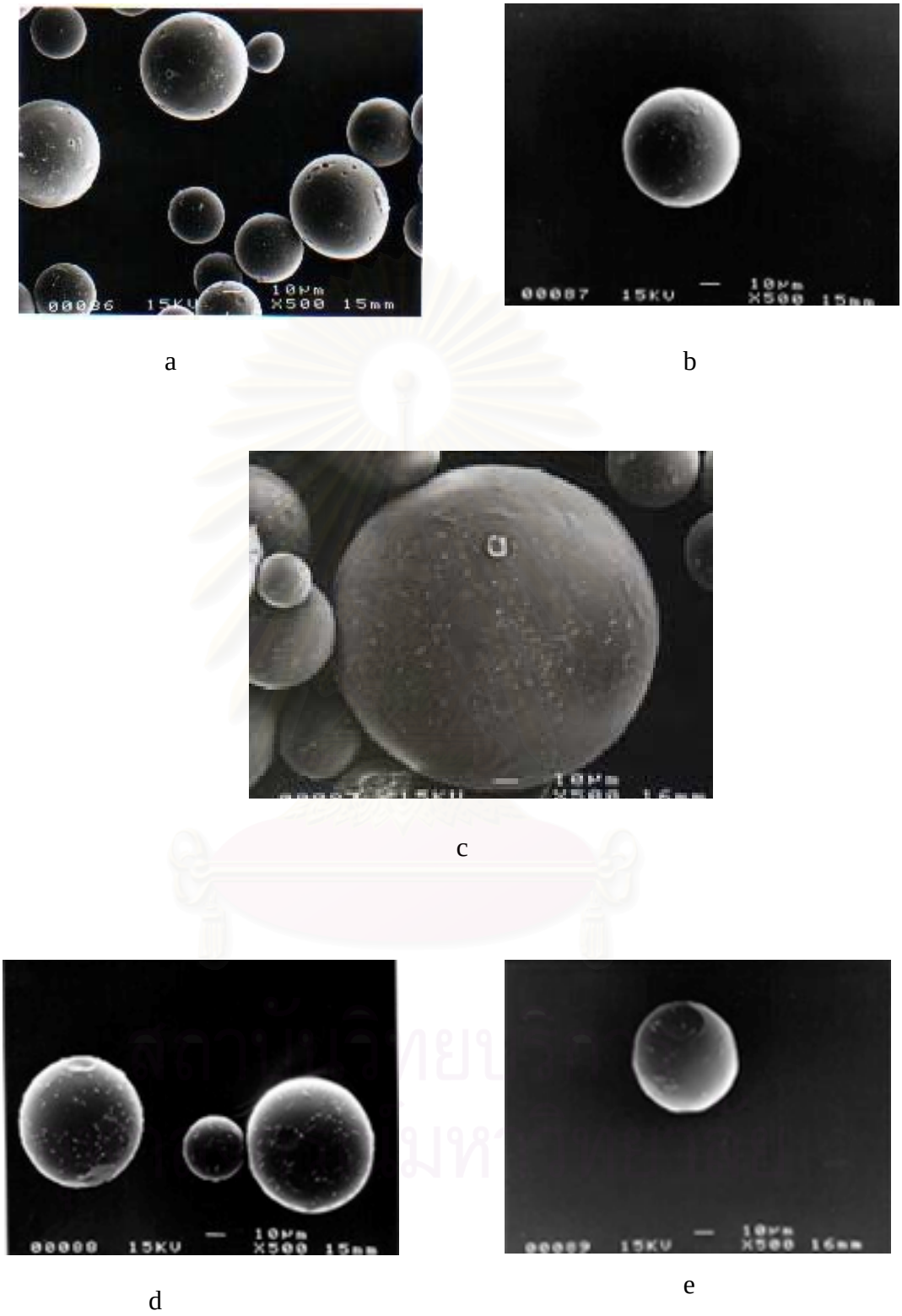
The effect of the emulsifier concentration on the *in vitro* drug release is shown in Figures 22 and 23. It can be observed that the drug release profile obtained from microcapsules exhibited burst effect. Among all the models tested, the Higuchi model appeared to provide the best fits for all the investigated formulations (Tables 20 and 21). The result suggested that the mechanism of drug release would probably be through the dissolution of drug in microcapsules followed by diffusion-controlled release.

The drug release rate constant of microcapsules with 0.5 % Span80 was significantly higher than those with other Span80 concentrations in the medium pH 1.2. In contrary, there was no significant difference between the rate constants of all microcapsules prepared with different concentrations of Span80 in the medium pH 6.8 ($p>0.05$) (Tables 2H and 7H Appendix H).

The dissolution efficiency (DE) of microcapsules prepared with 0.5% Span80 was significantly higher than that of the microcapsules prepared with 2.0% Span80 ($p<0.05$), whereas there was no significant difference of the DE values of microcapsules at pH 6.8 ($p>0.05$) (Table 22).

From the results obtained on yield, andrographolide content, dehydroandrographolide content, core entrapment, size, size distribution and rate constant, ranking scores were given from 1 to 5. The highest score of 5 was ranked to the best or most appropriate value of each parameter. In addition, the score of the highest drug release rate constant was given as 5 due to the comparison between the drug release profile of microcapsules with the release profile of the 1:1 mixture of andrographolide crystals and crude extract (core material). The percentage drug release of microcapsules was much less than that shown from the latter (Figures 1G and 2G, Appendix G). Thus, the appreciation of the relatively high drug release rate constant was given with the ranking score of 5.

Thus from all results obtained, the ranking scores of the parameter of microcapsules were given as shown in Table 2I (Appendix I). The 0.5 % concentration of Span80 gave the highest score, thus it was chosen for further study.



a b

c

d e

Figure 21 Scanning electron microphotographs of *Andrographis paniculata* extract microcapsules prepared with 0 (a), 0.5 (b), 1.0 (c), 1.5 (d) and 2% Span80 (e).

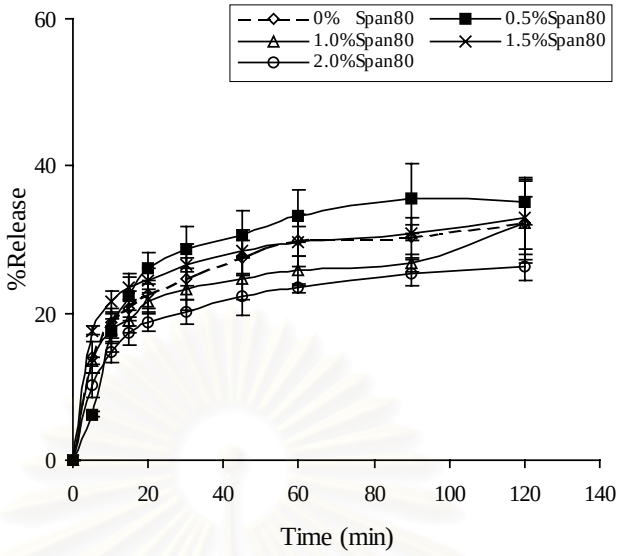


Figure 22 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 0.5, 1.0, 1.5 and 2.0% Span 80 in simulated gastric fluid without pepsin pH 1.2.

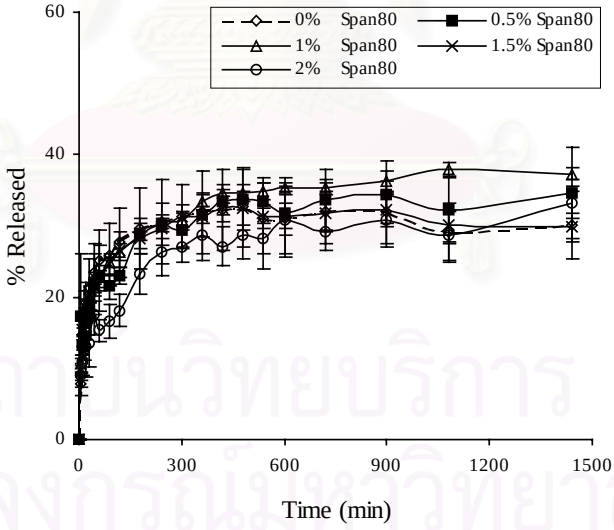


Figure 23 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 0.5, 1.0, 1.5 and 2.0% Span80 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

Table 20 The release rate constants of zero-order (k_0), first-order (k) and Higuchi-model (k_h) and the coefficient of determination (R^2) of microcapsules prepared with various concentrations of Span80 in pH 1.2.

Span80	Zero-order		First-order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
0 %	0.1377	0.7877	0.0018	0.8097	1.9770	0.9092
	±0.0182	±0.0765	±0.0003	±0.0791	±0.2263	±0.0497
0.5 %	0.1918	0.6225	0.0026	0.6689	2.8669	0.7781
	±0.0183	±0.237	±0.0003	±0.0255	±0.2813	±0.0180
1.0 %	0.1312	0.8220	0.0017	0.8373	1.8289	0.8990
	±0.0316	±0.0631	±0.0005	±0.0595	±0.3937	±0.0547
1.5 %	0.1116	0.8128	0.0015	0.8342	1.5910	0.9238
	±0.0063	±0.0313	±0.0001	±0.0279	±0.1001	±0.0225
2.0 %	0.1170	0.7621	0.0015	0.7854	1.6874	0.8877
	±0.0043	±0.0137	±0.0001	±0.0098	±0.0629	±0.0152

Table 21 The release rate constants of zero order (k_0), first order (k) and Higuchi-model (k_h) and the coefficient of determination (R^2) of microcapsules prepared with various concentrations of Span80 in pH 6.8±0.1.

Span80	Zero-order		First-order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
0 %	0.0273	0.5945	0.0004	0.6251	0.8304	0.7671
	±0.0046	±0.0206	±0.0001	±0.0203	±0.1452	±0.0168
0.5 %	0.0318	0.7554	0.0004	0.7724	0.9027	0.8492
	±0.0042	±0.0494	±0.0001	±0.0447	±0.1333	±0.0817
1.0 %	0.0339	0.8139	0.0005	0.8447	0.9738	0.9330
	±0.0012	±0.0107	±0.00002	±0.0097	±0.0350	±0.0094
1.5 %	0.0288	0.7025	0.0004	0.7282	0.8526	0.8591
	±0.0023	±0.0111	±0.00003	±0.0099	±0.0705	±0.0058
2.0 %	0.0336	0.8450	0.0004	0.8591	0.9458	0.9662
	±0.0039	±0.0586	±0.0001	±0.0592	±0.0960	±0.0167

Table 22 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and various concentrations of Span80.

Conc. Span80	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
0%	26.85±2.66	30.09±4.71
0.5%	29.88±3.32	31.54±3.61
1.0%	24.53±1.86	34.01±0.97
1.5%	27.85±1.33	30.05±1.97
2.0%	21.86±1.53	27.90±2.63

VI. Effect of core to wall ratio

From the investigation of emulsifier concentration, the 0.5 % Span80 was chosen to further studies. The microcapsules were prepared with various core to wall ratios such as 1:1, 1:2, 1:3 and 2:3 . The microcapsules obtained had spherical shape with different sizes as shown in Table 23.

It was found that the microcapsules prepared with 1:1 core to wall ratio gave the highest percentage yield while the microcapsules prepared with 1:2 ratio exhibited the highest core entrapment.

Table 23 The percentage yield, mean particle size, polydispersibility index (P.I.) and the percentage andrographolide (AG) and dehydroandrographolide (DAG) contents of *Andrographis paniculata* extracts microcapsules prepared at different core to wall ratios.

Ratio	Yield (%)	Size Mean ± SD (μm) [a]	P.I.	AG content Mean ± SD (%) [b]	DAGcontent Mean ± SD (%) [b]	Core entrapment Mean ± SD (%) [b]
1:1	99.82	42.23± 23.88	0.57	22.06± 1.08	2.74± 0.04	88.38+3.73
1:2	88.14	53.07± 33.41	0.63	16.04± 0.91	1.90± 0.04	95.62+4.94
1:3	82.98	120.06± 69.89	0.58	11.43± 0.34	1.59± 0.01	92.13+2.50
2:3	92.03	381.32±316.06	0.83	16.53± 0.20	2.38± 0.18	84.13+1.03

a n = 600; b n = 3

The effect of core to wall ratio on the size of microcapsules is displayed in Table 23 and Figure 24. From the 2:3 core to wall ratio, the cumulative % undersize curve showed the larger size while the 1:1 core to wall ratio shifted to smaller size. The largest particle size of 381.32 μm was obtained from the 2:3 ratio, whereas the smallest microcapsules size of 42.23 μm was resulted from the 1:1 ratio. The polydispersibility index of the microcapsules prepared with the 2:3 core to wall ratio was highest, thus the size distribution of the microcapsules prepared with this ratio was widest while the microcapsules prepared with the 1:1 core to wall ratio had narrow size distribution. Since the volumes of internal and external phases in this process were kept constant, thus, the increase of amount of core and wall materials increased the concentration of internal phase. This showed the influence on the viscosity of the system. The more viscous internal phase was more difficult to be dispersed in external phase during emulsification. This resulted in larger emulsified droplets and consequently larger solid microcapsules (Amperiadou and Georgarakis, 1995 ; Barkai, Pathak and Benita, 1990).

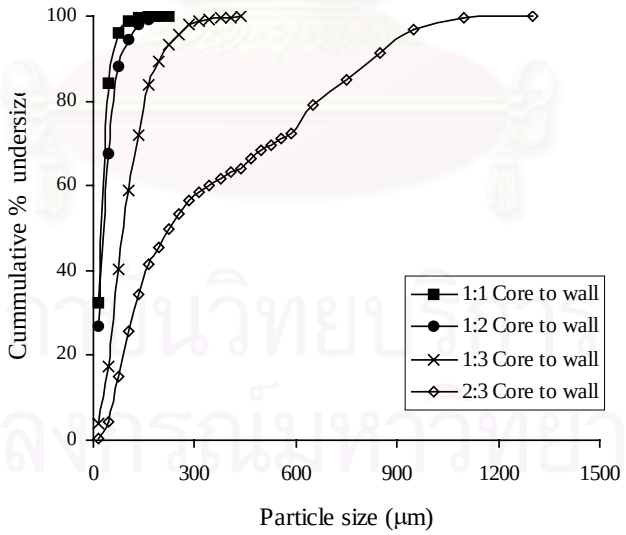


Figure 24 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 on 1:1, 1:2, 1:3 and 2:3 core to wall.

The effect of core to wall ratio on the *in vitro* drug release is shown in Figures 25 and 26. It was observed that the initial drug release profile showed a small burst effect in the some ratios. At the pH 1.2, the 2:3 core to wall showed the slowest release profile due to the largest particle and increasing polymer content in the microcapsules. However, It would be observed that the microcapsules of 2:3 ratio, inspite of large particle size, they had abound rough surface (Figure 27). Inspite of high polymer content, the 1:3 core to wall microcapsules showed fast release at pH 6.8 due to the presence of drug crystals on the surface and relatively smaller particle size (Figure 27).

The release profile of 1:1 and 1:2 core to wall microcapsules showed very similar characteristics due to similarly small particle sizes as shown in Figure 24.

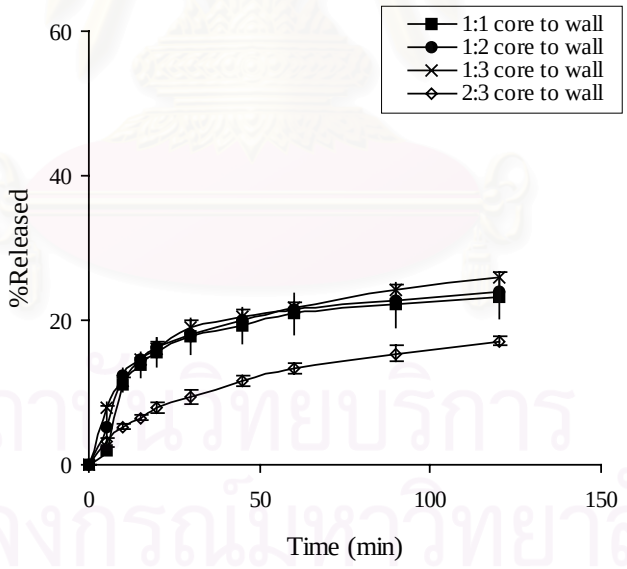


Figure 25 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared with core to wall ratio of 1:1, 1:2 , 1:3 and 2:3 in simulated gastric fluid without pepsin pH 1.2.

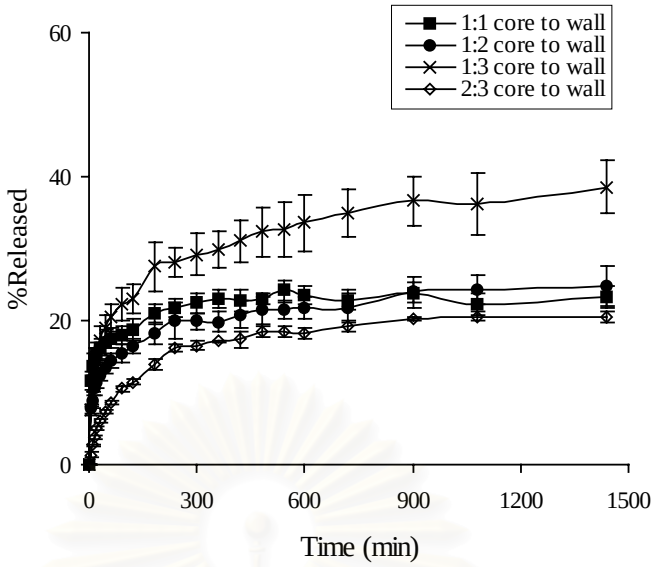
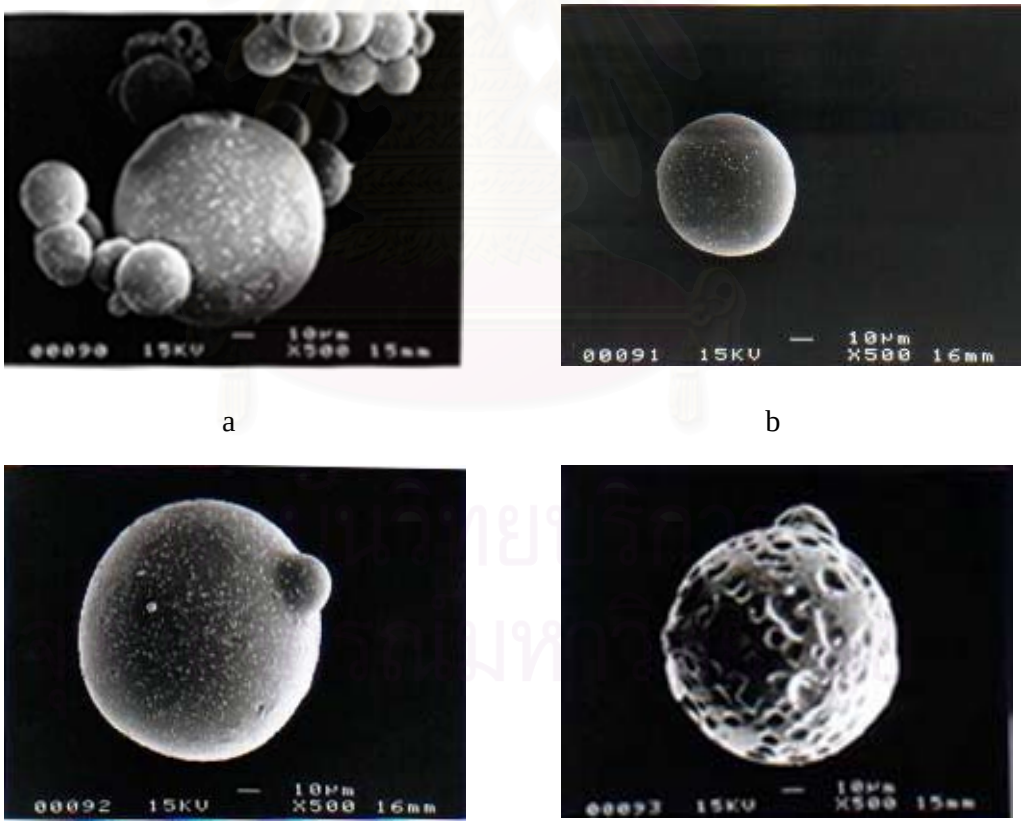


Figure 26 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared with core to wall ratio of 1:1, 1:2, 1:3 and 2:3 in simulated intestinal fluid without pancreatin pH 6.8±0.1.



a b

c d

Figure 27 Scanning electron microphotographs of *Andrographis paniculata* extract microcapsules prepared with 1:1 (a), 1:2 (b), 1:3 (c) and 2:3 (d) core to wall ratio.

The drug release rate constant of the microcapsules having various ratios were obtained from Higuchi equation (Tables 24 and 25, Figures 7F and 8F in Appendix F). The Higuchi rate constants were not significantly different in the medium pH 1.2 ($p>0.05$) (Table 3H). But in the medium pH 6.8, the Higuchi rate constant of microcapsules having 1:3 ratio was significantly higher than those of other core to wall ratios ($p< 0.05$) (Table 8H, Appendix H).

Table 24 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 in various core to wall ratios in pH 1.2.

Core to	Zero-order		First-order		Higuchi-Model	
wall ratio	k_0	R^2	k	R^2	k_h	R^2
1:1	0.1337 ±0.0219	0.6375 ±0.0168	0.0016 ±0.0003	0.6716 ±0.0142	1.9857 ±0.3310	0.7860 ±0.0137
1:2	0.1251 ±0.0071	0.7052 ±0.0679	0.0015 ±0.0001	0.7347 ±0.0616	1.8298 ±0.1270	0.8420 ±0.0602
1:3	0.1361 ±0.0033	0.8230 ±0.0283	0.0017 ±0.0001	0.8462 ±0.0262	1.9384 ±0.0611	0.9341 ±0.0183
2:3	0.1147 ±0.0036	0.9123 ±0.0165	0.0013 ±0.00005	0.9237 ±0.0158	1.5938 ±0.0516	0.9847 ±0.0066

Table 25 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 in various core to wall ratios in pH 6.8±0.1.

Core to	Zero-order		First-order		Higuchi-Model	
wall ratio	k_0	R^2	k	R^2	k_h	R^2
1:1	0.0173 ±0.0010	0.8146 ±0.0235	0.0002 ±0.00001	0.8268 ±0.0227	0.4958 ±0.0265	0.9344 ±0.0146
1:2	0.0207 ±0.0015	0.8001 ±0.0208	0.0002 ±0.00002	0.8140 ±0.0226	0.5966 ±0.0450	0.9291 ±0.0150
1:3	0.0363 ±0.0044	0.8019 ±0.0261	0.0005 ±0.0001	0.8325 ±0.0288	1.0469 ±0.1211	0.9280 ±0.0155
2:3	0.0271 ±0.0008	0.8216 ±0.0088	0.0003 ±0.00001	0.8355 ±0.0088	0.7784 ±0.0234	0.9453 ±0.0047

In Table 26, the dissolution efficiency (DE) of the *in vitro* drug release was determined from microcapsules after 2 h in medium pH 1.2 and after 24 h in medium

pH 6.8. The DE was used to compare between the microcapsules. It was found that the microcapsules prepared with 2:3 core to wall ratio was significantly lower than those of other ratios at pH 1.2 ($p<0.05$). In the medium pH 6.8, the DE of the microcapsules with 1:3 core to wall ratio was higher than those of other ratios ($p<0.05$) (Tables 13H and 18H, Appendix H).

Table 26 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 in various core to wall ratios.

Core to wall ratio	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
1:1	18.72±2.44	22.32±1.33
1:2	19.41±1.04	21.62±1.86
1:3	20.31±0.76	32.74±3.30
2:3	12.04±0.80	17.92±0.44

From the results obtained on yield, andrographolide content, dehydroandrographolide content, core entrapment, size, size distribution and rate constant, ranking scores were given from 1 to 5. The highest score of 5 was ranked to the best or most appropriate value of each parameter. In addition, the score of the highest drug release rate constant was given as 5 due to the comparison between the drug release profile of microcapsules with the release profile of the 1:1 mixture of andrographolide crystals and crude extract (core material). The percentage drug release of microcapsules was much less than that shown from the latter (Figures 1G and 2G, Appendix G). Thus, the appreciation of the relatively high drug release rate constant was given with the ranking score of 5

Thus from all results obtained, the ranking scores of the parameter of microcapsules were given as shown in Table 3I (Appendix I), the 1:1 ratio showed the highest ranking score. Thus, it was chosen in the further study.

VII. Effect of polymer type

In this study, Eudragit RL100 and Eudragit RS100 were compared for their contributions on the microencapsulation. From the previous step, the 1:1 core to wall was chosen for this study. The results of the microcapsules obtained are in Table 27.

Table 27 The percentage yield, mean particle size, polydispersibility index (P.I.) and the percentages andrographolide (AG) and dehydroandrographolide (DAG) contents of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100) in 1: 1 core to wall.

Polymer	Yield (%)	Size Mean ± SD (μm) [a]	P.I.	AG content Mean ± SD (%) [b]	DAG content Mean ± SD (%) [b]	Core entrapment Mean ± SD (%) [b]
ERL100	99.82	42.23±23.98	0.57	22.06±1.08	2.74±0.04	88.38±3.73
ERS100	94.71	377.79±271.82	0.72	21.21±0.31	2.50±0.02	85.26±1.15

a n = 600 ; b n = 3

The percentage yield, the drug content and the core entrapment in microcapsules prepared with Eudragit RL100 were higher than the microcapsules prepared with Eudragit RS100.

Eudragit RL100 microcapsules had markedly larger size and wider size distribution than those prepared from Eudragit RL100 as shown in Table 27 and Figure 29. The possible explanation to this observation might be due to the more hydrophobicity of Eudragit RS100 than Eudragit RL100 that resulted in the different encapsulation.

Figures 30 and 31 show the *in vitro* drug release of the microcapsules prepared from two polymers. The result agreed with the previous studies that the drug release of microcapsules prepared with Eudragit RL100 was markedly faster than microcapsules prepared with Eudragit RS100 due to greater permeability and swellibility of Eudragit RL100 polymer to water than Eudragit RS100 (Bodmerier and Chen, 1989; Kristmundsdóttir, Gudmundsson and Ingvasdóttir 1996; Ammar and Khalil, 1997). Moreover, the main cause of the faster release of Eudragit RL100 was the much smaller particle size than those of Eudragit RS100. However, a small burst effect at the initial drug release was observed in the Eudragit RL100 microcapsules

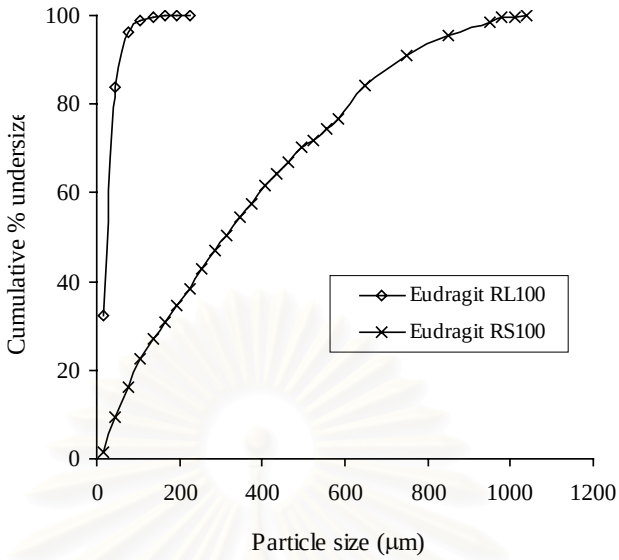


Figure 28 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 and Eudragit RS100.

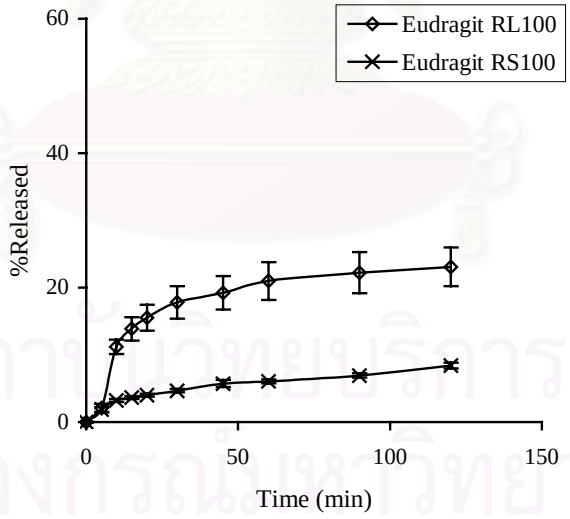


Figure 29 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated gastric fluid without pepsin pH 1.2.

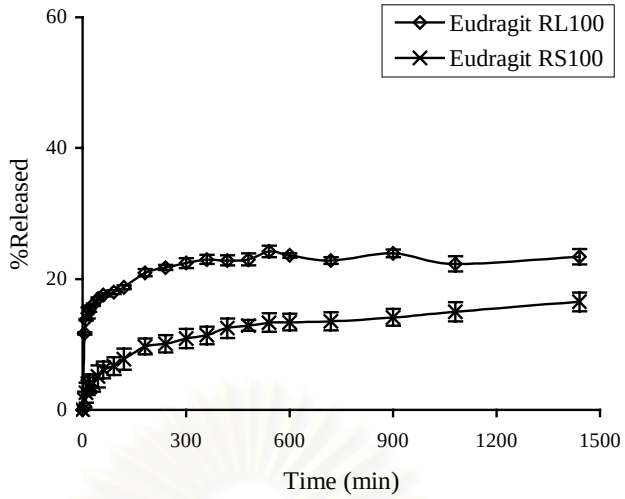
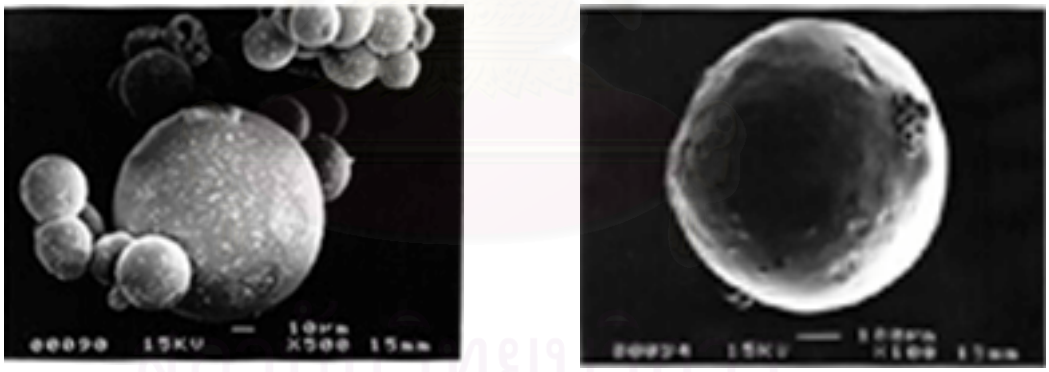


Figure 30 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

whereas it was absent in Eudragit RS100 microcapsules. This might be due to uncoated drug crystals on the surface of Eudragit RL100 microcapsules as shown in Figure 32.



a b

Figure 31 Scanning electron microphotographs of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 (a) and Eudragit RS100 (b).

The drug release rate constant of the microcapsules prepared from both polymers were obtained from Higuchi equation (Tables 28 and 29). The Higuchi rate constant of the microcapsules prepared from Eudragit RL100 was significantly

higher than that prepared from Eudragit RS100 in both media, pH 1.2 and pH 6.8 ($p < 0.05$) (Tables 21H and 22H, Appendix H).

Table 28 The release rate constants of zero-order (k_0) , first-order (k) and Higuchi plots (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100) in1:1 core to wall in pH 1.2.

Polymer	Zero-order		First -order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
ERL100	0.1337 ±0.0219	0.6375 ±0.0168	0.0016 ±0.0003	0.6716 ±0.0142	1.9857 ±0.3310	0.7860 ±0.0137
ERS100	0.0497 ±0.0040	0.9235 ±0.0055	0.0005 ±0.00004	0.9285 ±0.0058	0.6840 ±0.0534	0.9785 ±0.0010

Table 29 The release rate constants of zero-order (k_0) , first-order (k) and Higuchi plots (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100) in1:1 core to wall in pH 6.8±0.1.

Polymer	Zero-order		First-order		Higuchi-Model	
	k_0	R^2	k	R^2	k_h	R^2
ERL100	0.0173 ±0.0010	0.8146 ±0.0235	0.0002 ±0.00001	0.8268 ±0.0227	0.4958 ±0.0265	0.9344 ±0.0146
ERS100	0.0187 ±0.0010	0.8870 ±0.0277	0.0002 ±0.00001	0.8959 ±0.0277	0.5260 ±0.0260	0.9748 ±0.0146

The dissolution efficiency (DE) of microcapsules prepared with Eudragit RL100 and Eudragit RS100 is presented in Table 30. The DE values of microcapsules prepared with Eudragit RL100 was higher than those of Eudragit RS100 in pH1.2 and 6.8 ($p<0.05$) (Table 23H and 24H, Appendix H).

Table 30 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and Eudragit RS100 with1:1 core to wall.

Polymer	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
Eudragit RL100	18.72±2.44	22.32±1.33
Eudragit RS100	5.72±0.30	12.88±0.68

IX. Effect of Poloxamer188 concentration

In this study, the addition of Poloxamer188 in the preparation of microcapsules at 0, 10 and 20% of total polymer was performed. Poloxamer188 is a water-soluble polymer that has demonstrated an outstanding dissolution enhancement of nifedipine, a practically insoluble drug (Chutimaworapan et al., 2000) and appropriate controlled release of nifedipine when incorporated Poloxamer188 with Eudragit RS100 (Chutimaworapan et al., 2001). The microcapsules were prepared using preparation parameters as shown in Table 7. Both Eudragit RL100 and Eudragit RS100 were used as the polymer in the preparation.

The percentage yield , particle size and drug content are illustrated in Table 31. The microcapsules prepared with Eudragit RL100 without Poloxamer188 had high percentage yield and highest drug content. Similarly, the microcapsules prepared with Eudragit RS100 without Poloxamer188 had highest percentage yield.

Table 31 The percentage yield, mean particle size, polydispersibility index (P.I.) and the percentage andrographolide (AG) and dehydroandrographolide (DAG) contents of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100) containing varied Poloxamer188 (PXM) concentrations.

Wall	PXM (%)	Yield (%)	Size Mean ± SD (μm) [a]	P.I.	AG content Mean± SD (%)[b]	DAG content Mean ± SD (%)[b]	Core entrapment Mean ± SD (%)[b]
ERL100							
	0	99.82	42.23±23.98	0.57	22.06±1.08	2.74±0.04	88.38±3.73
	10	93.94	101.71±64.59	0.64	20.87±0.90	1.64±0.10	85.62±3.18
	20	101.36	149.06±110.17	0.74	19.76±0.08	1.08±0.34	80.24±0.20
ERS100							
	0	94.71	377.79±271.82	0.72	21.21±0.31	2.50±0.02	85.26±1.15
	10	90.53	100.38±67.16	0.67	23.53±1.23	2.36±0.11	91.76±2.83
	20	84.90	106.46±68.01	0.64	21.21±0.77	2.22±0.07	85.35±2.67

a n = 600 b n = 3

Since Poloxamer188 was a nonionic surfactant, it was added into the internal phase with the core material. It might change the solubility of the core in light liquid paraffin (external phase) during the emulsifier step, and thus the core was removed from the internal phase to the external phase. During solidification of emulsion droplets, certain portions of core were not encapsulated. Consequently, the addition of Poloxamer188 trended to decrease the yield, drug content and core entrapment of microcapsules.

The microcapsules prepared from Eudragit RL 100 with 20 % Poloxamer188 markedly had larger size and wide size distribution (Table 31, Figure 32). In contrary, the effect of the addition Poloxamer188 into the Eudragit RS100 microcapsules was slightly different from into Eudragit RL100. The particle size and size distribution of the microcapsules were improved by the addition of 10 and 20 % Poloxamer188.

The effect of Polaxamer188 on the morphology of microcapsules is depicted in Figure 34. The microcapsules with Poloxamer188 were agglomerated and rough-surfaced. The increase of Poloxamer188 concentration resulted in the increased roughness of the surfaces. The roughness might represent the surface pores of the microcapsules and thus this surface characteristic showed the influence on drug release.

From the *in vitro* release profile of the microcapsules (Figures 35-38), the microcapsules with 10 and 20 % Polxamer188 showed higher release than those without Poloxamer188. This might also be attributed to the solubilizing and wetting properties of Polxamer188.

The drug release rate constant of these microcapsules could be obtained from Higuchi equation (Tables 32-35). In the medium pH 1.2, unexpectedly, the release rate constant of Eudragit RL100 microcapsules without Poloxamer188 was significantly higher than those with 10 and 20% Poloxamer188 ($p<0.05$) (Table 4H, Appendix H). This might be due to the effect of the smaller particle size of the microcapsules without Poloxamer188. However, for Eudragit RS100 microcapsules in pH 1.2 and 6.8, the addition of Poloxamer188 significantly increased the release rate constant ($p<0.05$) (Tables 5H and 10H, Appendix H.).

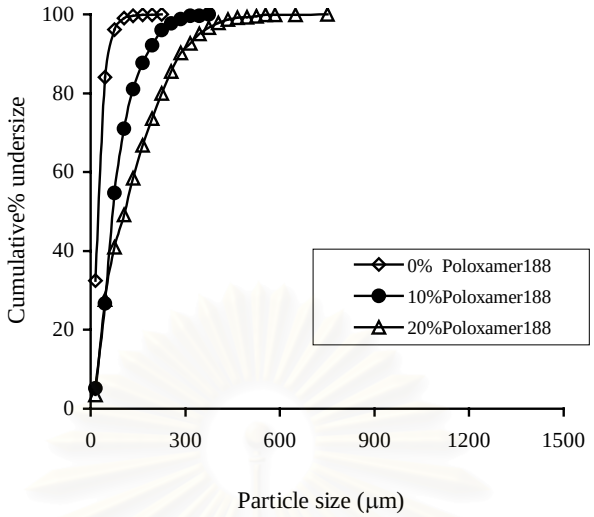


Figure 32 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 10 and 20%Poloxamer188.

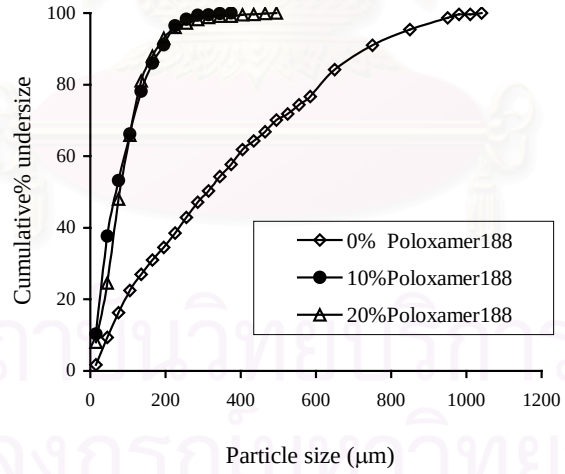
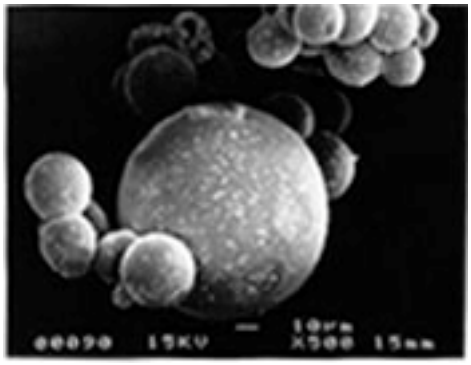
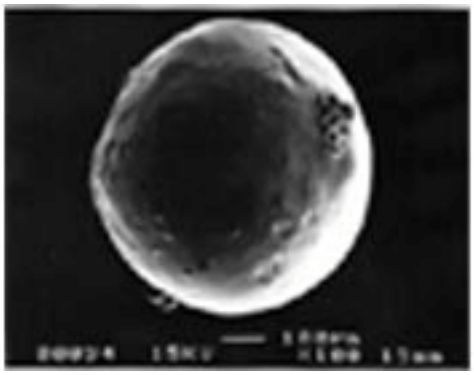


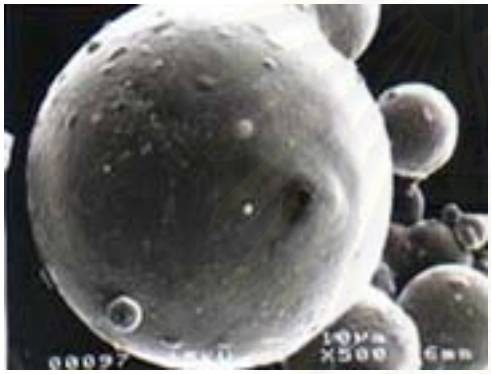
Figure 33 Cumulative % undersize of *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 with 0, 10 and 20% Poloxamer188.



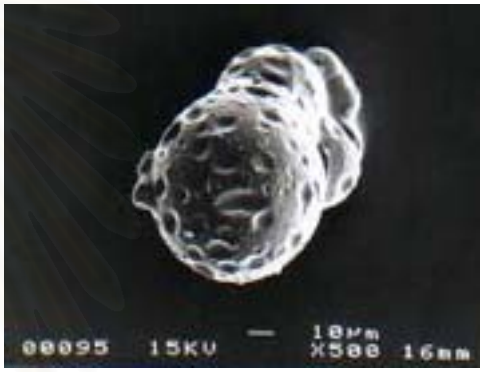
a



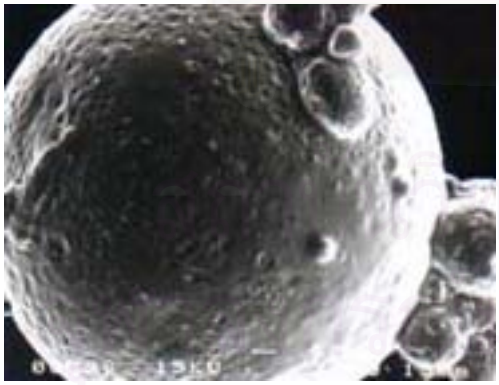
d



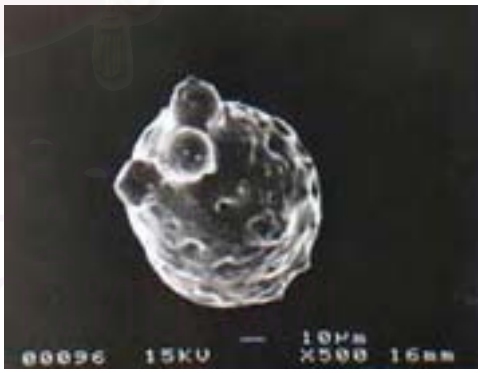
b



e



c



f

Figure 34 Scanning electron microphotographs of *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0% (a), 10% (b), 20% (c) Poloxamer188 and Eudragit RS100 with 0% (d), 10% (e), 20% (f) Poloxamer188.

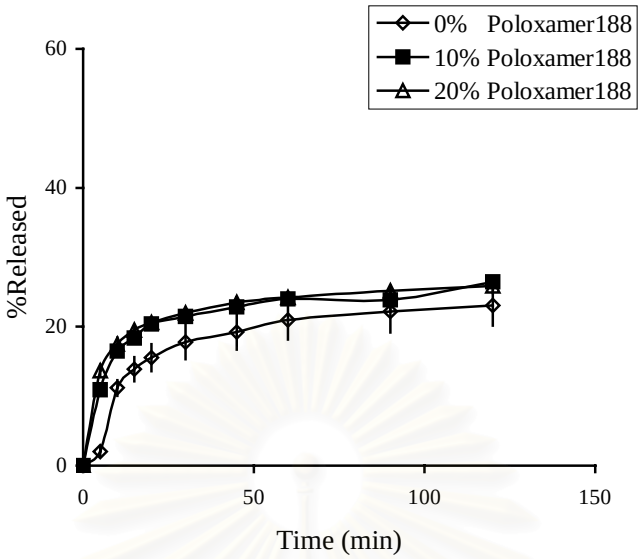


Figure 35 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 10 and 20% Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

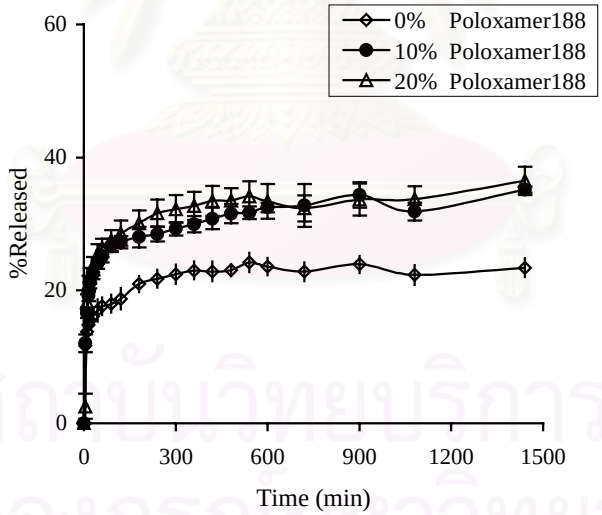


Figure 36 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0, 10 and 20% Poloxamer 188 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

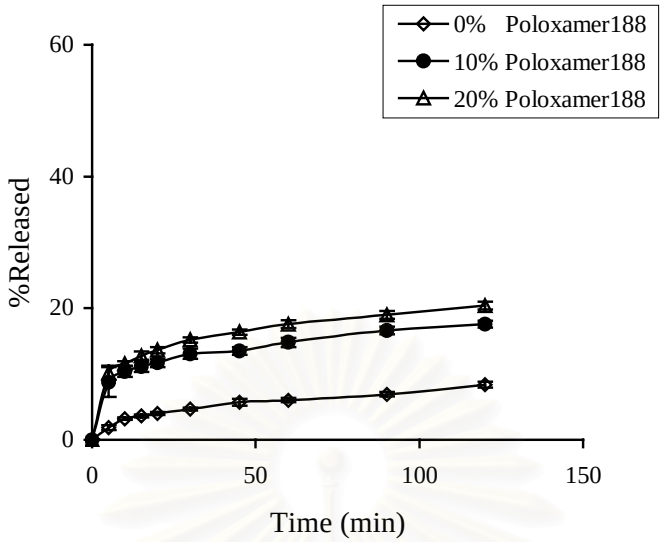


Figure 37 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 with 0, 10 and 20% Poloxamer 188 in simulated gastric fluid without pepsin pH 1.2.

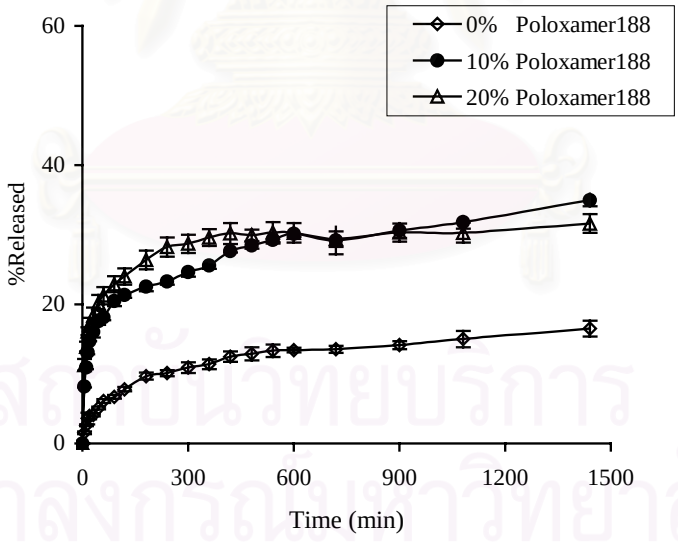


Figure 38 The release profiles of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 with 0, 10 and 20% Poloxamer 188 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

Table 32 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 and various concentrations of Poloxamer188 in pH 1.2.

Poloxamer	Zero-order		First -order		Higuchi-Model	
188	k_0	R^2	k	R^2	k_h	R^2
0%	0.1337 ±0.0219	0.6375 ±0.0168	0.0016 ±0.0003	0.6716 ±0.0142	1.9857 ±0.3310	0.7860 ±0.0137
10%	0.0987 ±0.0026	0.6737 ±0.0744	0.0012 ±0.00004	0.6978 ±0.0752	1.4473 ±0.0210	0.8094 ±0.0713
20%	0.0850 ±0.0033	0.7140 ±0.0147	0.0011 ±0.00004	0.7328 ±0.0158	1.2444 ±0.0464	0.9252 ±0.0057

Table 33 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RL100 and various concentrations of Poloxamer188 in pH 6.8±0.1.

Poloxamer	Zero-order		First -order		Higuchi-Model	
188	k_0	R^2	k	R^2	k_h	R^2
0%	0.0173 ±0.0010	0.8146 ±0.0235	0.0002 ±0.00001	0.8268 ±0.0227	0.4958 ±0.0265	0.9344 ±0.0146
10%	0.0233 ±0.0004	0.6822 ±0.0142	0.0003 ±0.00001	0.7152± 0.0138	0.6884± 0.0137	0.8268 ±0.0153
20%	0.0282 ±0.0023	0.5229 ±0.0295	0.0004 ±0.00004	0.5816± 0.0331	0.8554 ±0.0654	0.6703 ±0.0305

The dissolution efficiency (DE) of these microcapsules is illustrated in Tables 36 and 37. It was found that microcapsules prepared with both polymers with 20% Polxamer188 had significantly higher DE values than those without Poloxamer188, in pH 1.2 and 6.8 ($p<0.05$, Tables 14H, 15H and 19H, 20H Appendix H). In addition, increasing Poloxamer188 concentration increased the DE value. These result were found in both media pH 1.2 and 6.8.

Table 34 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RS100 and various concentrations of Poloxamer188 in pH 1.2.

Poloxamer	Zero-order		First -order		Higuchi-Model	
188	k_0	R^2	k	R^2	k_h	R^2
	0.0497 ±0.0040	0.9235 ±0.0055	0.0005 ±0.00004	0.9285 ±0.0058	0.6840 ±0.0534	0.9785 ±0.0010
10%	0.0708 ±0.0089	0.9147 ±0.0449	0.0008 ±0.0001	0.9228 ±0.0400	0.9771 ±0.1393	0.9700 ±0.0117
20%	0.0834 ±0.0007	0.9046 ±0.0214	0.0010 ±0.00001	0.9143 ±0.0198	1.1612 ±0.0186	0.9807 ±0.0078

Table 35 The release rate constants of zero-order (k_0), first-order (k) and Higuchi model (k_h) and the coefficient of determination (R^2) of *Andrographis paniculata* microcapsules prepared with Eudragit RS100 and various concentrations of Poloxamer188 pH 6.8±0.1.

Poloxamer	Zero-order		First -order		Higuchi-Model	
188	k_0	R^2	k	R^2	k_h	R^2
0%	0.0187 ±0.0010	0.8870 ±0.0277	0.0002 ±0.00001	0.8959 ±0.0277	0.5260 ±0.0260	0.9748 ±0.0146
10%	0.0298 ±0.0003	0.8464 ±0.0116	0.0004 ±0.00001	0.8725 ±0.0102	0.8445 ±0.0098	0.9462 ±0.0067
20%	0.0274 ±0.0012	0.7693 ±0.0323	0.0004 ±0.00002	0.7905 ±0.0316	0.7993± 0.0309	0.9112 ±0.0232

Table 36 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 in various concentrations of Poloxamer188.

Poloxamer188	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
0%	18.72±2.44	22.32±1.33
10%	22.04±0.28	31.37±1.27
20%	22.69±0.60	32.66±2.03

Table 37 Dissolution efficiency of *Andrographis paniculata* extract microcapsules prepared with Eudragit RS100 in various concentrations of Poloxamer188.

Poloxamer188	$DE_{0\text{-}2hr}$ (pH 1.2)	$DE_{0\text{-}24hr}$ (pH 6.8±0.1)
0%	5.72±0.30	12.88±0.68
10%	14.17±0.65	28.52±0.79
20%	16.52±0.53	29.01±1.26

CHAPTER V
CONCLUSIONS

The present study was to develop sustained release microcapsules of *Andrographis paniculata* extract using nonaqueous or oil in oil solvent evaporation technique. The factors influencing on microencapsulation, i.e. the stirring rate, emulsifier (Span80) concentration, core to wall ratio, type of polymer and addition of the solubility enhancer (Poloxamer188) were investigated. The physicochemical properties of the microcapsules were evaluated including percentage yield, size distribution, drug content, core entrapment and the drug release properties.

The results of the investigation are concluded as follows:

1. *Andrographis paniculata* extract could be prepared by extraction, yielding as andrographolide crystals (81.32% andrographolide) and crude extract containing andrographolide (2.62%) and dehydroandrographolide (13.04%). The 1:1 mixture of andrographolide crystals and crude extract was used as the core material in the microencapsulation process.

2. Due to the insolubility property to water of Eudragit polymer, the release of andrographolide from the microcapsules was suppressed and much lower than that of the intact core material. Thus the consideration for the relatively high release rate constant of the microcapsules was applied.

3. The higher stirring rate used in the microencapsulation process resulted in the smaller microcapsule size, and consequently, the faster andrographolide release. The high stirring rate increased the evaporation rate that cause andrographolide crystallization and adhered on the microcapsule surface. This result could be related to the burst effect observed in the drug release. The drug release rate constant of the microcapsule prepared at 1000 rpm was significantly higher than other stirring rates at pH 6.8. In addition, the microcapsules prepared at 1000 rpm had highest drug content and core entrapment. The appropriate stirring rate obtained in this study was 1000 rpm.

4 The increase of Span80 concentration from 0 to 2.0% gave the larger particle size. The 0.5% Span80 was the appropriate concentration since it resulted in small microparticle size and highest core entrapment. The release rate constant and dissolution efficiency at pH 1.2 of the microcapsules with 0.5% Span80 was significantly highest. However, at pH 6.8, there was no significant difference in release rate constant and dissolution efficiency.

5. The core to wall ratio dramatically effected the particle size, size distribution of microcapsules and microcapsule morphology. The 2:3 core to wall ratio gave the biggest size, wide size distribution and porous surface. At pH 6.8, the release rate constant and dissolution efficiency of the microcapsules of 1:3 core to wall ratio was significantly higher than other core to wall ratios.

6. Microcapsules prepared from Eudragit RL100 had higher yield, smaller particle size, higher drug contents and higher core entrapment than those from Eudragit RS100. At pH 1.2 and 6.8, Eudragit RL100 microcapsules gave significantly high release rate constants and dissolution efficiency values.

7. The addition of Poloxamer188 at 10 and 20% in Eudragit RL100 microcapsules increased the microcapsule size due to agglomeration of particles and decreased the core entrapment. At pH 1.2, the Eudragit RL100 microcapsules with 20% Polxamer188 had the lowest release rate constant that might be due to the larger particle size. However, the different effect was observed in Eudragit RS100 microcapsules. The presence of Poloxamer188 at 20% significantly increased the release rate constants and dissolution efficiency value.

8. The release kinetics of andrographolide from microcapsules was demonstrated to follow Higuchi-Model, diffusion-controlled mechanism.

9. Since the results obtained in this study were based on one batch of the production of each microcapsule formulation and the factorial design was not applied, the validation of the microencapsulation process with processing parameter obtained from the study might be needed. Moreover, further *in vivo* study of the product should be carried out.

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APPENDICES

APPENDIX A
DETAILS OF EUDRAGIT AND POLOXAMER

POLYMETHACRYLATES

Nonproprietary Names:

Ammonio methacrylate copolymer, Methacrylic acid copolymer

Synonyms:

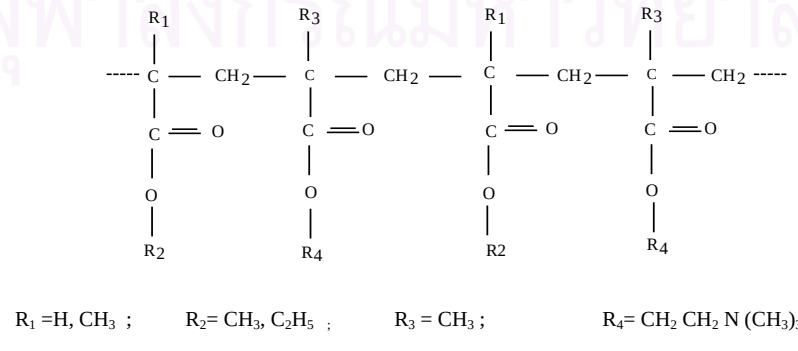
Eudragit, polymeric methacrylates.

Description:

Polymethacrylates are primarily used in oral capsule and tablet formulation as film coating agent. Depending on the type of polymer used, Eudragit RL100 and Eudragit RS100 are used to form water insoluble films coats for sustained release products.

Polymethacrylates are synthetic cationic and anionic polymers of dimethylaminoethylmethacrylate, methacrylic acid and methacrylic acid ester in varying ratio. Eudragit RL100 and Eudragit RS100 are copolymers synthesized from acrylic acid and methacrylic acid ester with Eudragit RL100 (type A) having 10 % of functional quaternary ammonium groups and Eudragit RS100 (type B) having 5 % of functional quaternary ammonium groups. The ammonium group are present as salts and give rise to pH-independent permeability of the polymers. Both polymers are water–insoluble and film prepared from Eudragit RL100 are freely permeable to water, whereas, films prepared from Eudragit RS100 are only slightly permeable to water.

The structure formula are shown in Figure 1A



R_1 =H, CH_3 ; R_2= CH_3, C_2H_5 ; R_3 = CH_3 ; R_4= CH_2 CH_2 N $(CH_3)_3$ $^+Cl^-$

Figure 1A Eudragit structural formula.

Dry powder of polymer forms are stable at temperature less than 30°C. Above this temperature , powder trends to form clumps although this does not affect the quality of the substance and the clumps can be readily broken up. Dry powder are stable for at least two years if store in tightly closed container at less than 30°C.

Polymethacrylate copolymer are widely used as film coating materials in oral pharmaceutical formulations. They are also used to a lesser extent in topical formulations and are generally regarded as nontoxic and nonirritant materials (Wade and Weller, 1994a).

Table 1A Summary of properties of Eudragit RL100 and Eudragit RS100.

Chemical name	MW	Type	Behavior in digestive juices	Soluble in
Poly (ethylcrylate,methyl methacrylate, trimethyl ammonioethyl methacrylate, trimethylammonioethyl methacrylate chloride)1:2:0.2	150000	Eudragit RL	Insoluble films of high permeability	Acetone, alcohol, dichloromethan, solvent ethylecetate
Poly (ethylcrylate,methyl methacrylate, trimethyl ammonioethyl methacrylate, trimethylammonioethyl methacrylate chloride)1:2:0.1	150000	Eudragit RS	Insoluble films of low permeability	Acetone, alcohol, dichloromethan, solvent ethylecetate

POLOXAMERS

The poloxamer polyols are a series of closely related block copolymer of ethylene oxide and propylene oxide conforming to the general formula

$$OH(C_2H_4O)_a\ (C_3H_6O)_b(C_2H_4O)_aH$$

Table 2A The general properties of Poloxamer.

Poloxamer	Physical form	a	b	Average MW
188	solid	80	27	7680-9516
237	solid	64	37	6840-8830
338	solid	141	44	12700-17400
407	solid	101	56	9840-14600

-Poloxamer are nonionic polyoxyethylene-polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agent.

-The polyoxyethylene segment is hydrophilic, polyoxypropylene segment is hydrophobic.

-Generally occur as white-colored, waxy, freeflowing granules.

-Melting point 52°C for poloxamer188, 56°C for poloxamer 407.

-Freely soluble in ethanol 95% and water.

-Poloxamer188 is administered orally as a wetting agent and stool lubricant in the treatment of constipation.

-Poloxamer may also used therapeutically as a wetting agent in eye drop formulation, in the treatment of kidney stones and as wound cleansers.

-$LD50_{(rat,oral)}$ of poloxamer188 is 9.4g/kg (Wade and Weller, 1994b).

APPENDIX B

DETERMINATION *ANDROGRAPHIS PANICULATA* NEES MICROCAPSULES BY HPLC

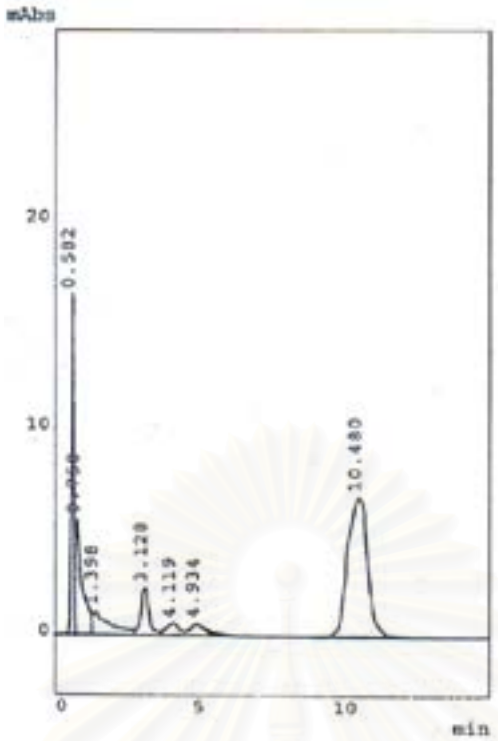


Figure 1B Chromatograms of crude extract of *Andrographis paniculata* assayed by HPLC method

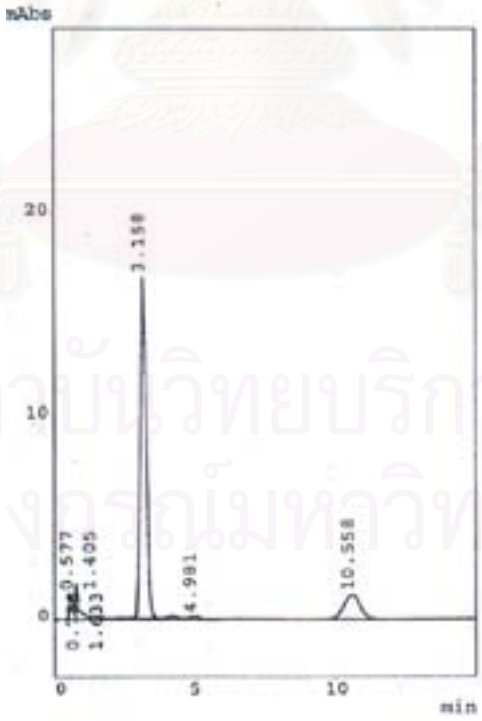


Figure 2B Chromatograms of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and 0.5% Span80 in 1:2 core to wall at 1000 rpm assayed by HPLC method.

Table 1B Calibration curve data of andrographolide assayed by HPLC method.

Standard solution no.	Concentration (μg/mL)	Peak area (x 10^{-4})
1	0	0
2	16.20	5.591
3	32.40	11.463
4	48.60	17.093
5	81.00	28.440
6	121.50	43.790
7	162.00	57.364

Table 2B Calibration curve data of dehydroandrographolide assayed by HPLC method.

Standard solution no.	Concentration (μg/mL)	Peak area (x 10^{-4})
1	0	0
2	8.08	4.448
3	16.16	9.220
4	32.32	18.375
5	48.48	27.601
6	80.80	46.208
7	121.20	69.963

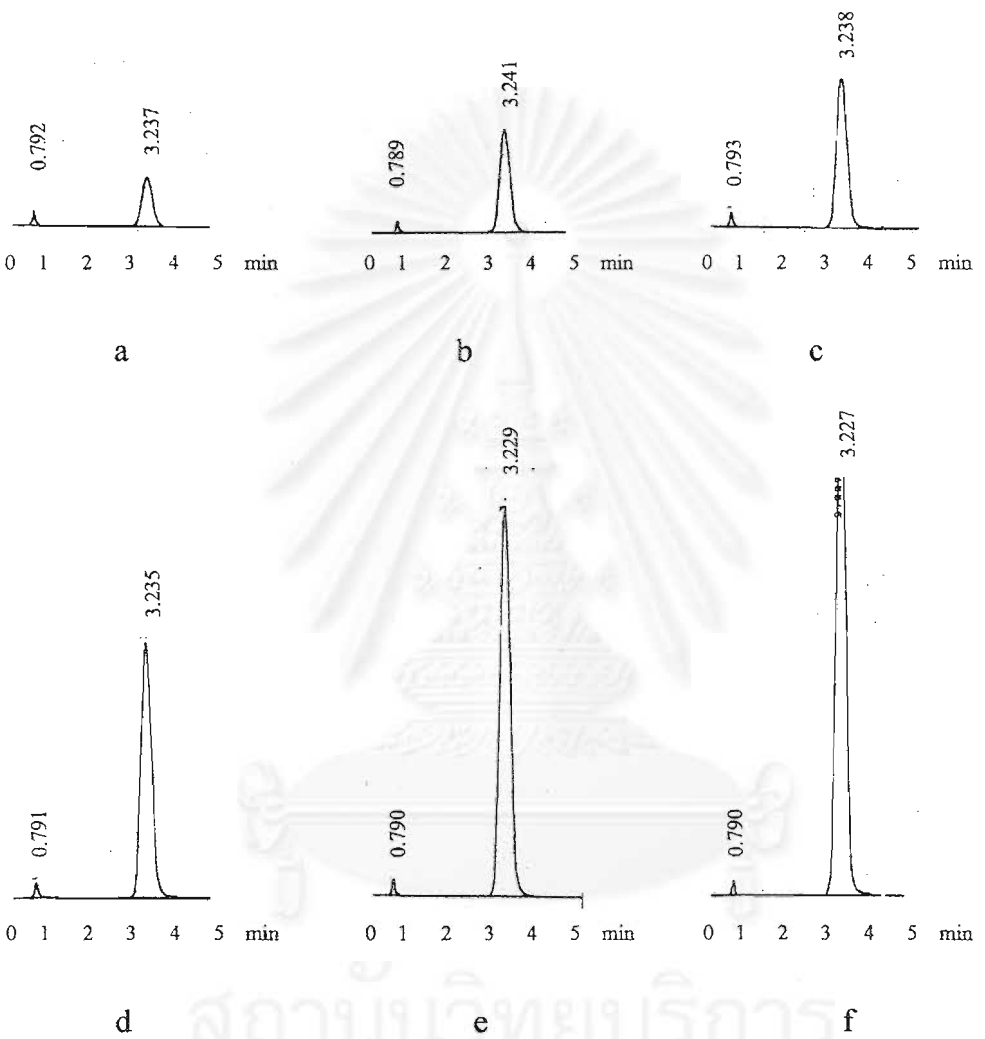


Figure 3B Chromatograms of standard solution of andrographolide at the concentrations of 16.20 (a), 32.40 (b), 48.60 (c), 81.00 (d), 121.50 (e), 162.00 (f)μg/mL, respectively (retention time = 3.2) assayed by HPLC method.

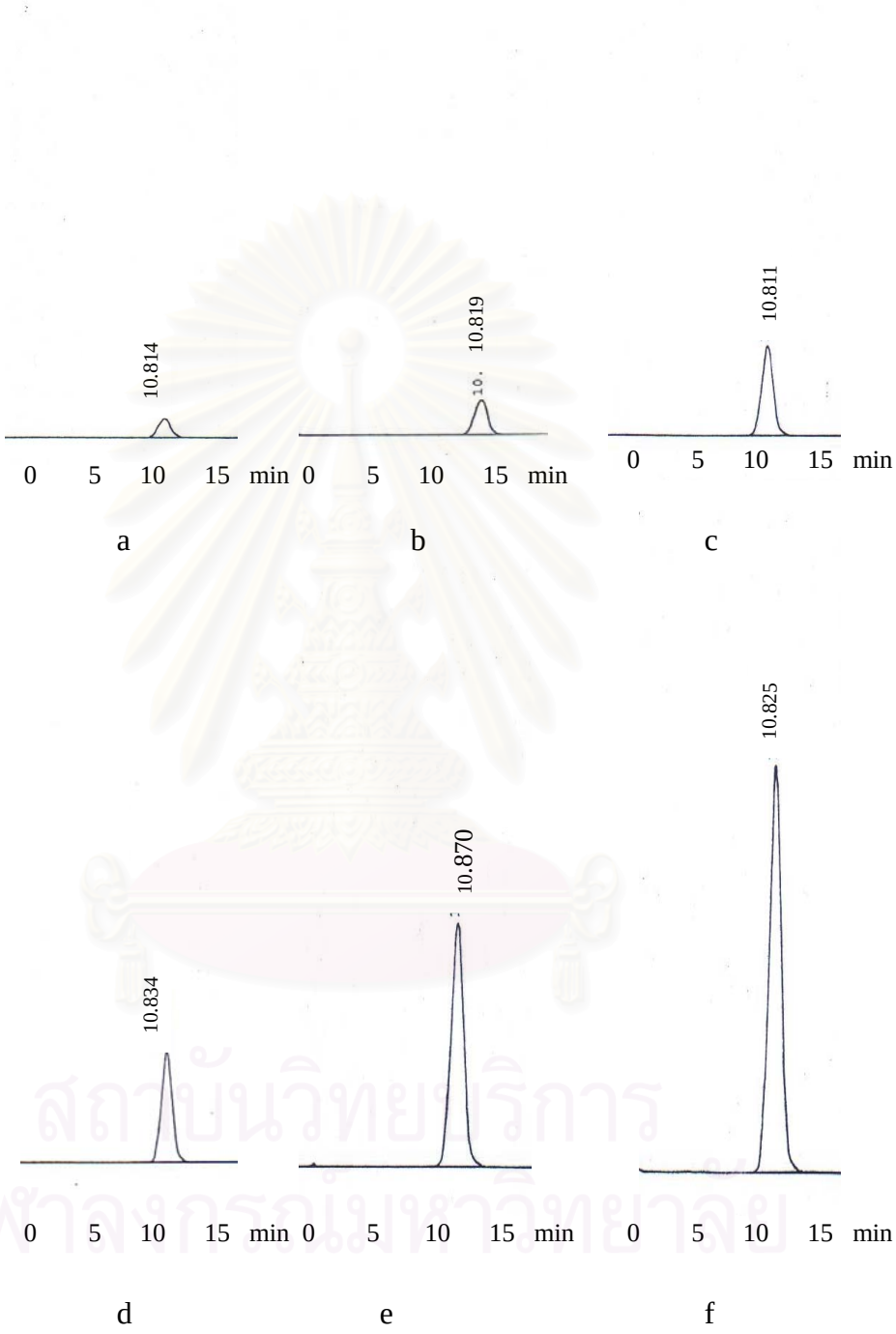


Figure 4B Chromatograms of standard solution of dehydroandrographolide at the concentrations of 8.08 (a), 16.16 (b), 32.32 (c), 48.48 (d), 80.80 (e), 121.20 (f) μg/mL, respectively (retention time = 10.8) assayed by HPLC method.

APPENDIX C
ANALYSIS OF ANDROGRAPHOLIDE FROM DRUG RELEASE BY UV/VISIBLE SPECTROPHOTOMETRIC METHOD

Analysis of andrographolide from drug release by UV/Visible spectrophotometric method

In the dissolution study of *Andrographis paniculata* extract microcapsules, two dissolution media were performed, i.e., simulated gastric fluid without pepsin pH 1.2 and simulated intestinal fluid without pancreatin pH 6.8±0.1. The sample from the dissolution study was assayed for the andrographolide dissolved by UV/Visible spectrophotometer at the wavelength 223 nm which is the maximum absorption in both media (Figures 1C and 2C). The calibration curve was plotted between andrographolide concentration in μg/mL and absorbance at 223 nm.

Both calibration curves of andrographolide in the gastric fluid pH 1.2 and the intestonal fluid pH 6.8, the straight line from the linear regression was obtained with the coefficient of determination (R^2).

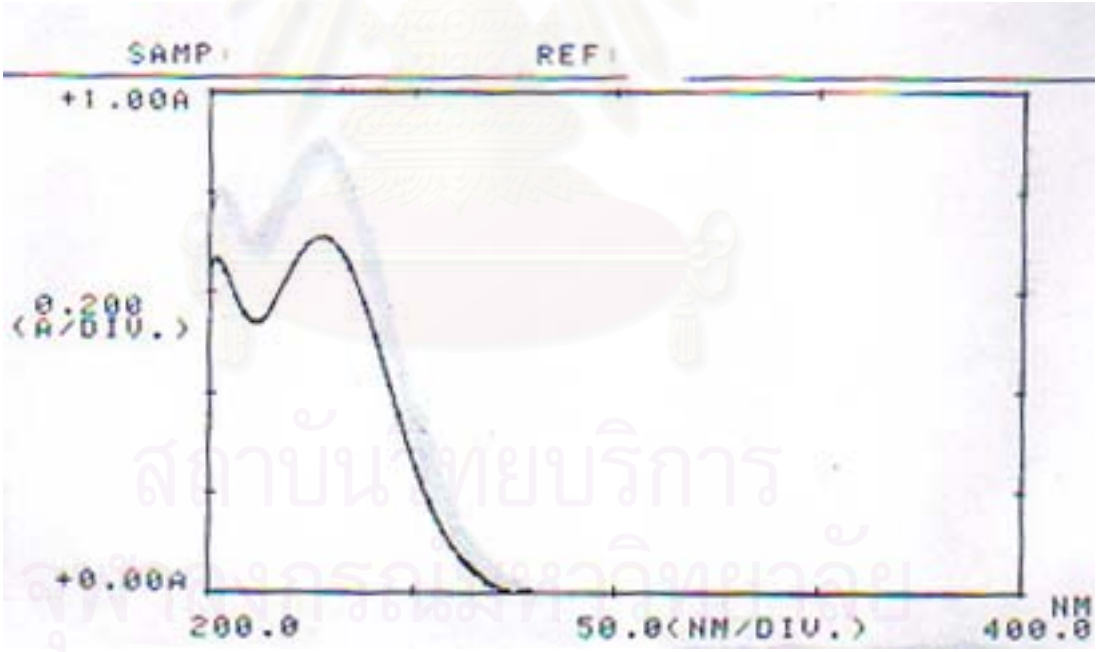


Figure 1C Spectrum of andrographolide standard solution in simulated gastric fluid without pepsin pH 1.2 assayed by UV/Visible spectrophotometer.

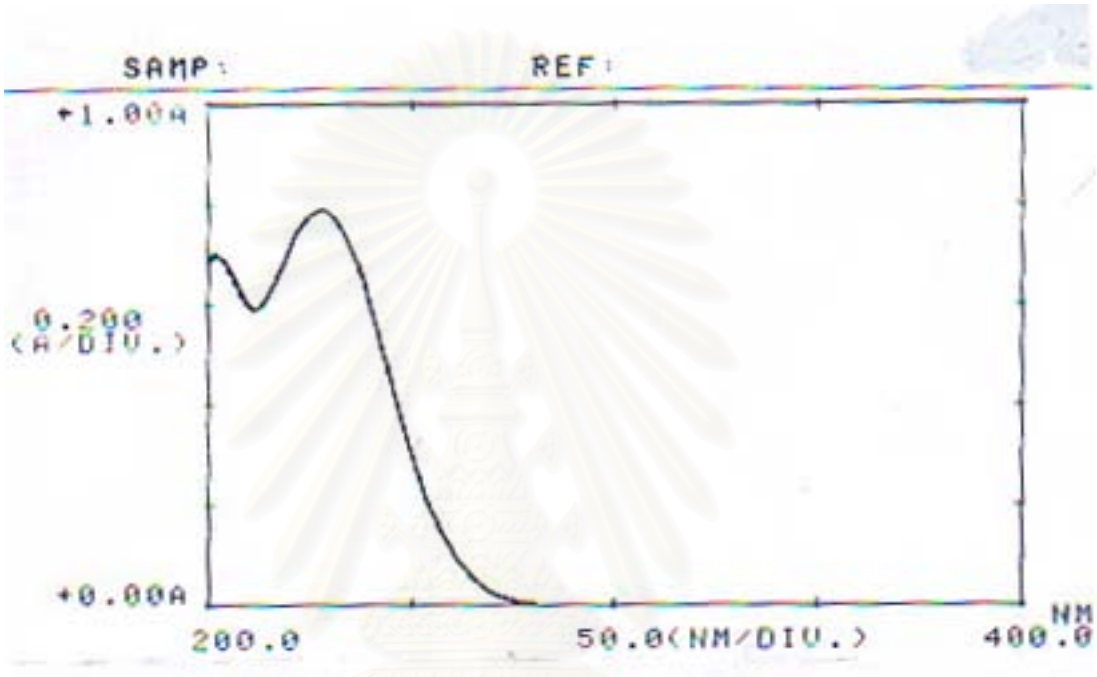


Figure 2C Spectrum of andrographolide standard solution in simulated intestinal fluid without pancreatin pH 6.8 assayed by UV/Visible spectrophotometer.

Table 1C Calibration curve data of andrographolide in simulated gastric fluid without pepsin pH 1.2 assayed by UV/Visible spectrophotometer.

Standard solution no.	Concentration (μg/mL)	Absorbance at 223 nm
1	0	0
2	4.06	0.133
3	8.12	0.276
4	10.15	0.360
5	12.18	0.424
6	16.24	0.583
7	20.30	0.709

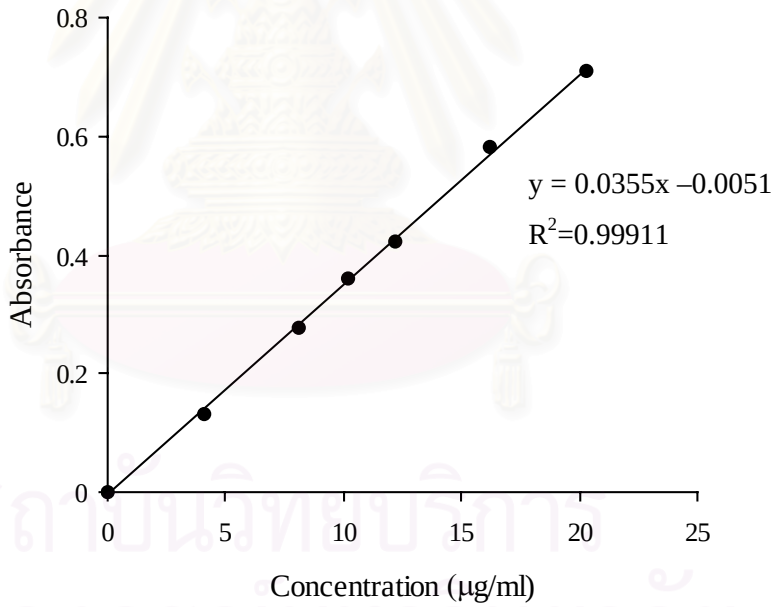


Figure 3C The calibration curve of andrographolide standard in simulated gastric fluid without pepsin assayed by UV/Visible spectrophotometer.

Table 2C Calibration curve data of andrographolide in simulated intestinal fluid without pancreatin pH 6.8±0.1 assayed by UV/Visible spectrophotometer.

Standard solution no.	Concentration (μg/mL)	Absorbance at 223 nm
1	0	0
2	4.04	0.137
3	8.08	0.276
4	10.10	0.362
5	12.12	0.421
6	16.16	0.560
7	20.20	0.701

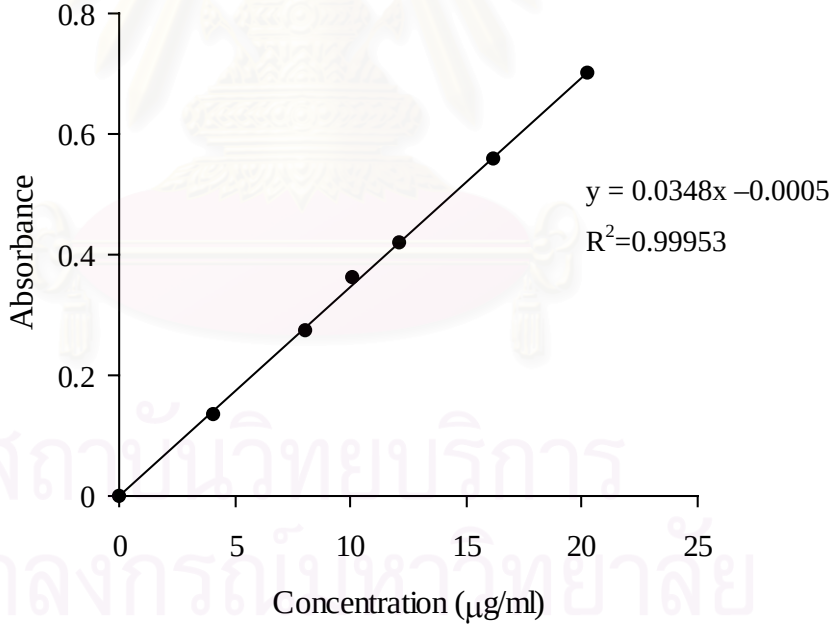


Figure 4C The calibration curve of andrographolide standard in simulated intestinal fluid without pancreatin assayed by UV/Visible spectrophotometer.

APPENDIX D
DRUG CONTENTS OF MICROCAPSULES

Table 1D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 at various stirring rates.

rpm	core		wall	wt.theo.	wt.prod.	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)	ERL100 (g)	(g)	(g)							
250	2.507	2.515	10.119	15.141	14.392	95.05	11.67	10.71	12.65	1.88	1.79	1.71
500	2.542	2.503	10.127	15.173	15.065	99.29	10.96	11.74	9.41	2.01	1.90	1.88
800	2.540	2.522	10.197	15.259	14.843	97.28	12.70	11.39	11.86	1.84	1.76	1.83
1000	2.541	2.575	10.102	15.218	14.972	98.38	12.28	14.09	14.01	1.91	1.79	1.73
1200	2.553	2.560	10.149	15.262	15.196	99.57	12.97	12.47	11.10	1.69	1.65	1.77

Table 2D The amount of andrographolide (AG) and dehydroandrographolide (DAG) in microcapsules prepared with Eudragit RL100 at various stirring rates.

rpm	theo total AG+ DAG	AG in product 1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG) 1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
250	2.44	1.68	1.54	1.82	0.27	0.26	0.25	1.95	1.80	2.07
500	2.43	1.65	1.77	1.42	0.30	0.29	0.28	1.95	2.06	1.70
800	2.45	1.88	1.69	1.76	0.27	0.26	0.27	2.16	1.95	2.03
1000	2.49	1.84	2.11	2.10	0.29	0.27	0.26	2.12	2.38	2.36
1200	2.48	1.97	1.89	1.69	0.26	0.25	0.27	2.23	2.15	1.96

Table 3D The percentage core entrapment in microcapsules prepared with Eudragit RL100 at various stirring rates.

rpm	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
250	33.166	27.923	25.748	29.586	84.192	77.635	89.205	83.68	5.80
500	33.253	26.895	28.291	23.419	80.879	85.079	70.426	78.79	7.55
800	33.173	30.059	27.188	28.309	90.612	81.958	85.337	85.97	4.36
1000	33.616	29.122	32.595	32.315	86.632	96.964	96.129	93.24	5.74
1200	33.500	30.217	29.093	26.518	90.200	86.846	79.160	85.40	5.66

Table 4D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 and various concentrations of Span 80.

Span 80 (%)	core		wall	wt.theo.	wt.prod.	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)	ERL100 (g)	(g)	(g)							
0	2.560	2.509	10.065	15.134	14.443	95.43	13.57	12.51	14.83	1.99	1.93	1.94
0.5	2.528	2.500	10.021	15.049	13.943	92.65	14.57	15.88	16.02	1.88	1.79	1.86
1.0	2.541	2.575	10.102	15.218	14.972	98.39	12.28	14.09	14.01	1.91	1.79	1.73
1.5	2.502	2.534	10.079	15.115	14.890	98.51	11.75	13.56	13.44	1.73	1.85	1.81
2.0	2.518	2.515	10.008	15.041	15.293	101.68	12.92	16.11	13.74	1.21	1.60	1.16

Table 5D The amount andrographolide (AG) and dehydroandrographolide (DAG) in microcapsules prepared with Eudragit RL100 and various concentrations of Span 80.

Span 80 (%)	theo total AG+ DAG	AG in product 1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG) 1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
0	2.44	1.96	1.81	2.14	0.29	0.28	0.28	2.25	2.08	2.42
0.5	2.43	2.03	2.21	2.23	0.26	0.25	0.26	2.29	2.46	2.49
1.0	2.49	1.84	2.11	2.10	0.29	0.27	0.26	2.12	2.38	2.36
1.5	2.45	1.75	2.02	2.00	0.26	0.28	0.27	2.01	2.30	2.27
2.0	2.44	1.98	2.46	2.10	0.18	0.25	0.18	2.16	2.71	2.28

Table 6D The percentage core entrapment in microcapsules prepared with Eudragit RL100 and various concentrations of Span 80.

Span 80 (%)	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
0	33.49	32.32	29.98	34.82	96.49	89.49	103.95	96.64	7.23
0.5	33.41	34.05	36.57	37.01	101.91	109.45	110.78	107.38	4.79
1.0	33.62	29.12	32.60	32.31	86.63	96.96	96.13	93.24	5.74
1.5	33.32	27.66	31.65	31.31	83.03	95.00	93.98	90.67	6.64
2.0	33.46	29.14	36.53	30.75	87.08	109.17	91.88	96.04	11.62

Table 7D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared at different core to wall ratios

Ratio	core		wall	wt.theo.	wt.prod.	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)	ERL100 (g)	(g)	(g)							
1: 1	2.599	2.514	5.032	10.145	10.127	99.823	21.80	21.12	23.24	2.73	2.79	2.71
2 : 3	5.070	5.011	15.140	25.221	23.211	92.031	16.43	16.39	16.75	2.22	2.57	2.35
1: 2	2.509	2.511	10.057	15.077	13.288	88.137	15.39	15.65	17.09	1.93	1.85	1.93
1: 3	2.532	2.513	15.027	20.072	16.656	82.981	11.21	11.25	11.82	1.58	1.58	1.59

Table 8D The amount andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared at different core to wall ratios.

Ratio	theo total AG+ DAG	AG in product 1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG) 1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
1: 1	2.85	2.21	2.14	2.35	0.28	0.28	0.27	2.48	2.42	2.63
2 : 3	5.66	3.81	3.80	3.89	0.52	0.60	0.55	4.33	4.40	4.43
1: 2	2.83	2.05	2.08	2.27	0.26	0.25	0.26	2.30	2.33	2.53
1: 3	2.84	1.87	1.87	1.97	0.26	0.26	0.27	2.13	2.14	2.24

Table 9D The percentage core entrapment in microcapsules prepared at different core to wall ratios.

Ratio	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
1: 1	50.403	44.073	42.949	46.615	87.440	85.211	92.483	88.38	3.73
2 : 3	39.973	33.250	33.799	34.054	83.181	84.554	85.194	84.31	1.03
1: 2	33.298	30.736	31.053	33.731	92.306	93.257	101.298	95.62	4.94
1: 3	25.136	22.764	22.828	23.882	90.566	90.821	95.013	92.13	2.50

Table 10D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100).

Polymer	core		Wt.wall	wt.theo.	wt.prod.	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)	(g)	(g)	(g)							
ERL100	2.599	2.514	5.032	10.145	10.127	99.823	21.80	21.12	23.24	2.73	2.79	2.71
ERS100	2.510	2.514	5.160	10.184	9.648	94.741	21.54	21.17	20.92	2.52	2.48	2.51

Table 11D The amount andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100).

Polymer	theo total AG+DAG	AG in product1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG)1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
ERL100	2.85	2.21	2.14	2.35	0.28	0.28	0.27	2.48	2.42	2.63
ERS100	2.83	2.08	2.04	2.02	0.24	0.24	0.24	2.32	2.28	2.26

Table 12D The percentage core entrapment in microcapsules prepared with Eudragit RL100 (ERL100) and Eudragit RS100 (ERS100).

Polymer	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
ER L100	50.403	44.073	42.949	46.615	87.440	85.211	92.483	88.38	3.73
ERS100	49.33	42.68	41.95	41.56	86.51	85.03	84.24	85.26	1.15

Table 13D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 with various Poloxamer188 (PXM) concentrations.

PXM (%)	core		wall	wt.theo.	wt.prod.	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)	ERL100 (g)	(g)	(g)							
0	2.599	2.514	5.032	10.145	10.127	99.823	21.80	21.12	23.24	2.73	2.79	2.71
10	2.568	2.531	5.464	10.563	9.923	93.943	21.11	21.63	19.87	2.28	2.26	2.34
20	2.558	2.509	5.317	10.384	10.525	101.359	19.77	19.82	19.67	2.15	2.13	2.17

Table 14D The amount of andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RL100 with various Poloxamer188 (PXM) concentrations.

PXM (%)	theo total AG+ DAG	AG in product 1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG) 1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
0	2.85	2.21	2.14	2.35	0.28	0.28	0.27	2.48	2.42	2.63
10	2.86	2.09	2.15	1.97	0.23	0.22	0.23	2.32	2.37	2.20
20	2.84	2.08	2.09	2.07	0.23	0.22	0.23	2.31	2.31	2.30

Table 15D The percentage core entrapment in microcapsules prepared with Eudragit RL100 with various Poloxamer188 concentrations.

Poloxamer (%)	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
0	50.403	44.073	42.949	46.615	87.440	85.211	92.483	88.38	3.73
10	48.273	41.732	42.630	39.637	86.449	88.311	82.110	85.62	3.18
20	48.799	39.179	39.240	39.045	80.286	80.411	80.012	80.24	0.20

Table 16D The percentage andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RS100 with various Poloxamer188 (PXM) concentrations.

PXM (%)	core		wall ERS100 (g)	wt.theo. (g)	wt.prod. (g)	% yield	%AG content 1	%AG. content2	%AG content3	%DAG content1	%DAG content2	%DAG content3
	cru.pow. (g)	crystal (g)										
0	2.510	2.514	5.160	10.184	9.648	94.741	21.54	21.17	20.92	2.52	2.48	2.51
10	2.518	2.516	5.196	10.230	9.261	90.528	23.88	23.26	22.16	2.33	2.19	2.48
20	2.501	2.526	5.327	10.353	8.790	84.901	21.59	21.70	20.32	2.15	2.26	2.27

Table 17D The amount of andrographolide (AG) and dehydroandrographolide (DAG) contents in microcapsules prepared with Eudragit RS100 with various Poloxamer188 (PXM) concentrations.

PXM (%)	theo total AG+ DAG	AG in product 1 (g)	AG in product 2 (g)	AG in product 3 (g)	DAG in product 1 (g)	DAG in product 2 (g)	DAG in product 3 (g)	Total (AG+DAG) 1 (g)	Total (AG+DAG)2 (g)	Total (AG+DAG)3 (g)
0	2.83	2.08	2.04	2.02	0.24	0.24	0.24	2.32	2.28	2.26
10	2.84	2.21	2.15	2.05	0.22	0.20	0.23	2.43	2.36	2.28
20	2.84	1.90	1.91	1.79	0.19	0.20	0.20	2.09	2.11	1.99

Table 18D The percentage core entrapment in microcapsules prepared with Eudragit RS100 with various Poloxamer188 concentrations.

Poloxamer (%)	% theore. crud in microcap	%obser. crud in in microcap 1	%obser. crud in in microcap 2	%obser. crud in in microcap 3	% core entrap1	% core entrap2	% core entrap3	mean %core	SD
0	49.33	42.68	41.95	41.56	86.51	85.03	84.24	85.26	1.15
10	49.21	46.53	45.19	43.74	94.56	91.82	88.89	91.76	2.83
20	48.55	41.99	42.37	39.96	86.49	87.27	82.30	85.35	2.6

APPENDIX E
CUMULATIVE PERCENTAGE UNDERSIZE OF MICROCAPSULES

Table 1E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules prepared by solvent evaporation at stirring rate 250, 500, 800, 1000 and 1200 rpm.

Size interval (μm)	Mid size (μm)	Stirring rate (rpm).				
		250	500	800	1000	1200
0-30	15	0.00	10.33	12.83	10.83	22.17
30-60	45	4.33	42.50	52.67	56.50	69.33
60-90	75	17.33	64.50	76.83	78.00	88.50
90-120	105	30.67	76.50	87.00	92.67	95.17
120-150	135	44.17	81.50	94.00	97.83	98.17
150-180	165	58.33	85.83	96.50	98.67	99.67
180-210	195	69.50	90.17	98.83	99.83	99.83
210-240	225	76.17	92.50	99.83	99.83	100.00
240-270	255	80.17	95.33	100.00	100.00	100.00
270-300	285	83.83	97.00	100.00	100.00	100.00
300-330	315	87.17	98.67	100.00	100.00	100.00
330-360	345	89.67	99.00	100.00	100.00	100.00
360-390	375	92.17	99.67	100.00	100.00	100.00
390-420	405	93.83	99.83	100.00	100.00	100.00
420-450	435	95.17	100.00	100.00	100.00	100.00
450-480	465	96.17	100.00	100.00	100.00	100.00
480-510	495	97.00	100.00	100.00	100.00	100.00
510-540	525	97.67	100.00	100.00	100.00	100.00
540-570	555	98.33	100.00	100.00	100.00	100.00
570-600	585	99.00	100.00	100.00	100.00	100.00
600-630	615	99.33	100.00	100.00	100.00	100.00
630-660	645	99.83	100.00	100.00	100.00	100.00
660-1000	830	100.00	100.00	100.00	100.00	100.00
Mean		192.16	95.67	69.88	65.38	53.42
SD		121.05	76.51	43.05	34.83	32.439
Polydispersibility index		0.63	0.80	0.62	0.53	0.61

Table 2E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and varied Span80 concentrations.

Size interval (μm)	Mid size (μm)	Span 80 concentration(%)				
		0	0.5	1	1.5	2
0-30	15	26.67	20.83	10.83	17.67	18.67
30-60	45	84.50	79.17	56.50	57.50	57.33
60-90	75	96.50	95.50	78.00	87.83	85.00
90-120	105	99.50	98.83	92.67	97.67	93.67
120-150	135	100.00	99.67	97.83	99.50	97.50
150-180	165	100.00	100.00	98.67	99.83	99.00
180-210	195	100.00	100.00	99.83	99.83	99.50
210-240	225	100.00	100.00	99.83	99.83	99.67
240-270	255	100.00	100.00	100.00	100.00	100.00
Mean		42.42	46.37	65.39	56.58	60.13
SD		20.25	21.63	34.86	29.17	35.72
Polydispersibility index		0.48	0.47	0.53	0.52	0.59

Table 3E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules in 1:1, 2:3, 1:2 and 1:3 core to wall prepared by solvent evaporation.

Size interval (μm)	Mid size (μm)	Core to wall ratio			
		1:1	2:3	1:2	1:3
0-30	15	32.50	0.33	26.83	4.00
30-60	45	84.00	4.33	67.50	17.33
60-90	75	96.17	15.17	88.00	40.17
90-120	105	99.00	25.67	94.33	58.83
120-150	135	99.67	34.50	98.00	71.83
150-180	165	99.83	41.67	99.17	83.83
180-210	195	99.83	45.50	100.00	89.33
210-240	225	100.00	49.83	100.00	93.17
240-270	255	100.00	53.17	100.00	95.50
270-300	285	100.00	56.67	100.00	98.00
300-330	315	100.00	58.33	100.00	98.83
330-360	345	100.00	60.17	100.00	99.17
360-390	375	100.00	61.67	100.00	99.50
390-420	405	100.00	63.17	100.00	99.67
420-450	435	100.00	64.17	100.00	100.00
450-480	465	100.00	66.33	100.00	100.00
480-510	495	100.00	68.33	100.00	100.00
510-540	525	100.00	69.50	100.00	100.00
540-570	555	100.00	71.00	100.00	100.00
570-600	585	100.00	72.33	100.00	100.00
600-700	650	100.00	79.00	100.00	100.00
700-800	750	100.00	85.17	100.00	100.00
800-900	850	100.00	91.33	100.00	100.00
900-1000	950	100.00	96.83	100.00	100.00
1000-1200	1100	100.00	99.50	100.00	100.00
1200-1400	1300	100.00	100.00	100.00	100.00
Mean		42.23	381.32	53.07	120.06
SD		23.98	316.06	33.41	69.89
Polydispersibility index		0.57	0.83	0.63	0.58

Table 4E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules with Eudragit RS100 and Eudragit RL100.

Size interval (μm)	Mid size (μm)	Polymer type	
		EudragitRS100	EudragitRL100
0-30	15	1.67	32.50
30-60	45	9.33	84.00
60-90	75	16.33	96.17
90-120	105	22.50	99.00
120-150	135	27.00	99.67
150-180	165	31.00	99.83
180-210	195	34.50	99.83
210-240	225	38.50	100.00
240-270	255	42.83	100.00
270-300	285	47.17	100.00
300-330	315	50.33	100.00
330-360	345	54.33	100.00
360-390	375	57.67	100.00
390-420	405	61.83	100.00
420-450	435	64.33	100.00
450-480	465	66.83	100.00
480-510	495	70.17	100.00
510-540	525	71.83	100.00
540-570	555	74.33	100.00
570-600	585	76.67	100.00
600-700	650	84.17	100.00
700-800	750	91.00	100.00
800-900	850	95.33	100.00
900-1000	950	98.67	100.00
1000-1100	1050	99.67	100.00
1100-1200	1150	99.67	100.00
1200-1300	1250	100.00	100.00
Mean		377.79	42.23
SD		271.82	23.88
Polydispersibility index		0.57	0.72

Table 5E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules prepared with Eudragit RL100 and varied Poloxamer188 concentrations.

Size interval (μm)	Mid size (μm)	Poloxamer188 concentration(%).		
		0%	10%	20%
0-30	15	32.50	5.17	3.50
30-60	45	84.00	26.67	27.67
60-90	75	96.17	54.67	41.00
90-120	105	99.00	71.00	49.17
120-150	135	99.67	81.00	58.50
150-180	165	99.83	87.67	66.83
180-210	195	99.83	92.17	73.50
210-240	225	100.00	96.00	80.00
240-270	255	100.00	97.67	85.50
270-300	285	100.00	98.83	90.17
300-330	315	100.00	99.67	92.67
330-360	345	100.00	99.67	95.00
360-390	375	100.00	100.00	96.67
390-420	405	100.00	100.00	97.83
420-450	435	100.00	100.00	98.67
450-480	465	100.00	100.00	99.17
480-510	495	100.00	100.00	99.33
510-540	525	100.00	100.00	99.50
540-570	555	100.00	100.00	99.83
570-600	585	100.00	100.00	99.83
600-630	615	100.00	100.00	99.83
630-660	645	100.00	100.00	99.83
660-690	830	100.00	100.00	99.83
690-720	705	100.00	100.00	99.83
720-750	735	100.00	100.00	99.83
750-780	765	100.00	100.00	100.00
Mean		42.23	101.71	149.06
SD		23.98	64.59	110.17
Polydispersibility index		0.57	0.64	0.74

Table 6E Cumulative percentage undersize of *Andrographis paniculata* extract microcapsules prepared with Eudragit RS100 and varied Poloxamer188 concentrations.

Size interval (μm)	Mid size (μm)	Poloxamer188 concentration(%)		
		0%	10%	20%
0-30	15	1.67	10.33	8.00
30-60	45	9.33	37.67	24.50
60-90	75	16.33	53.17	48.00
90-120	105	22.50	66.17	65.83
120-150	135	27.00	78.17	81.00
150-180	165	31.00	86.00	88.00
180-210	195	34.50	91.17	93.00
210-240	225	38.50	96.50	96.00
240-270	255	42.83	98.33	97.17
270-300	285	47.17	99.33	98.17
300-330	315	50.33	99.50	98.83
330-360	345	54.33	99.83	99.17
360-390	375	57.67	100.00	99.17
390-420	405	61.83	100.00	99.50
420-450	435	64.33	100.00	99.67
450-480	465	66.83	100.00	99.83
480-510	495	70.17	100.00	100.00
510-540	525	71.83	100.00	100.00
540-570	555	74.33	100.00	100.00
570-600	585	76.67	100.00	100.00
600-700	650	84.17	100.00	100.00
700-800	750	91.00	100.00	100.00
800-900	850	95.33	100.00	100.00
900-1000	950	98.67	100.00	100.00
1000-1100	980	99.67	100.00	100.00
1100-1200	1010	99.67	100.00	100.00
1200-1300	1040	100.00	100.00	100.00
Mean		377.79	100.38	106.46
SD		271.82	67.16	68.01
Polydispersibility index		0.72	0.67	0.64

APPENDIX F

THE ANDROGRAPHOLIDE RELEASE FROM MICROCAPSULES

Table 1F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 250 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	5.69	5.39	4.76	5.28	0.47
10	6.04	5.87	6.74	6.22	0.46
15	8.17	6.92	9.39	8.16	1.24
20	8.97	8.64	10.00	9.20	0.71
30	9.69	9.92	11.70	10.44	1.10
45	11.66	12.70	13.75	12.70	1.04
60	11.91	13.53	15.54	13.66	1.82
90	16.17	16.75	19.12	17.35	1.56
120	17.22	18.27	19.65	18.38	1.22
$k_{h\ 5\text{-}120\ min}$	1.3626	1.5835	1.7386	1.5616	0.1889

Table 2F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 500 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	8.44	8.87	7.89	8.40	0.49
10	10.82	8.92	11.67	10.47	1.41
15	13.00	10.60	13.10	12.23	1.42
20	14.62	13.43	13.50	13.85	0.67
30	18.88	14.82	15.99	16.57	2.09
45	18.02	16.74	18.11	17.63	0.77
60	19.22	18.74	19.69	19.22	0.47
90	21.08	19.89	21.18	20.72	0.71
120	21.33	20.82	20.17	20.77	0.58
$k_{h\ 5\text{-}120\ min}$	1.4552	1.5209	1.4294	1.4685	0.0471

Table 3F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 800 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	16.46	14.66	17.55	16.22	1.46
10	17.90	21.72	19.84	19.82	1.91
15	21.62	19.46	23.10	21.39	1.83
20	20.88	20.42	21.89	21.06	0.75
30	23.24	21.75	23.46	22.82	0.93
45	24.94	25.20	24.26	24.80	0.48
60	25.37	25.57	26.99	25.97	0.88
90	28.07	26.90	28.76	27.91	0.94
120	29.94	30.26	28.94	29.71	0.69
$k_{h\ 5\text{-}120\ min}$	1.4756	1.4763	1.2635	1.4051	0.1227

Table 4F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	13.13	14.12	13.21	13.48	0.55
10	16.68	19.44	17.01	17.71	1.51
15	18.35	20.70	17.70	18.92	1.58
20	22.96	21.95	19.65	21.52	1.70
30	24.90	24.91	20.00	23.27	2.83
45	24.56	27.88	21.87	24.77	3.01
60	25.43	27.90	23.91	25.75	2.01
90	26.56	27.99	25.87	26.81	1.08
120	38.30	31.43	27.31	32.35	5.55
$k_{h\ 5\text{-}120\ min}$	2.2640	1.7253	1.4973	1.8289	0.3937

Table 5F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 1200 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	14.80	14.17	14.61	14.53	0.32
10	20.01	16.38	18.63	18.34	1.83
15	19.47	18.53	20.67	19.56	1.07
20	20.89	19.31	22.12	20.77	1.41
30	22.70	21.02	24.05	22.59	1.52
45	24.22	22.65	24.64	23.84	1.05
60	27.17	24.62	25.60	25.80	1.29
90	27.95	27.62	28.59	28.05	0.49
120	29.66	27.38	28.12	28.39	1.16
$k_{h\ 5\text{-}120\ min}$	1.5607	1.5626	1.4407	1.5213	0.0698

Table 6F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0% Span80 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	12.48	13.14	16.35	13.99	2.07
10	17.23	17.71	21.02	18.65	2.06
15	18.94	19.44	23.38	20.59	2.43
20	21.30	21.52	24.85	22.56	1.99
30	22.72	23.33	27.87	24.64	2.82
45	25.54	26.76	30.22	27.51	2.43
60	27.71	27.95	33.93	29.86	3.53
90	27.53	30.47	32.98	30.33	2.73
120	28.37	33.27	35.12	32.25	3.48
$k_{h\ 5\text{-}120\ min}$	1.7221	2.1542	2.0547	1.9770	0.2263

Table 7F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0.5% Span80 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	5.78	6.46	6.45	6.23	0.39
10	14.34	19.37	18.70	17.47	2.73
15	19.12	22.92	24.98	22.34	2.98
20	23.69	27.12	27.46	26.09	2.09
30	25.13	29.92	30.80	28.62	3.05
45	27.13	31.50	33.49	30.71	3.25
60	29.13	34.30	36.05	33.16	3.60
90	30.27	37.45	39.07	35.59	4.68
120	31.61	37.11	36.75	35.16	3.08
$k_{h\ 5\text{-}120\ min}$	2.5445	2.9938	3.0624	2.8669	0.2813

Table 8F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 1.0% Span80 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	13.13	14.12	13.21	13.48	0.55
10	16.68	19.44	17.01	17.71	1.51
15	18.35	20.70	17.70	18.92	1.58
20	22.96	21.95	19.65	21.52	1.70
30	24.90	24.91	20.00	23.27	2.83
45	24.56	27.88	21.87	24.77	3.01
60	25.43	27.90	23.91	25.75	2.01
90	26.56	27.99	25.87	26.81	1.08
120	38.30	31.43	27.31	32.35	5.55
$k_{h\ 5\text{-}120\ min}$	2.2640	1.7253	1.4973	1.8289	0.394

Table 9F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 1.5% Span80 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	19.30	16.99	16.60	17.63	1.46
10	22.67	21.09	20.84	21.53	0.99
15	24.57	23.60	22.11	23.43	1.24
20	25.27	24.95	23.20	24.47	1.12
30	27.33	27.62	25.03	26.66	1.42
45	28.45	29.06	27.61	28.37	0.73
60	31.03	30.31	27.62	29.66	1.80
90	32.85	30.76	29.03	30.88	1.91
120	33.29	33.79	31.53	32.87	1.19
$k_{h\ 5\text{-}120\ min}$	1.5590	1.7032	1.5108	1.5910	0.1001

Table 10F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 2.0% Span80 at 1000 rpm in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	10.58	8.26	11.63	10.16	1.72
10	13.35	14.56	15.97	14.63	1.31
15	15.98	16.60	19.20	17.26	1.71
20	18.20	17.86	20.42	18.83	1.39
30	20.15	18.62	21.77	20.18	1.57
45	20.93	20.62	25.51	22.35	2.74
60	23.21	22.80	24.06	23.36	0.64
90	24.24	24.65	27.53	25.47	1.79
120	25.26	25.16	28.35	26.26	1.81
$k_{h\ 5\text{-}120\ min}$	1.6205	1.6965	1.7453	1.6874	0.0629

Table 11F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ratio 1:1 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.42	2.00	2.70	2.04	0.64
10	12.10	9.96	11.45	11.17	1.10
15	15.73	12.29	13.57	13.86	1.74
20	17.60	13.78	15.24	15.54	1.93
30	20.45	15.69	17.21	17.78	2.43
45	21.94	17.03	18.67	19.21	2.50
60	24.11	18.61	20.18	20.97	2.83
90	25.66	19.81	21.12	22.20	3.07
120	26.35	20.81	22.11	23.09	2.90
$k_{h\ 5\text{-}120\ min}$	2.3674	1.7795	1.8102	1.9857	0.3310

Table 12F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ratio 1:2 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.22	6.71	7.78	5.24	3.52
10	11.58	13.44	12.26	12.43	0.94
15	13.60	14.81	15.12	14.51	0.80
20	14.70	16.92	17.02	16.21	1.31
30	16.94	18.80	18.41	18.05	0.98
45	18.91	20.91	19.88	19.90	1.00
60	20.34	22.01	21.92	21.43	0.94
90	21.64	23.27	23.24	22.71	0.93
120	22.73	24.67	24.48	23.96	1.07
$k_{h\ 5\text{-}120\ min}$	1.9740	1.7811	1.7344	1.8298	0.1270

Table 13F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ratio 1:3 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	7.88	8.07	7.83	7.93	0.13
10	12.20	11.41	11.54	11.72	0.43
15	14.88	14.48	14.00	14.45	0.44
20	17.21	15.40	15.48	16.03	1.02
30	20.13	18.37	18.50	19.00	0.98
45	21.69	20.19	19.66	20.51	1.05
60	22.63	21.27	21.30	21.73	0.78
90	25.04	23.42	23.83	24.09	0.84
120	26.76	25.90	25.31	25.99	0.73
$k_{h\ 5\text{-}120min}$	2.0089	1.9063	1.9000	1.9384	0.0611

Table 14F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ratio 2:3 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	2.44	3.57	3.45	3.15	0.62
10	4.94	5.13	5.68	5.25	0.38
15	6.08	6.95	6.60	6.54	0.44
20	7.21	8.01	8.48	7.90	0.64
30	8.23	9.83	10.18	9.41	1.04
45	10.96	11.66	12.47	11.69	0.75
60	12.40	13.49	14.04	13.31	0.84
90	14.06	16.15	15.99	15.40	1.16
120	16.32	17.49	17.37	17.06	0.64
$k_{h\ 5\text{-}120\ min}$	1.5346	1.6299	1.6168	1.5938	0.0516

Table 15F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 in core to wall ratio1:1 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.49	1.76	2.22	1.83	0.37
10	2.99	3.26	3.36	3.20	0.19
15	3.35	3.73	3.78	3.62	0.24
20	3.83	3.87	4.24	3.98	0.23
30	4.41	4.84	4.71	4.65	0.22
45	5.11	6.01	6.05	5.72	0.53
60	5.73	6.34	6.05	6.04	0.30
90	6.56	7.22	7.01	6.93	0.34
120	8.10	8.89	8.19	8.39	0.43
$k_{h\ 5\text{-}120\ min}$	0.6711	0.7427	0.6382	0.6840	0.0534

Table 16F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 and 10%Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	6.56	8.64	11.08	8.76	2.26
10	9.51	10.55	11.19	10.42	0.85
15	10.22	11.64	11.43	11.09	0.77
20	11.00	11.89	12.23	11.71	0.63
30	12.30	13.85	13.08	13.08	0.78
45	12.86	13.67	14.03	13.52	0.60
60	14.02	15.34	15.07	14.81	0.70
90	15.92	16.91	16.96	16.60	0.59
120	17.13	18.10	17.49	17.57	0.49
$k_{h\ 5\text{-}120\ min}$	1.0906	1.0191	0.8216	0.9771	0.1393

Table 17F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 and 20%Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	9.25	11.38	9.90	10.18	1.09
10	11.21	11.98	11.51	11.57	0.39
15	12.36	13.53	12.47	12.79	0.65
20	13.04	13.98	13.85	13.62	0.51
30	14.90	15.67	14.99	15.19	0.42
45	15.96	16.70	16.41	16.36	0.37
60	17.05	18.21	17.52	17.59	0.58
90	18.55	19.67	18.80	19.01	0.59
120	19.88	21.03	20.31	20.41	0.58
$k_{h\ 5\text{-}120\ min}$	1.1766	1.1405	1.1665	1.1612	0.0186

Table 18F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 and 10%Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	10.08	11.37	11.18	10.88	0.70
10	16.48	16.62	16.36	16.48	0.13
15	18.95	18.29	17.95	18.40	0.51
20	20.54	20.25	20.39	20.39	0.14
30	21.85	21.64	21.01	21.50	0.44
45	23.15	22.91	22.38	22.82	0.40
60	24.35	24.14	23.45	23.98	0.47
90	22.19	24.99	24.37	23.85	1.47
120	26.84	26.11	26.50	26.48	0.37
$k_{h\ 5\text{-}120\ min}$	1.4233	1.4557	1.4628	1.4473	0.0210

Table 19F The percentage release of Andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 and 20%Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	12.86	14.53	13.64	13.68	0.84
10	16.54	17.68	18.31	17.51	0.90
15	18.68	19.76	20.12	19.52	0.75
20	19.85	21.04	20.97	20.62	0.67
30	21.30	22.15	22.46	21.97	0.60
45	23.08	23.53	23.90	23.50	0.41
60	23.43	24.60	24.65	24.22	0.69
90	24.69	25.34	25.49	25.17	0.43
120	24.84	25.87	26.83	25.85	1.00
$k_{h\ 5\text{-}120\ min}$	1.2568	1.1930	1.2834	1.2444	0.0464

Table 20F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 250 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	0.70	1.60	0.10	0.80	0.75
10	2.11	1.97	1.85	1.98	0.13
15	2.78	2.27	2.07	2.37	0.37
20	3.89	3.29	4.31	3.83	0.51
30	4.64	4.75	4.32	4.57	0.22
45	6.49	6.62	6.77	6.63	0.14
60	7.53	8.92	7.63	8.03	0.77
90	8.80	8.69	9.03	8.84	0.17
120	10.65	9.83	11.20	10.56	0.69
180	14.13	12.61	15.13	13.96	1.27
240	14.37	13.11	14.53	14.00	0.78
300	15.93	14.68	15.29	15.30	0.62
360	16.45	14.87	16.55	15.96	0.95
420	19.41	16.37	19.08	18.29	1.67
480	19.35	18.55	21.47	19.79	1.51
540	21.27	19.59	21.48	20.78	1.04
600	22.32	19.41	21.69	21.14	1.53
720	22.91	21.40	24.77	23.03	1.69
900	24.03	22.25	23.60	23.29	0.93
1080	24.99	21.83	26.95	24.59	2.58
1440	28.69	25.81	27.25	27.25	1.44
$k_{h\ 5\text{-}600\ min}$	0.9372	0.8153	0.9599	0.9041	0.0777

Table 21F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 500 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	%release 1	%release 2	%release 3	mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	3.75	4.25	4.51	4.17	0.39
10	9.76	7.01	7.62	8.13	1.45
15	10.74	10.43	9.80	10.33	0.48
20	12.40	9.58	10.31	10.76	1.46
30	12.96	11.25	11.71	11.97	0.89
45	15.10	12.86	13.67	13.88	1.13
60	16.29	13.68	14.95	14.97	1.31
90	18.59	15.82	16.42	16.94	1.45
120	20.33	16.23	17.83	18.13	2.07
180	21.92	19.11	19.73	20.25	1.48
240	23.27	20.00	21.00	21.42	1.68
300	24.22	20.33	21.35	21.97	2.02
360	25.49	21.67	23.59	23.59	1.91
420	25.66	23.82	25.85	25.11	1.12
480	25.42	22.49	23.76	23.89	1.47
540	26.21	22.82	23.82	24.28	1.74
600	25.82	26.37	24.59	25.59	0.91
720	26.29	22.84	23.61	24.25	1.81
900	28.03	23.36	24.52	25.30	2.43
1080	27.80	25.17	25.15	26.04	1.53
1440	28.90	24.44	24.88	26.07	2.46
$k_{h\ 5\text{-}600\ min}$	0.8548	0.8150	0.8297	0.8332	0.0201

Table 22F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 800 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	15.00	11.02	16.78	14.27	2.95
10	20.38	16.87	21.81	19.69	2.54
15	21.32	16.98	22.20	20.17	2.80
20	23.06	18.26	22.66	21.33	2.66
30	24.16	20.45	27.42	24.01	3.49
45	25.53	21.96	29.09	25.53	3.57
60	27.18	23.10	33.58	27.95	5.28
90	27.74	23.71	31.41	27.62	3.85
120	30.84	25.59	31.49	29.31	3.24
180	32.50	25.75	33.32	30.52	4.15
240	31.78	31.32	34.06	32.39	1.47
300	33.69	29.09	35.80	32.86	3.43
360	36.62	30.43	37.28	34.78	3.78
420	35.91	35.18	41.58	37.56	3.51
480	37.00	32.27	41.42	36.90	4.58
540	39.56	33.01	40.42	37.66	4.05
600	38.66	31.58	40.50	36.92	4.71
720	41.12	32.10	43.06	38.76	5.85
900	40.58	35.34	43.26	39.73	4.03
1080	41.86	35.06	43.26	40.06	4.39
1440	41.04	34.90	43.99	39.98	4.64
$k_{h\ 5\text{-}600\ min}$	0.9060	0.8496	0.9331	0.8962	0.0426

Table 23F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	9.49	10.93	11.59	10.67	1.08
10	14.67	15.60	15.25	15.18	0.47
15	18.35	17.22	18.12	17.90	0.60
20	18.37	19.66	18.97	19.00	0.65
30	19.53	19.51	20.40	19.81	0.51
45	21.69	22.69	22.42	22.26	0.52
60	22.20	23.55	23.43	23.06	0.75
90	24.02	25.48	25.11	24.87	0.76
120	25.11	26.58	27.46	26.38	1.19
180	27.93	28.93	29.07	28.64	0.62
240	29.11	31.04	31.00	30.38	1.10
300	29.78	31.55	31.09	30.81	0.92
360	32.18	34.49	33.86	33.51	1.19
420	33.11	35.09	35.46	34.55	1.27
480	33.36	35.18	35.39	34.64	1.11
540	33.61	35.60	35.55	34.92	1.13
600	34.28	35.26	36.14	35.23	0.93
720	34.78	35.51	35.89	35.39	0.57
900	34.61	36.86	37.15	36.21	1.39
1080	36.60	38.20	38.74	37.85	1.11
1440	36.28	37.79	37.75	37.27	0.86
$k_{h\ 5\text{-}600\ min}$	0.9333	0.9945	0.9935	0.9738	0.0350

Table 24F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 at 1200 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	14.85	13.24	16.37	14.82	1.57
10	17.17	17.21	18.58	17.66	0.80
15	20.47	17.39	22.32	20.06	2.49
20	23.85	20.58	25.67	23.36	2.58
30	22.91	21.15	25.48	23.18	2.18
45	25.30	23.62	28.10	25.67	2.27
60	25.56	25.54	29.63	26.91	2.36
90	27.48	24.99	32.56	28.34	3.86
120	27.41	32.23	32.88	30.84	2.99
180	32.29	27.58	35.70	31.86	4.08
240	29.76	27.55	38.24	31.85	5.64
300	30.06	27.71	35.33	31.03	3.90
360	28.70	28.59	37.23	31.51	4.95
420	30.46	28.91	39.35	32.91	5.64
480	30.55	29.55	38.86	32.98	5.11
540	30.07	30.74	36.54	32.45	3.56
600	30.54	30.75	36.53	32.61	3.40
720	29.27	33.61	39.85	34.24	5.32
900	29.58	31.56	41.08	34.07	6.15
1080	30.54	28.77	37.16	32.16	4.42
1440	30.87	29.63	37.03	32.51	3.97
$k_{h\ 5\text{-}600\ min}$	0.5510	0.6071	0.8479	0.6687	0.1578

Table 25F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0% Span80 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	7.53	9.33	6.34	7.73	1.50
10	16.71	15.68	11.61	14.67	2.70
15	18.98	20.02	13.87	17.62	3.30
20	20.84	20.71	15.92	19.16	2.81
30	23.72	23.62	17.01	21.45	3.84
45	26.04	25.62	18.80	23.49	4.06
60	28.18	27.13	20.22	25.17	4.32
90	28.28	28.79	20.80	25.96	4.48
120	30.87	30.21	22.14	27.74	4.86
180	34.58	30.96	23.36	29.63	5.73
240	34.88	33.12	23.81	30.60	5.95
300	34.42	33.14	26.05	31.20	4.51
360	36.54	33.80	25.42	31.92	5.79
420	36.18	34.72	25.67	32.19	5.69
480	37.01	34.55	26.44	32.67	5.53
540	33.32	34.64	25.36	31.10	5.02
600	34.96	33.64	24.78	31.13	5.54
720	35.52	33.64	26.82	31.99	4.58
900	34.60	34.13	26.95	31.90	4.29
1080	31.83	30.99	24.41	29.07	4.06
1440	33.84	31.72	24.77	30.11	4.74
$k_{h\ 5\text{-}600\ min}$	0.9535	0.8674	0.6703	0.8304	0.1452

Table 26F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 0.5% Span80 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	11.04	27.22	13.94	17.40	8.63
10	13.39	13.91	12.49	13.27	0.72
15	14.51	14.87	14.01	14.46	0.43
20	17.17	17.70	14.40	16.42	1.77
30	17.64	19.69	17.31	18.21	1.29
45	26.08	21.50	17.55	21.71	4.27
60	27.86	21.34	18.78	22.66	4.69
90	23.29	21.42	19.78	21.50	1.76
120	23.18	23.91	21.61	22.90	1.17
180	27.94	31.30	27.43	28.89	2.10
240	32.55	31.08	26.93	30.19	2.91
300	31.84	29.79	26.93	29.52	2.47
360	32.02	32.36	30.29	31.55	1.11
420	35.32	32.71	31.99	33.34	1.75
480	38.45	32.20	30.31	33.66	4.26
540	34.53	36.06	29.92	33.50	3.19
600	34.97	31.88	28.78	31.87	3.10
720	38.54	31.77	30.83	33.71	4.21
900	39.48	33.48	30.46	34.47	4.59
1080	37.65	30.66	28.55	32.29	4.76
1440	41.58	33.48	28.92	34.66	6.41
$k_{h\ 5\text{-}600\ min}$	1.0515	0.7942	0.8624	0.9027	0.1333

Table 27F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 1.0% Span80 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	9.49	10.93	11.59	10.67	1.08
10	14.67	15.60	15.25	15.18	0.47
15	18.35	17.22	18.12	17.90	0.60
20	18.37	19.66	18.97	19.00	0.65
30	19.53	19.51	20.40	19.81	0.51
45	21.69	22.69	22.42	22.26	0.52
60	22.20	23.55	23.43	23.06	0.75
90	24.02	25.48	25.11	24.87	0.76
120	25.11	26.58	27.46	26.38	1.19
180	27.93	28.93	29.07	28.64	0.62
240	29.11	31.04	31.00	30.38	1.10
300	29.78	31.55	31.09	30.81	0.92
360	32.18	34.49	33.86	33.51	1.19
420	33.11	35.09	35.46	34.55	1.27
480	33.36	35.18	35.39	34.64	1.11
540	33.61	35.60	35.55	34.92	1.13
600	34.28	35.26	36.14	35.23	0.93
720	34.78	35.51	35.89	35.39	0.57
900	34.61	36.86	37.15	36.21	1.39
1080	36.60	38.20	38.74	37.85	1.11
1440	36.28	37.79	37.75	37.27	0.86
$k_{h\ 5\text{-}600\ min}$	0.9333	0.9945	0.9935	0.9738	0.0350

Table 28F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 1.5% Span80 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	11.36	8.25	9.72	9.78	1.56
10	17.82	13.75	14.37	15.32	2.19
15	17.77	15.93	15.45	16.38	1.23
20	19.76	17.48	18.24	18.49	1.16
30	21.59	19.33	20.14	20.35	1.14
45	23.58	21.56	20.43	21.86	1.60
60	24.63	22.57	21.40	22.87	1.63
90	26.11	24.50	23.15	24.59	1.48
120	29.31	26.04	24.41	26.59	2.49
180	30.02	28.51	25.81	28.12	2.13
240	30.98	29.98	28.19	29.72	1.41
300	32.19	32.52	29.53	31.41	1.64
360	34.27	31.46	28.42	31.39	2.93
420	34.37	32.07	30.78	32.41	1.82
480	35.06	32.23	30.31	32.53	2.39
540	32.99	31.46	29.68	31.38	1.66
600	32.89	31.69	29.40	31.33	1.78
720	34.36	31.84	29.12	31.77	2.62
900	33.77	33.15	30.02	32.31	2.01
1080	31.00	31.62	27.52	30.04	2.21
1440	31.50	30.23	27.64	29.79	1.97
$k_{h\ 5\text{-}600\ min}$	0.8647	0.9163	0.7769	0.8526	0.0705

Table 29F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 with 2.0% Span80 at 1000 rpm in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	7.64	8.55	10.91	9.03	1.69
10	9.80	10.02	13.23	11.02	1.92
15	12.38	11.22	13.99	12.53	1.39
20	8.37	14.41	16.17	12.98	4.09
30	9.81	15.94	14.68	13.47	3.24
45	14.20	19.21	19.43	17.61	2.96
60	13.64	15.41	16.87	15.31	1.62
90	14.15	17.06	18.78	16.66	2.34
120	15.69	18.42	20.29	18.13	2.31
180	20.31	23.59	25.99	23.29	2.85
240	22.89	26.96	29.45	26.43	3.31
300	25.17	26.66	29.55	27.13	2.23
360	24.67	29.68	31.55	28.64	3.56
420	24.16	27.55	29.64	27.12	2.77
480	25.18	28.89	32.06	28.71	3.44
540	24.74	27.15	32.99	28.29	4.24
600	27.67	28.57	36.26	30.83	4.72
720	26.59	29.21	32.01	29.27	2.71
900	26.87	31.28	34.49	30.88	3.83
1080	25.27	28.59	32.08	28.65	3.41
1440	35.71	31.44	32.57	33.24	2.21
$k_{h\ 5\text{-}600\ min}$	0.8806	0.9007	1.0561	0.9458	0.0960

Table 30F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ration 1:1 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	11.98	12.70	10.36	11.68	1.20
10	14.09	15.17	12.08	13.78	1.57
15	15.14	15.94	13.33	14.80	1.34
20	15.93	16.56	13.88	15.46	1.40
30	16.35	17.27	14.62	16.08	1.35
45	17.19	18.55	15.22	16.99	1.67
60	17.82	18.76	16.19	17.59	1.30
90	18.14	19.27	16.57	17.99	1.36
120	18.92	20.24	17.03	18.73	1.62
180	20.91	22.18	19.79	20.96	1.20
240	22.44	22.60	20.22	21.75	1.33
300	23.18	23.37	20.77	22.44	1.45
360	23.96	23.57	21.46	23.00	1.35
420	23.86	23.52	21.14	22.84	1.48
480	23.34	23.63	22.06	23.01	0.83
540	24.80	25.26	22.57	24.21	1.44
600	24.44	24.25	22.12	23.60	1.29
720	23.08	24.04	21.29	22.80	1.40
900	24.75	24.55	22.43	23.91	1.28
1080	22.41	23.78	20.83	22.34	1.48
1440	24.12	24.34	21.78	23.41	1.42
$k_{h\ 5\text{-}600\ min}$	0.5207	0.4680	0.4987	0.4958	0.0265

Table 31F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ration 1:2 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	7.39	7.44	8.34	7.72	0.54
10	8.29	8.43	10.21	8.97	1.07
15	9.90	10.10	11.98	10.66	1.15
20	10.97	11.06	12.11	11.38	0.63
30	11.62	12.29	13.45	12.45	0.92
45	12.53	13.47	14.30	13.43	0.89
60	13.39	14.53	15.40	14.44	1.01
90	14.41	15.21	16.68	15.43	1.15
120	15.64	16.27	17.66	16.52	1.03
180	16.99	17.78	19.73	18.16	1.41
240	17.90	19.23	22.77	19.97	2.52
300	18.76	20.02	20.97	19.92	1.11
360	18.44	19.92	21.14	19.83	1.35
420	18.98	20.86	22.66	20.83	1.84
480	19.99	20.87	23.64	21.50	1.90
540	20.16	21.93	22.37	21.48	1.17
600	20.05	22.10	22.97	21.71	1.50
720	19.52	21.99	23.76	21.76	2.13
900	21.65	24.44	25.71	23.93	2.07
1080	22.36	24.01	26.32	24.23	1.99
1440	21.88	24.56	27.78	24.74	2.95
$k_{h\ 5\text{-}600\ min}$	0.5451	0.6167	0.6281	0.5966	0.0450

Table 32F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ration 1:3 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	7.33	8.27	6.98	7.53	0.67
10	9.72	12.52	10.61	10.95	1.43
15	12.73	14.36	12.12	13.07	1.16
20	13.24	15.52	14.85	14.53	1.17
30	15.43	19.35	16.71	17.16	2.00
45	18.19	20.99	18.04	19.07	1.66
60	19.59	22.63	19.01	20.41	1.94
90	20.89	25.02	21.29	22.40	2.28
120	21.46	25.42	22.54	23.14	2.05
180	25.76	31.44	25.26	27.49	3.43
240	26.35	30.33	27.73	28.14	2.02
300	26.44	32.23	28.71	29.13	2.92
360	27.57	32.72	29.25	29.85	2.63
420	28.87	34.45	29.77	31.03	2.99
480	28.64	35.41	32.85	32.30	3.42
540	29.45	36.85	31.64	32.64	3.80
600	30.18	37.81	32.68	33.56	3.89
720	32.21	38.49	33.83	34.84	3.26
900	33.76	40.40	35.85	36.67	3.40
1080	32.31	40.70	35.69	36.23	4.22
1440	35.30	42.52	37.97	38.60	3.65
$k_{h\ 5\text{-}600\ min}$	0.9274	1.1695	1.0437	1.0469	0.1211

Table 33F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 in core to wall ration 2:3 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.30	1.16	1.70	1.39	0.28
10	2.91	2.58	2.63	2.71	0.18
15	4.20	3.72	3.71	3.88	0.28
20	5.09	5.25	4.90	5.08	0.17
30	6.31	5.91	6.10	6.11	0.20
45	7.59	7.05	7.45	7.36	0.28
60	8.87	8.30	8.70	8.62	0.29
90	10.71	10.15	10.82	10.56	0.36
120	11.71	11.02	11.53	11.42	0.36
180	13.88	13.19	14.72	13.93	0.76
240	16.10	15.70	16.68	16.16	0.49
300	16.50	15.98	17.23	16.57	0.63
360	17.00	17.12	17.18	17.10	0.09
420	17.61	16.10	18.42	17.38	1.18
480	19.39	18.15	18.10	18.55	0.73
540	17.63	18.70	19.07	18.47	0.75
600	18.12	17.62	19.02	18.25	0.71
720	19.89	18.32	19.40	19.20	0.80
900	20.23	20.49	20.05	20.26	0.22
1080	21.01	19.80	20.92	20.58	0.67
1440	21.35	19.80	20.54	20.56	0.78
$k_{h\ 5\text{-}600\ min}$	0.7672	0.7627	0.8053	0.7784	0.0234

Table 34F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 in core to wall ration 1:1 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.43	1.46	1.80	1.56	0.20
10	2.50	2.73	2.69	2.64	0.12
15	4.58	3.20	3.03	3.60	0.85
20	3.62	3.80	4.52	3.98	0.47
30	4.15	3.90	4.25	4.10	0.18
45	5.31	4.92	5.08	5.11	0.20
60	6.09	5.86	6.41	6.12	0.28
90	6.77	6.42	6.97	6.72	0.28
120	7.89	7.45	8.02	7.79	0.30
180	10.21	9.17	9.69	9.69	0.52
240	10.47	9.65	10.19	10.10	0.42
300	11.48	10.02	11.25	10.92	0.79
360	11.68	10.58	11.92	11.39	0.71
420	13.23	11.70	12.53	12.48	0.77
480	12.22	12.49	13.97	12.89	0.94
540	13.23	12.50	14.25	13.33	0.88
600	13.14	13.29	13.81	13.41	0.35
720	13.38	13.11	14.08	13.52	0.50
900	13.82	13.80	14.80	14.14	0.57
1080	14.88	13.90	16.25	15.01	1.18
1440	16.19	15.53	17.80	16.51	1.17
$k_{h\ 5\text{-}600\ min}$	0.5232	0.5015	0.5533	0.5260	0.0260

Table 35F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 and 10%Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	8.15	8.29	8.09	8.18	0.10
10	10.83	11.21	10.75	10.93	0.24
15	13.34	13.18	12.79	13.10	0.29
20	14.73	14.73	14.68	14.71	0.03
30	16.99	15.81	15.38	16.06	0.83
45	18.12	17.57	17.08	17.59	0.52
60	18.57	18.14	17.68	18.13	0.44
90	20.63	21.12	19.66	20.47	0.74
120	21.67	21.44	20.91	21.34	0.39
180	22.99	22.83	21.78	22.54	0.66
240	23.39	23.66	22.80	23.28	0.44
300	25.06	24.95	23.86	24.62	0.66
360	25.85	25.78	25.01	25.55	0.46
420	27.86	27.68	27.45	27.66	0.21
480	29.00	28.41	28.01	28.47	0.49
540	29.78	29.18	28.79	29.25	0.50
600	30.52	30.16	29.62	30.10	0.45
720	29.50	30.99	27.06	29.18	1.98
900	30.62	31.66	29.52	30.60	1.07
1080	31.60	32.79	30.96	31.79	0.93
1440	35.38	35.62	33.95	34.98	0.91
$k_{h\ 5\text{-}600\ min}$	0.8545	0.8440	0.8350	0.8445	0.0098

Table 36F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RS100 and 20%Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	10.90	10.72	12.25	11.29	0.84
10	14.22	13.11	14.40	13.91	0.70
15	16.47	15.46	16.50	16.14	0.59
20	17.73	15.82	17.50	17.02	1.04
30	18.83	17.09	19.25	18.39	1.15
45	20.90	18.92	20.80	20.21	1.11
60	21.45	19.95	22.40	21.27	1.24
90	23.36	21.57	23.72	22.88	1.15
120	24.51	22.65	24.83	23.99	1.18
180	26.85	24.83	27.41	26.37	1.36
240	28.77	26.67	29.29	28.24	1.39
300	28.51	27.49	30.12	28.71	1.32
360	30.46	28.21	30.07	29.58	1.21
420	29.88	28.87	31.82	30.19	1.50
480	29.49	29.38	30.90	29.92	0.85
540	29.98	29.13	31.94	30.35	1.44
600	30.25	28.87	31.67	30.27	1.40
720	28.95	28.57	30.63	29.38	1.09
900	30.52	28.92	31.50	30.31	1.30
1080	30.53	28.72	31.61	30.29	1.46
1440	31.67	30.24	32.93	31.61	1.34
$k_{h\ 5\text{-}600\ min}$	0.7770	0.7862	0.8346	0.7993	0.0309

Table 37F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 and 10%Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	10.51	12.76	12.88	12.05	1.34
10	15.71	17.09	17.09	16.63	0.80
15	18.28	19.56	19.78	19.21	0.81
20	20.29	21.23	21.97	21.16	0.84
30	21.60	23.10	22.75	22.48	0.79
45	23.15	24.55	24.55	24.08	0.81
60	24.11	25.84	25.32	25.09	0.89
90	25.61	27.44	27.23	26.76	1.00
120	26.17	27.77	27.84	27.26	0.94
180	26.22	28.94	28.88	28.01	1.55
240	27.17	29.27	28.99	28.48	1.14
300	28.13	29.80	29.81	29.25	0.97
360	28.53	30.71	30.63	29.96	1.24
420	28.98	31.62	31.83	30.81	1.59
480	29.88	32.64	32.11	31.55	1.46
540	30.59	31.95	32.49	31.68	0.98
600	31.69	32.70	33.10	32.49	0.73
720	30.40	31.42	36.42	32.75	3.22
900	33.08	33.44	36.55	34.36	1.91
1080	30.35	32.33	32.90	31.86	1.34
1440	34.63	35.41	35.49	35.18	0.48
$k_{h\ 5\text{-}600\ min}$	0.6727	0.6949	0.6975	0.6884	0.0137

Table 38F The percentage release of andrographolide from *Andrographis paniculata* extract microcapsules prepared from Eudragit RL100 and 20%Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.00	0.00	0.00	0.00	0.00
5	1.04	1.93	4.66	2.54	1.89
10	16.92	18.78	19.02	18.24	1.15
15	19.59	21.51	21.87	20.99	1.22
20	20.82	22.86	22.90	22.19	1.19
30	22.09	24.27	24.66	23.67	1.38
45	23.82	25.96	26.64	25.47	1.47
60	24.79	27.14	27.22	26.38	1.38
90	25.65	28.32	28.52	27.50	1.60
120	26.47	29.50	29.88	28.62	1.87
180	27.94	30.96	31.47	30.12	1.91
240	29.26	32.93	32.72	31.64	2.06
300	29.83	33.61	33.29	32.24	2.10
360	30.23	33.84	33.97	32.68	2.12
420	30.89	35.19	34.26	33.45	2.26
480	31.50	35.02	34.15	33.56	1.83
540	31.51	35.70	35.22	34.14	2.29
600	30.39	35.08	34.66	33.38	2.59
720	30.09	33.79	33.13	32.34	1.98
900	31.04	34.23	35.78	33.69	2.41
1080	31.56	35.02	34.72	33.77	1.92
1440	34.04	37.60	37.82	36.48	2.12
$k_{h\ 5\text{-}600\ min}$	0.7927	0.9232	0.8503	0.8554	0.0654

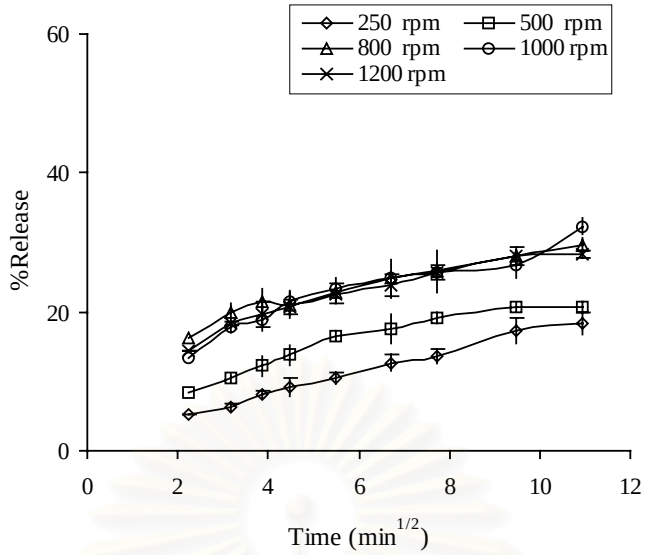


Figure 1F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 at 250, 500, 800, 1000 and 1200 rpm in simulated gastric fluid without pepsin pH 1.2.

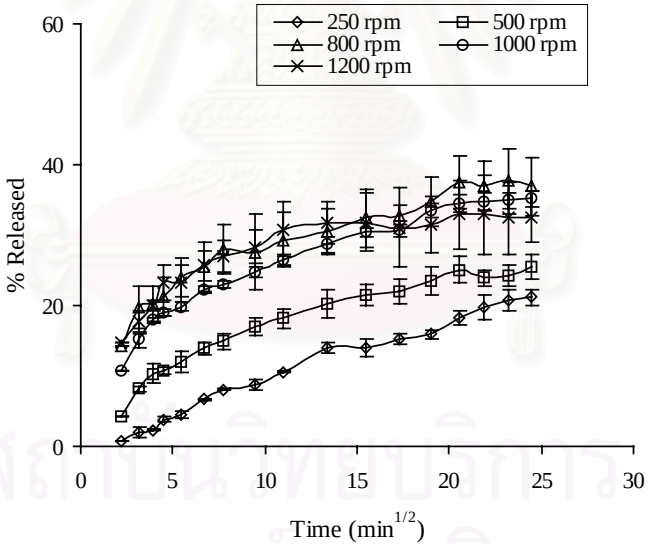


Figure 2F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 at 250, 500, 800, 1000 and 1200 rpm in simulated intestinal fluid without pancreatin pH 6.8±0.1.

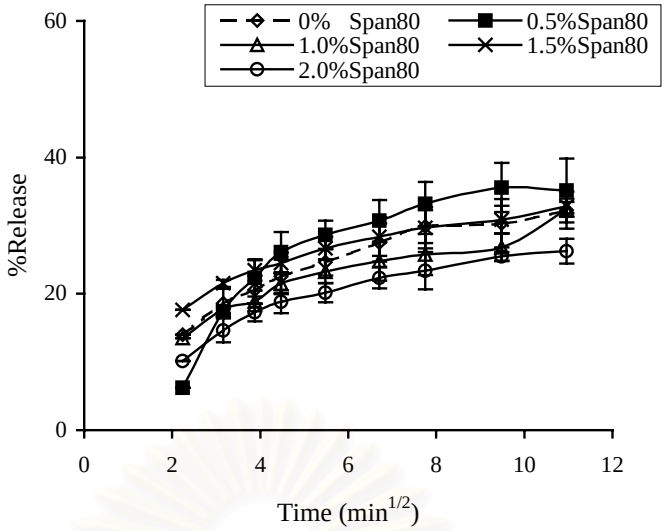


Figure 3F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 with 0, 0.5, 1, 1.5 and 2% Span80 in simulated gastric fluid without pepsin pH 1.2.

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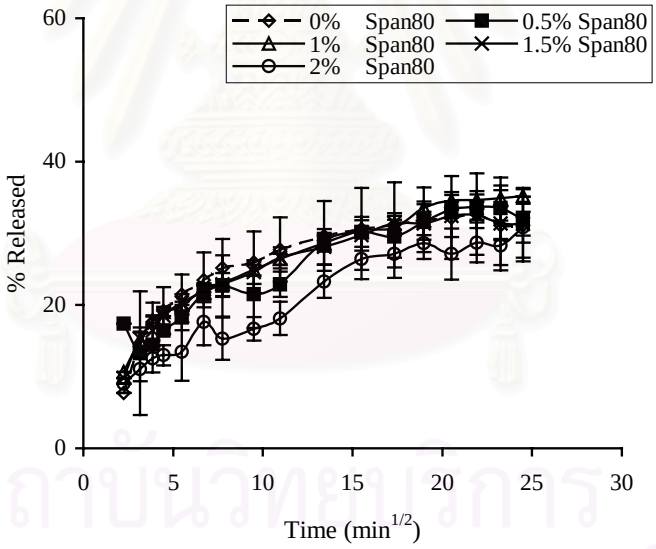

Figure 4F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 with 0, 0.5, 1, 1.5 and 2% Span80 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

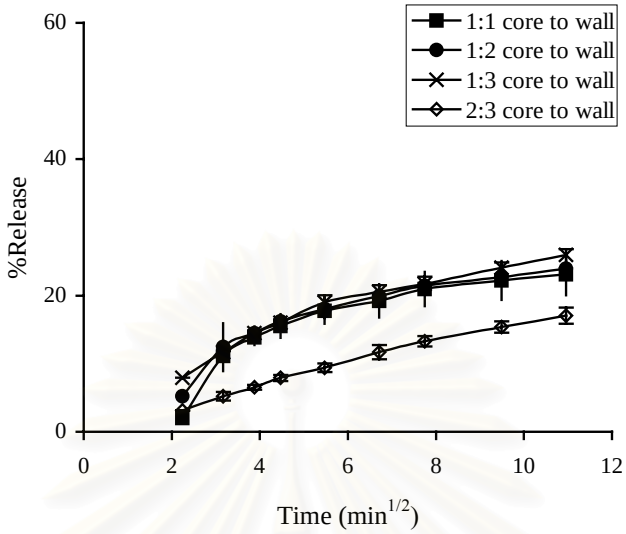

Figure 5F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 at 1:1, 1:2, 1:3 and 2:3 core to wall ration in simulated gastric fluid without pepsin pH 1.2.

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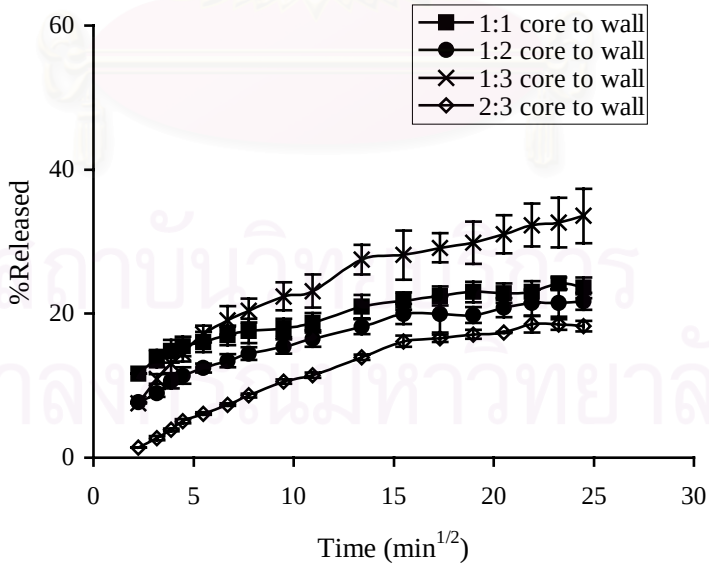

Figure 6F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 at 1:1, 1:2, 1:3 and 2:3 core to wall ration in simulated intestinal fluid without pancreatin pH 6.8±0.1.

.

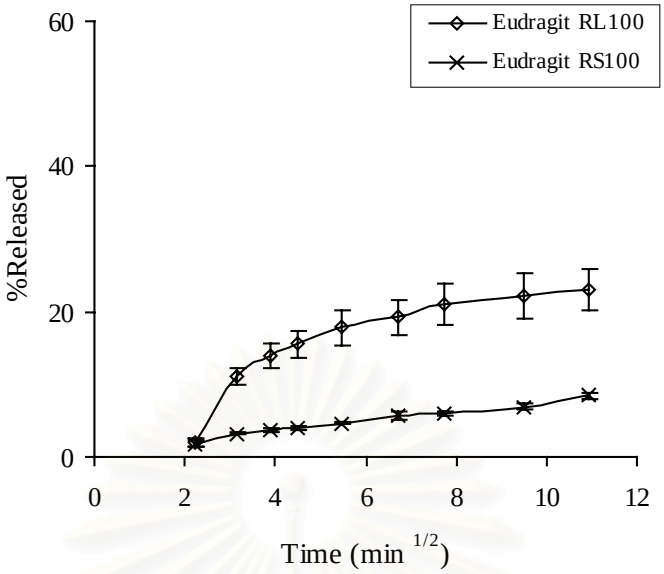

Figure 7F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 and Eudragit RS100 in simulated gastric fluid without pepsin pH 1.2.

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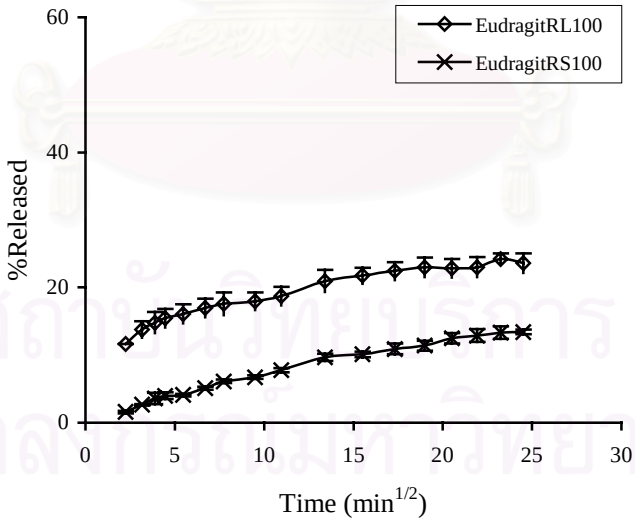


Figure 8F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 and Eudragit RS100 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

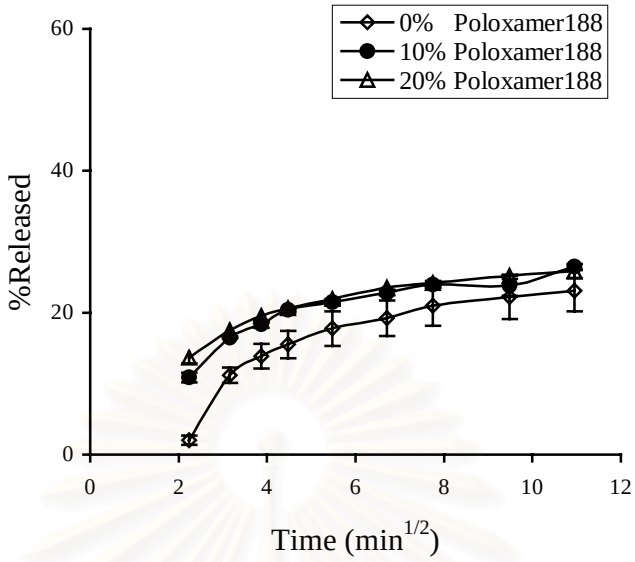


Figure 9F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 with 0, 10 and 20% Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

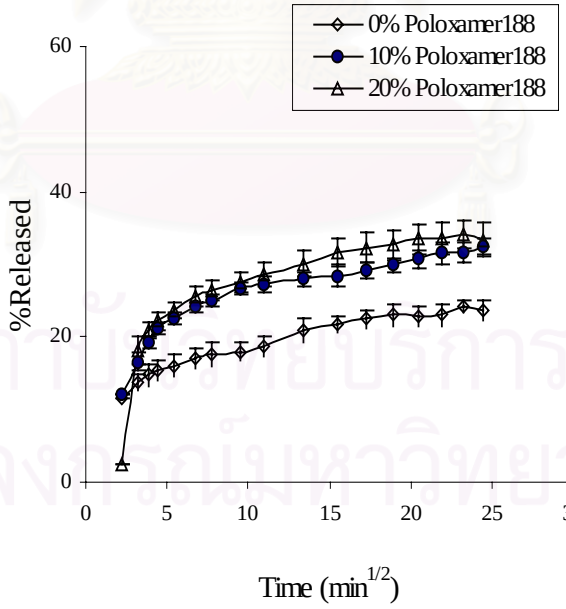


Figure 10F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RL100 with 0, 10 and 20% Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8±0.1.

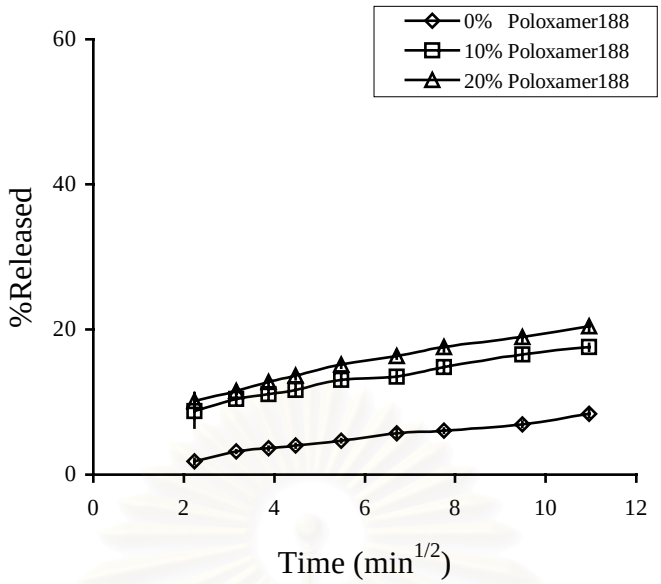


Figure 11F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RS100 with 0, 10 and 20% Poloxamer188 in simulated gastric fluid without pepsin pH 1.2.

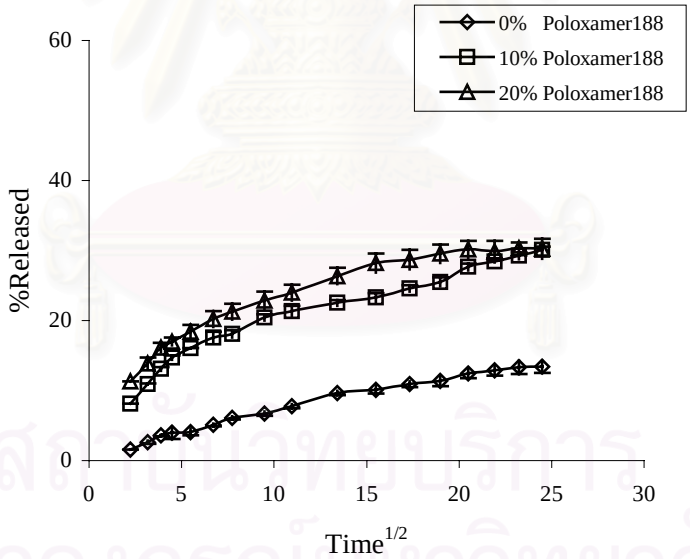
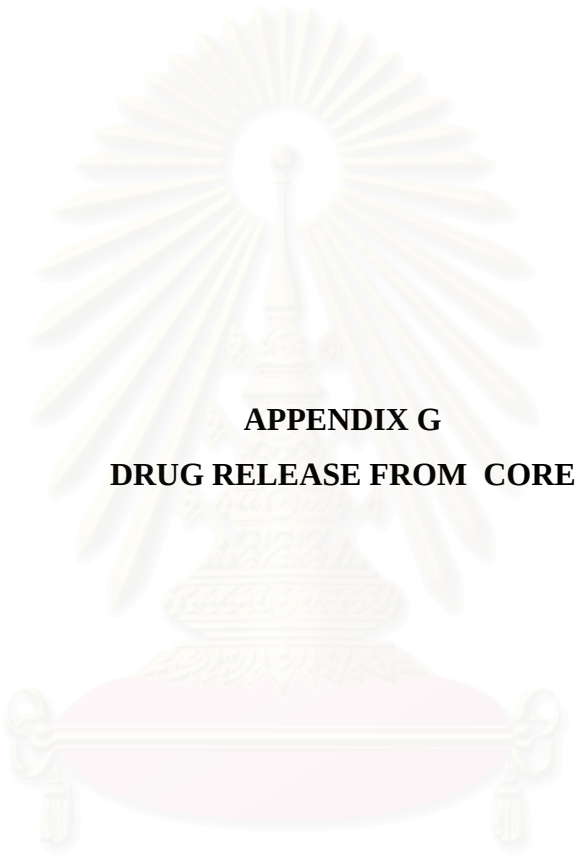


Figure 12F The Higuchi plots of the release of andrographolide from *Andrographis paniculata* microcapsules prepared from Eudragit RS100 with 0, 10 and 20% Poloxamer188 in simulated intestinal fluid without pancreatin pH 6.8±0.1.



APPENDIX G
DRUG RELEASE FROM CORE

Table 1G The percentage release of andrographolide crystals I and crude extract in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.000	0.000	0.000	0.000	0.000
5	13.063	11.753	15.773	13.530	2.050
10	25.371	26.498	24.281	25.383	1.109
15	33.596	28.569	27.998	30.054	3.080
20	36.088	30.379	34.820	33.762	2.998
30	42.511	34.651	40.580	39.247	4.096
45	50.703	40.546	43.850	45.033	5.181
60	57.624	45.504	50.994	51.374	6.069
90	59.293	49.130	57.295	55.239	5.384
120	78.297	66.576	66.398	70.424	6.819
$k_{h\ 5\text{-}120\ min}$	6.5524	5.1762	5.4870	5.7385	0.722

Table 2G The percentage release of andrographolide crystals II and crude extract in simulated gastric fluid without pepsin pH 1.2.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.000	0.000	0.000	0.000	0.000
5	14.784	14.514	20.254	16.517	3.239
10	27.318	25.799	33.395	28.837	4.019
15	34.879	34.112	42.189	37.060	4.458
20	40.830	39.543	45.790	42.055	3.299
30	49.727	47.998	54.747	50.824	3.506
45	56.741	53.690	60.719	57.050	3.524
60	61.528	59.281	67.535	62.781	4.268
90	66.198	63.915	72.310	67.474	4.341
120	70.233	68.196	75.459	71.296	3.746
$k_{h\ 5\text{-}120\ min}$	6.1090	5.9063	6.0556	6.0236	0.105

Table 3G The percentage release of andrographolide crystal I and crude extract in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.000	0.000	0.000	0.000	0.000
5	21.497	26.930	24.976	24.468	2.752
10	31.606	38.495	33.698	34.600	3.532
15	34.991	43.382	38.248	38.874	4.230
20	38.628	46.303	42.354	42.428	3.838
30	42.412	47.444	46.035	45.297	2.596
45	46.342	51.470	50.980	49.597	2.830
60	49.983	56.476	54.103	53.521	3.286
90	53.187	61.006	58.764	57.652	4.026
120	58.417	65.372	63.151	62.313	3.553
180	61.341	67.647	64.723	64.570	3.156
240	65.700	71.518	68.953	68.724	2.916
300	68.619	72.665	69.962	70.415	2.061
360	69.649	72.993	69.545	70.729	1.961
420	69.799	73.799	69.965	71.187	2.263
480	70.813	74.607	71.093	72.171	2.114
540	72.122	75.254	71.381	72.919	2.056
600	76.617	76.062	73.071	75.250	1.907
720	74.036	76.066	73.643	74.582	1.301
900	75.759	79.121	74.772	76.550	2.280
1080	75.768	78.173	75.200	76.381	1.578
1440	74.465	76.078	71.122	73.888	2.528
$k_{h\ 5\text{-}600\ min}$	2.0730	1.8490	1.8267	1.9162	0.1362

Table 4G The percentage release of andrographolide crystal II and crude extract in simulated intestinal fluid without pancreatin pH 6.8 ± 0.1.

Time (min)	% Release 1	% Release 2	% Release 3	Mean	SD
0	0.000	0.000	0.000	0.000	0.000
5	10.727	12.375	19.485	14.196	4.654
10	41.901	35.245	40.200	39.115	3.458
15	52.672	43.730	48.706	48.370	4.481
20	59.057	49.619	53.655	54.110	4.735
30	63.708	53.952	58.419	58.693	4.884
45	69.888	60.143	64.428	64.819	4.884
60	71.973	63.342	66.538	67.284	4.364
90	74.549	66.280	69.790	70.206	4.150
120	76.700	68.488	73.215	72.801	4.122
180	73.076	66.854	79.039	72.989	6.093
240	84.579	70.014	75.075	76.556	7.394
300	78.393	68.414	72.565	73.124	5.013
360	76.650	69.757	68.120	71.509	4.527
420	76.355	69.223	68.926	71.502	4.206
480	71.510	68.950	71.147	70.536	1.385
540	74.332	69.489	68.666	70.829	3.062
600	75.203	70.845	69.760	71.936	2.881
720	82.616	76.262	79.183	79.354	3.180
900	82.657	72.235	76.189	77.027	5.261
1080	78.668	71.401	75.618	75.229	3.649
1440	77.221	71.937	80.047	76.402	4.116
$k_{h\ 5\text{-}600\ min}$	1.5121	1.5678	1.3102	1.4634	0.1356

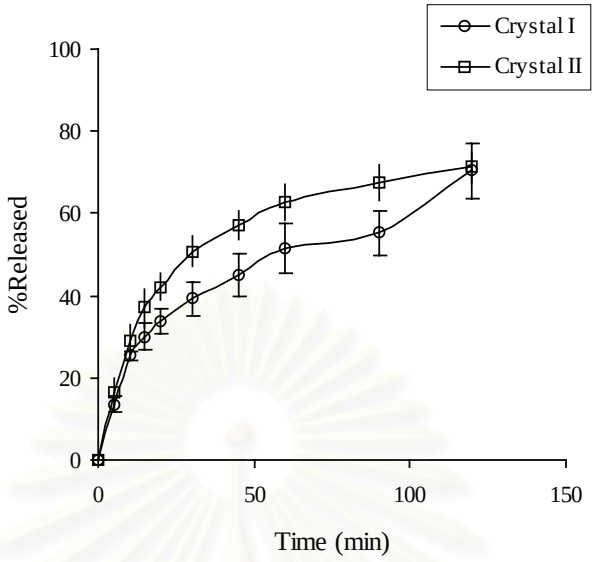


Figure 1G The release profile of core (andrographolide crystal and dry crude extract) in simulated gastric fluid without pepsin pH 1.2.

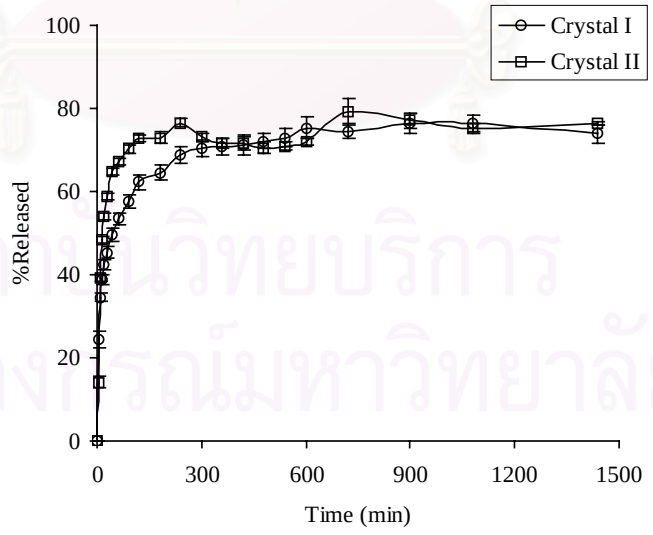


Figure 2G The release profile of core (andrographolide crystal and dry crude extract) in simulated intestinal fluid without pepsin pH 6.8±0.1.

APPENDIX H
STATISTICS FOR COMPARISON OF RELEASE RATE CONSTANT AND DISSOLUTION EFFICIENCY

Table 1H Test of statistics on the effect of the stirring rate on the drug release rate from the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KRPM	Between Groups	.318	4	7.958E-02	1.869	.193
	Within Groups	.426	10	4.257E-02		
	Total	.744	14			

Dependent Variable: KRPM
Scheffe

(I) RPM	(J) RPM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
250	500	9.307E-02	.168	.988	-.535293	.721426
	800	.156447	.168	.924	-.471913	.784806
	1000	-.267300	.168	.653	-.895659	.361059
	1200	4.023E-02	.168	1.000	-.588126	.668593
500	250	-9.31E-02	.168	.988	-.721426	.535293
	800	6.338E-02	.168	.997	-.564979	.691739
	1000	-.360367	.168	.391	-.988726	.267993
	1200	-5.28E-02	.168	.999	-.681193	.575526
800	250	-.156447	.168	.924	-.784806	.471913
	500	-6.34E-02	.168	.997	-.691739	.564979
	1000	-.423747	.168	.253	-1.052106	.204613
	1200	-.116213	.168	.973	-.744573	.512146
1000	250	.267300	.168	.653	-.361059	.895659
	500	.360367	.168	.391	-.267993	.988726
	800	.423747	.168	.253	-.204613	1.052106
	1200	.307533	.168	.534	-.320826	.935893
1200	250	-4.02E-02	.168	1.000	-.668593	.588126
	500	5.283E-02	.168	.999	-.575526	.681193
	800	.116213	.168	.973	-.512146	.744573
	1000	-.307533	.168	.534	-.935893	.320826

RPM	N	Subset for alpha = .05
		1
800	3	1.405120
500	3	1.468500
1200	3	1.521333
250	3	1.561567
1000	3	1.828867
Sig.		.253

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 2H Test of statistics on the effect of the concentration of Span 80 on the drug release rate from the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KSPAN	Between Groups	3.138	4	.784	13.103	.001
	Within Groups	.599	10	5.986E-02		
	Total	3.736	14			

Dependent Variable: KSPAN
Scheffe

(I) SPAN80	(J) SPAN80	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-.889900*	.200	.018	-1.635034	-.144766
	1.00	.148133	.200	.965	-.597001	.893267
	1.50	.386000	.200	.483	-.359134	1.131134
	2.00	.289603	.200	.720	-.455531	1.034737
.50	.00	.889900*	.200	.018	.144766	1.635034
	1.00	1.038033*	.200	.007	.292899	1.783167
	1.50	1.275900*	.200	.001	.530766	2.021034
	2.00	1.179503*	.200	.003	.434369	1.924637
1.00	.00	-.148133	.200	.965	-.893267	.597001
	.50	-1.038033*	.200	.007	-1.783167	-.292899
	1.50	.237867	.200	.835	-.507267	.983001
	2.00	.141470	.200	.970	-.603664	.886604
1.50	.00	-.386000	.200	.483	-1.131134	.359134
	.50	-1.275900*	.200	.001	-2.021034	-.530766
	1.00	-.237867	.200	.835	-.983001	.507267
	2.00	-9.64E-02	.200	.993	-.841531	.648737
2.00	.00	-.289603	.200	.720	-1.034737	.455531
	.50	-1.179503*	.200	.003	-1.924637	-.434369
	1.00	-.141470	.200	.970	-.886604	.603664
	1.50	9.640E-02	.200	.993	-.648737	.841531

*. The mean difference is significant at the .05 level.

SPAN80	N	Subset for alpha = .05	
		1	2
1.50	3	1.591000	
2.00	3	1.687397	
1.00	3	1.828867	
.00	3	1.977000	
.50	3		2.866900
Sig.		.483	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 3H Test of statistics on the effect of the core to wall ratio on the drug release rate from the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KRATIO	Between Groups	.275	3	9.161E-02	2.775	.110
	Within Groups	.264	8	3.301E-02		
	Total	.539	11			

Dependent Variable: KRATIO
Scheffe

(I) RATIO	(J) RATIO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	.155870	.148	.778	-.362270	.674010
	13	4.730E-02	.148	.991	-.470840	.565440
	23	.391933	.148	.151	-.126207	.910073
12	11	-.155870	.148	.778	-.674010	.362270
	13	-.108570	.148	.908	-.626710	.409570
	23	.236064	.148	.507	-.282077	.754204
13	11	-4.73E-02	.148	.991	-.565440	.470840
	12	.108570	.148	.908	-.409570	.626710
	23	.344633	.148	.225	-.173507	.862773
23	11	-.391933	.148	.151	-.910073	.126207
	12	-.236064	.148	.507	-.754204	.282077
	13	-.344633	.148	.225	-.862773	.173507

RATIO	N	Subset for alpha = .05
		1
23	3	1.593767
12	3	1.829830
13	3	1.938400
11	3	1.985700
Sig.		.151

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 4H Test of statistics on the effect of the concentration of Poloxamer 188 on the drug release rate from the microcapsules prepared with Eudragit RL100 in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KPXMRL	Between Groups	.881	2	.440	11.782	.008
	Within Groups	.224	6	3.737E-02		
	Total	1.105	8			

Dependent Variable: KPXMRL
Scheffe

(I) PXMRL	(J) PXMRL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	.538441*	.158	.039	3.221E-02	1.044669
	20	.741300*	.158	.010	.235072	1.247528
10	0	-.538441*	.158	.039	-1.044669	-3.22E-02
	20	.202859	.158	.482	-.303369	.709087
20	0	-.741300*	.158	.010	-1.247528	-.235072
	10	-.202859	.158	.482	-.709087	.303369

*. The mean difference is significant at the .05 level.

KPXMRL

Scheffe[a]

PXMRL	N	Subset for alpha = .05	
		1	2
20	3	1.244400	
10	3	1.447259	
0	3		1.985700
Sig.		.482	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 5H Test of statistics on the effect of the concentration of Poloxamer 188 on the drug release rate from the microcapsules prepared with Eudragit RS100 in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KPXMRS	Between Groups	.348	2	.174	23.051	.002
	Within Groups	4.523E-02	6	7.538E-03		
	Total	.393	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: KPXMRS
Scheffe

(I) PXMRS	(J) PXMRS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-.293100*	.071	.018	-.520467	-6.57E-02
	20	-.477209*	.071	.002	-.704576	-.249842
10	0	.293100*	.071	.018	6.573E-02	.520467
	20	-.184109	.071	.104	-.411476	4.326E-02
20	0	.477209*	.071	.002	.249842	.704576
	10	.184109	.071	.104	-4.33E-02	.411476

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

KPXMRS

Scheffe[a]

PXMRS	N	Subset for alpha = .05	
		1	2
0	3	.684000	
10	3		.977100
20	3		1.161209
Sig.		1.000	.104

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 6H Test of statistics on the effect of the stirring rate on the drug release rate from the microcapsules in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KH	Between Groups	.160	4	4.006E-02	5.826	.011
	Within Groups	6.877E-02	10	6.877E-03		
	Total	.229	14			

Dependent Variable: KH
Scheffe

(I) RPM	(J) RPM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
250	500	7.097E-02	.068	.888	-.181584	.323517
	800	7.889E-03	.068	1.000	-.244661	.260440
	1000	-6.96E-02	.068	.894	-.322180	.182922
	1200	.235476	.068	.071	-1.71E-02	.488027
500	250	-7.10E-02	.068	.888	-.323517	.181584
	800	-6.31E-02	.068	.923	-.315628	.189473
	1000	-.140596	.068	.417	-.393147	.111955
	1200	.164509	.068	.281	-8.80E-02	.417060
800	250	-7.89E-03	.068	1.000	-.260440	.244661
	500	6.308E-02	.068	.923	-.189473	.315628
	1000	-7.75E-02	.068	.853	-.330069	.175032
	1200	.227587	.068	.083	-2.50E-02	.480137
1000	250	6.963E-02	.068	.894	-.182922	.322180
	500	.140596	.068	.417	-.111955	.393147
	800	7.752E-02	.068	.853	-.175032	.330069
	1200	.305105*	.068	.017	5.255E-02	.557656
1200	250	-.235476	.068	.071	-.488027	1.707E-02
	500	-.164509	.068	.281	-.417060	8.804E-02
	800	-.227587	.068	.083	-.480137	2.496E-02
	1000	-.305105*	.068	.017	-.557656	-5.26E-02

*. The mean difference is significant at the .05 level.

RPM	N	Subset for alpha = .05	
		1	2
1200	3	.668657	
500	3	.833167	.833167
800	3	.896244	.896244
250	3	.904133	.904133
1000	3		.973763
Sig.		.071	.417

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 7H Test of statistics on the effect of the concentration of Span 80 on the drug release rate from the microcapsules in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KHSPAN80	Between Groups	4.389E-02	4	1.097E-02	1.005	.449
	Within Groups	.109	10	1.092E-02		
	Total	.153	14			

Dependent Variable: KHSPAN80
Scheffe

(I) SPAN80	(J) SPAN80	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-6.96E-02	.085	.951	-.387863	.248620
	1.00	-.143384	.085	.606	-.461625	.174858
	1.50	-2.22E-02	.085	.999	-.340470	.296013
	2.00	-.115412	.085	.765	-.433654	.202829
.50	.00	6.962E-02	.085	.951	-.248620	.387863
	1.00	-7.38E-02	.085	.940	-.392004	.244479
	1.50	4.739E-02	.085	.988	-.270849	.365634
	2.00	-4.58E-02	.085	.989	-.364032	.272451
1.00	.00	.143384	.085	.606	-.174858	.461625
	.50	7.376E-02	.085	.940	-.244479	.392004
	1.50	.121155	.085	.734	-.197086	.439397
	2.00	2.797E-02	.085	.998	-.290270	.346213
1.50	.00	2.223E-02	.085	.999	-.296013	.340470
	.50	-4.74E-02	.085	.988	-.365634	.270849
	1.00	-.121155	.085	.734	-.439397	.197086
	2.00	-9.32E-02	.085	.873	-.411425	.225058
2.00	.00	.115412	.085	.765	-.202829	.433654
	.50	4.579E-02	.085	.989	-.272451	.364032
	1.00	-2.80E-02	.085	.998	-.346213	.290270
	1.50	9.318E-02	.085	.873	-.225058	.411425

SPAN80	N	Subset for alpha = .05
		1
.00	3	.830379
1.50	3	.852607
.50	3	.900000
2.00	3	.945791
1.00	3	.973763
Sig.		.606

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 8H Test of statistics on the effect of the core to wall ratio on the drug release rate from the microcapsules in simulated intestinal fluid without pancreatin pH 6.8± 0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KHRATIO	Between Groups	.526	3	.175	39.126	.000
	Within Groups	3.586E-02	8	4.483E-03		
	Total	.562	11			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: KHRATIO
Scheffe

(I) RATIO	(J) RATIO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	-.100861	.055	.392	-.291792	9.007E-02
	13	-.551087*	.055	.000	-.742019	-.360156
	23	-.282630*	.055	.006	-.473561	-9.17E-02
12	11	.100861	.055	.392	-9.01E-02	.291792
	13	-.450226*	.055	.000	-.641157	-.259295
	23	-.181768	.055	.062	-.372699	9.163E-03
13	11	.551087*	.055	.000	.360156	.742019
	12	.450226*	.055	.000	.259295	.641157
	23	.268458*	.055	.008	7.753E-02	.459389
23	11	.282630*	.055	.006	9.170E-02	.473561
	12	.181768	.055	.062	-9.16E-03	.372699
	13	-.268458*	.055	.008	-.459389	-7.75E-02

*. The mean difference is significant at the .05 level.

Scheffe[a]

RATIO	N	Subset for alpha = .05		
		1	2	3
11	3	.495783		
12	3	.596645	.596645	
23	3		.778413	
13	3			1.046871
Sig.		.392	.062	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 9H Test of statistics on the effect of the concentration of Poloxamer 188 on the drug release rate from the microcapsules prepared with Eudragit RL100 in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KHPXRL	Between Groups	.194	2	9.716E-02	56.452	.000
	Within Groups	1.033E-02	6	1.721E-03		
	Total	.205	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: KHPXRL
Scheffe

(I) PXMRL	(J) PXMRL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-.192586*	.034	.004	-.301225	-8.39E-02
	20	-.359621*	.034	.000	-.468260	-.250981
10	0	.192586*	.034	.004	8.395E-02	.301225
	20	-.167035*	.034	.008	-.275675	-5.84E-02
20	0	.359621*	.034	.000	.250981	.468260
	10	.167035*	.034	.008	5.840E-02	.275675

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

KHPXRL

Scheffe[a]

PXMRL	N	Subset for alpha = .05		
		1	2	3
0	3	.495783		
10	3		.688369	
20	3			.855404
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 10H Test of statistics on the effect of the concentration of Poloxamer 188 on the drug release rate from the microcapsules prepared with Eudragit RS100 in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KHPXRS	Between Groups	.178	2	8.907E-02	154.602	.000
	Within Groups	3.457E-03	6	5.761E-04		
	Total	.182	8			

Dependent Variable: KHPXRS
Scheffe

(I) PXMRS	(J) PXMRS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-.318495*	.020	.000	-.381352	-.255638
	20	-.273238*	.020	.000	-.336095	-.210381
10	0	.318495*	.020	.000	.255638	.381352
	20	4.526E-02	.020	.148	-1.76E-02	.108113
20	0	.273238*	.020	.000	.210381	.336095
	10	-4.53E-02	.020	.148	-.108113	1.760E-02

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

KHPXRS

Scheffe[a]

PXMRS	N	Subset for alpha = .05	
		1	2
0	3	.526022	
20	3		.799260
10	3		.844516
Sig.		1.000	.148

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 11H Test of statistics on the effect of the stirring rate on the dissolution efficiency of the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DERPM	Between Groups	326.964	4	81.741	65.478	.000
	Within Groups	12.484	10	1.248		
	Total	339.448	14			

Dependent Variable: DERPM
Scheffe

(I) RPM	(J) RPM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
250	500	-4.203852*	.912	.015	-7.606564	-.801140
	800	-11.482623*	.912	.000	-14.885335	-8.079911
	1000	-11.261679*	.912	.000	-14.664391	-7.858967
	1200	-10.948673*	.912	.000	-14.351386	-7.545961
500	250	4.203852*	.912	.015	.801140	7.606564
	800	-7.278771*	.912	.000	-10.681483	-3.876059
	1000	-7.057827*	.912	.000	-10.460539	-3.655115
	1200	-6.744821*	.912	.000	-10.147533	-3.342109
800	250	11.482623*	.912	.000	8.079911	14.885335
	500	7.278771*	.912	.000	3.876059	10.681483
	1000	.220944	.912	.999	-3.181768	3.623656
	1200	.533950	.912	.985	-2.868762	3.936662
1000	250	11.261679*	.912	.000	7.858967	14.664391
	500	7.057827*	.912	.000	3.655115	10.460539
	800	-.220944	.912	.999	-3.623656	3.181768
	1200	.313006	.912	.998	-3.089706	3.715718
1200	250	10.948673*	.912	.000	7.545961	14.351386
	500	6.744821*	.912	.000	3.342109	10.147533
	800	-.533950	.912	.985	-3.936662	2.868762
	1000	-.313006	.912	.998	-3.715718	3.089706

*. The mean difference is significant at the .05 level.

		Subset for alpha = .05		
RPM	N	1	2	3
250	3	13.264490		
500	3		17.468343	
1200	3			24.213164
1000	3			24.526169
800	3			24.747113
Sig.		1.000	1.000	.985

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000

Table 12H Test of statistics on the effect of the concentration of Span 80 on the dissolution efficiency of the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DESPAN	Between Groups	115.032	4	28.758	5.601	.012
	Within Groups	51.341	10	5.134		
	Total	166.373	14			

Dependent Variable: DESPAN
Scheffe

(I) SPAN80	(J) SPAN80	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-3.033054	1.850	.626	-9.933614	3.867506
	1.00	2.319701	1.850	.809	-4.580859	9.220261
	1.50	-1.004002	1.850	.989	-7.904561	5.896558
	2.00	4.989700	1.850	.202	-1.910860	11.890260
.50	.00	3.033054	1.850	.626	-3.867506	9.933614
	1.00	5.352755	1.850	.157	-1.547805	12.253315
	1.50	2.029052	1.850	.871	-4.871508	8.929612
	2.00	8.022754*	1.850	.022	1.122194	14.923314
1.00	.00	-2.319701	1.850	.809	-9.220261	4.580859
	.50	-5.352755	1.850	.157	-12.253315	1.547805
	1.50	-3.323703	1.850	.548	-10.224262	3.576857
	2.00	2.669999	1.850	.723	-4.230561	9.570559
1.50	.00	1.004002	1.850	.989	-5.896558	7.904561
	.50	-2.029052	1.850	.871	-8.929612	4.871508
	1.00	3.323703	1.850	.548	-3.576857	10.224262
	2.00	5.993702	1.850	.098	-.906858	12.894262
2.00	.00	-4.989700	1.850	.202	-11.890260	1.910860
	.50	-8.022754*	1.850	.022	-14.923314	-1.122194
	1.00	-2.669999	1.850	.723	-9.570559	4.230561
	1.50	-5.993702	1.850	.098	-12.894262	.906858

*. The mean difference is significant at the .05 level.

SPAN80	N	Subset for alpha = .05	
		1	2
2.00	3	21.856170	
1.00	3	24.526169	24.526169
.00	3	26.845870	26.845870
1.50	3	27.849872	27.849872
.50	3		29.878924
Sig.		.098	.157

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 13H Test of statistics on the effect of the core to wall ratio on the dissolution efficiency of the microcapsules in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DERATIO	Between Groups	128.526	3	42.842	20.744	.000
	Within Groups	16.522	8	2.065		
	Total	145.048	11			

Dependent Variable: DERATIO
Scheffe

(I) RATIO	(J) RATIO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	-.693053	1.173	.948	-4.791327	3.405221
	13	-1.593866	1.173	.624	-5.692140	2.504407
	23	6.682107*	1.173	.003	2.583833	10.780381
12	11	.693053	1.173	.948	-3.405221	4.791327
	13	-.900814	1.173	.896	-4.999088	3.197460
	23	7.375160*	1.173	.002	3.276886	11.473434
13	11	1.593866	1.173	.624	-2.504407	5.692140
	12	.900814	1.173	.896	-3.197460	4.999088
	23	8.275973*	1.173	.001	4.177700	12.374247
23	11	-6.682107*	1.173	.003	-10.780381	-2.583833
	12	-7.375160*	1.173	.002	-11.473434	-3.276886
	13	-8.275973*	1.173	.001	-12.374247	-4.177700

*. The mean difference is significant at the .05 level.

Scheffe[a]

RATIO	N	Subset for alpha = .05	
		1	2
23	3	12.037362	
11	3		18.719469
12	3		19.412522
13	3		20.313336
Sig.		1.000	.624

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 14H Test of statistics on the effect of the concentration of Poloxamer 188 on the dissolution efficiency of the microcapsules prepared with Eudragit RL100 in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DEPXMRL	Between Groups	27.253	2	13.627	6.402	.032
	Within Groups	12.771	6	2.129		
	Total	40.024	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: DEPXMRL
Scheffe

(I) PXMRL	(J) PXMRL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-3.321706	1.191	.083	-7.142275	.498863
	20	-3.974142*	1.191	.043	-7.794711	-.153572
10	0	3.321706	1.191	.083	-.498863	7.142275
	20	-.652436	1.191	.864	-4.473005	3.168134
20	0	3.974142*	1.191	.043	.153572	7.794711
	10	.652436	1.191	.864	-3.168134	4.473005

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

DEPXMRL

Scheffe[a]

PXMRL	N	Subset for alpha = .05	
		1	2
0	3	18.719469	
10	3	22.041175	22.041175
20	3		22.693611
Sig.		.083	.864

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 15H Test of statistics on the effect of the concentration of Poloxamer188 on the dissolution efficiency of the microcapsules prepared with Eudragit RS100 in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DEPXMRS	Between Groups	193.342	2	96.671	369.174	.000
	Within Groups	1.571	6	.262		
	Total	194.913	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: DEPXMRS
Scheffe

(I) PXMRS	(J) PXMRS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-8.444776*	.418	.000	-9.784825	-7.104727
	20	-10.793933*	.418	.000	-12.133982	-9.453883
10	0	8.444776*	.418	.000	7.104727	9.784825
	20	-2.349157*	.418	.004	-3.689206	-1.009107
20	0	10.793933*	.418	.000	9.453883	12.133982
	10	2.349157*	.418	.004	1.009107	3.689206

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

DEPXMRS

Scheffe[a]

PXMRS	N	Subset for alpha = .05		
		1	2	3
0	3	5.724084		
10	3		14.168860	
20	3			16.518017
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 16H Test of statistics on the effect of the stirring rate on the dissolution efficiency of the microcapsules in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DERPM	Between Groups	586.254	4	146.564	17.714	.000
	Within Groups	82.740	10	8.274		
	Total	668.994	14			

Dependent Variable: DERPM
Scheffe

(I) RPM	(J) RPM	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
250	500	-3.299669	2.349	.741	-12.059802	5.460465
	800	-16.277479*	2.349	.001	-25.037613	-7.517345
	1000	-13.650951*	2.349	.003	-22.411085	-4.890817
	1200	-11.707766*	2.349	.009	-20.467900	-2.947632
500	250	3.299669	2.349	.741	-5.460465	12.059802
	800	-12.977811*	2.349	.004	-21.737944	-4.217677
	1000	-10.351283*	2.349	.019	-19.111416	-1.591149
	1200	-8.408097	2.349	.062	-17.168231	.352036
800	250	16.277479*	2.349	.001	7.517345	25.037613
	500	12.977811*	2.349	.004	4.217677	21.737944
	1000	2.626528	2.349	.863	-6.133606	11.386662
	1200	4.569713	2.349	.477	-4.190421	13.329847
1000	250	13.650951*	2.349	.003	4.890817	22.411085
	500	10.351283*	2.349	.019	1.591149	19.111416
	800	-2.626528	2.349	.863	-11.386662	6.133606
	1200	1.943185	2.349	.948	-6.816949	10.703319
1200	250	11.707766*	2.349	.009	2.947632	20.467900
	500	8.408097	2.349	.062	-.352036	17.168231
	800	-4.569713	2.349	.477	-13.329847	4.190421
	1000	-1.943185	2.349	.948	-10.703319	6.816949

*. The mean difference is significant at the .05 level.

RPM	N	Subset for alpha = .05		
		1	2	3
250	3	20.354438		
500	3	23.654107	23.654107	
1200	3		32.062204	32.062204
1000	3			34.005390
800	3			36.631918
Sig.		.741	.062	.477

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 17H Test of statistics on the effect of the concentration of Span 80 on the dissolution efficiency of the microcapsules in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DESPAN	Between Groups	60.839	4	15.210	1.619	.244
	Within Groups	93.945	10	9.394		
	Total	154.784	14			

Dependent Variable: DESPAN
Scheffe

(I) SPAN80	(J) SPAN80	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-1.448434	2.503	.986	-10.782891	7.886023
	1.00	-3.916292	2.503	.663	-13.250749	5.418165
	1.50	4.038E-02	2.503	1.000	-9.294080	9.374834
	2.00	2.192042	2.503	.937	-7.142415	11.526499
.50	.00	1.448434	2.503	.986	-7.886023	10.782891
	1.00	-2.467859	2.503	.907	-11.802316	6.866598
	1.50	1.488810	2.503	.984	-7.845647	10.823267
	2.00	3.640476	2.503	.717	-5.693981	12.974933
1.00	.00	3.916292	2.503	.663	-5.418165	13.250749
	.50	2.467859	2.503	.907	-6.866598	11.802316
	1.50	3.956669	2.503	.655	-5.377788	13.291126
	2.00	6.108335	2.503	.277	-3.226122	15.442792
1.50	.00	-4.04E-02	2.503	1.000	-9.374834	9.294080
	.50	-1.488810	2.503	.984	-10.823267	7.845647
	1.00	-3.956669	2.503	.655	-13.291126	5.377788
	2.00	2.151666	2.503	.941	-7.182791	11.486123
2.00	.00	-2.192042	2.503	.937	-11.526499	7.142415
	.50	-3.640476	2.503	.717	-12.974933	5.693981
	1.00	-6.108335	2.503	.277	-15.442792	3.226122
	1.50	-2.151666	2.503	.941	-11.486123	7.182791

SPAN80	N	Subset for alpha = .05
		1
2.00	3	27.897055
1.50	3	30.048721
.00	3	30.089097
.50	3	31.537531
1.00	3	34.005390
Sig.		.277

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 18H Test of statistics on the effect of the core to wall ratio on the dissolution efficiency of the microcapsules in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DERATIO	Between Groups	364.336	3	121.445	29.743	.000
	Within Groups	32.665	8	4.083		
	Total	397.002	11			

Dependent Variable: DERATIO
Scheffe

(I) RATIO	(J) RATIO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
11	12	.698644	1.650	.980	-5.063807	6.461096
	13	-10.426703*	1.650	.002	-16.189154	-4.664251
	23	4.398101	1.650	.147	-1.364350	10.160553
12	11	-.698644	1.650	.980	-6.461096	5.063807
	13	-11.125347*	1.650	.001	-16.887799	-5.362895
	23	3.699457	1.650	.248	-2.062995	9.461908
13	11	10.426703*	1.650	.002	4.664251	16.189154
	12	11.125347*	1.650	.001	5.362895	16.887799
	23	14.824804*	1.650	.000	9.062352	20.587255
23	11	-4.398101	1.650	.147	-10.160553	1.364350
	12	-3.699457	1.650	.248	-9.461908	2.062995
	13	-14.824804*	1.650	.000	-20.587255	-9.062352

*. The mean difference is significant at the .05 level.

DERATIO

Scheffe[a]

RATIO	N	Subset for alpha = .05	
		1	2
23	3	17.920118	
12	3	21.619575	
11	3	22.318220	
13	3		32.744922
Sig.		.147	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 19H Test of statistics on the effect of the concentration of Poloxamer 188 on the dissolution efficiency of the microcapsules prepared with Eudragit RL100 in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DEPXMRL	Between Groups	190.591	2	95.295	37.992	.000
	Within Groups	15.050	6	2.508		
	Total	205.641	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: DEPXMRL
Scheffe

(I) PXMRL	(J) PXMRL	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-9.052774*	1.293	.001	-13.200215	-4.905333
	20	-10.342808*	1.293	.001	-14.490249	-6.195366
10	0	9.052774*	1.293	.001	4.905333	13.200215
	20	-1.290034	1.293	.631	-5.437475	2.857408
20	0	10.342808*	1.293	.001	6.195366	14.490249
	10	1.290034	1.293	.631	-2.857408	5.437475

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

DEPXMRL

Scheffe[a]

PXMRL	N	Subset for alpha = .05	
		1	2
0	3	22.318220	
10	3		31.370994
20	3		32.661027
Sig.		1.000	.631

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 20H Test of statistics on the effect of the concentration of Poloxamer 188 on the dissolution efficiency of the microcapsules prepared with Eudragit RS100 in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DEPXMRS	Between Groups	505.330	2	252.665	285.038	.000
	Within Groups	5.319	6	.886		
	Total	510.648	8			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: DEPXMRS
Scheffe

(I) PXMRS	(J) PXMRS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	-15.649257*	.769	.000	-18.114781	-13.183734
	20	-16.130674*	.769	.000	-18.596198	-13.665150
10	0	15.649257*	.769	.000	13.183734	18.114781
	20	-.481417	.769	.827	-2.946941	1.984107
20	0	16.130674*	.769	.000	13.665150	18.596198
	10	.481417	.769	.827	-1.984107	2.946941

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

DEPXMRS

Scheffe[a]

PXMRS	N	Subset for alpha = .05	
		1	2
0	3	12.875111	
10	3		28.524368
20	3		29.005785
Sig.		1.000	.827

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 21H Test of statistics on the drug release rate of microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated gastric fluid without pepsin pH1.2 by ONE-WAY ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
KPOLYMER	Between Groups	2.542	1	2.542	45.240	.003
	Within Groups	.225	4	5.618E-02		
	Total	2.766	5			

Table 22H Test of statistics on the drug release rate of microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated intestinal fluid without pancreatin without pepsin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
KPOLY68	Between Groups	4.15E+17	1	4.15E+17	1227.028	.000
	Within Groups	1.35E+15	4	3.38E+14		
	Total	4.16E+17	5			

Table 23H Test of statistics on the the dissolution efficiency of microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated gastric fluid without pepsin pH 1.2 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DEPOLYME	Between Groups	253.320	1	253.320	83.882	.001
	Within Groups	12.080	4	3.020		
	Total	265.400	5			

Table 24H Test of statistics on the the dissolution efficiency of microcapsules prepared with Eudragit RL100 and Eudragit RS100 in simulated intestinal fluid without pancreatin pH 6.8±0.1 by ONE-WAY ANOVA.

		Sum of Squares	df	Mean Square	F	Sig.
DEPOLYME	Between Groups	133.758	1	133.758	119.821	.000
	Within Groups	4.465	4	1.116		
	Total	138.224	5			

APPENDIX I
RANKING SCORES FOR MICROCAPSULES

Table 1I Ranking score of parameters of microcapsules prepared with varied stirring rates.

Stirrng rate (rpm)	250	500	800	1000	1200
% Yield	95.054	99.290	97.278	98.385	99.568
(score)	(1)	(4)	(2)	(3)	(5)
AG content	11.68±0.97	10.71±1.18	11.98±0.66	13.46±1.02	12.18±0.97
(score)	(3)	(1)	(4)	(5)	(2)
D AGcontent	1.79±0.09	1.93±0.07	1.81±0.04	1.81±0.09	1.70±0.06
(score)	(2)	(5)	(3)	(4)	(1)
% Core entrapment	83.68±5.80	78.79±7.55	85.97±4.36	93.24±5.74	85.40±5.66
(score)	(2)	(1)	(4)	(5)	(3)
Mean size (μm)±SD	192.16±121.05	95.67±76.51	69.88±43.05	65.38±34.83	53.42±32.39
(score)	(1)	(2)	(3)	(4)	(5)
P.I.	0.63	0.80	0.62	0.53	0.61
(score)	(2)	(1)	(3)	(5)	(4)
K Higuchi	1.5616	1.4685	1.4051	1.8289	1.5213
(pH 1.2)	±0.1889	±0.0471	±0.1227	±0.3937	±0.0698
(score)	(4)	(2)	(1)	(5)	(3)
K Higuchi	0.9041	0.8332	0.8962	0.9738	0.6687
(pH 6.8±0.1)	±0.0777	±0.0201	±0.0426	±0.0350	±0.1578
(score)	(4)	(2)	(3)	(5)	(1)
Total score	19	18	23	**36**	24

Table 2I Ranking score of parameters of microcapsules prepared with varied Span80 concentrations

Span80	0%	0.5%	1.0%	1.5%	2.0%
% Yield	95.43	92.65	98.38	98.51	101.68
(score)	(2)	(1)	(3)	(4)	(5)
AG content	13.64±1.16	15.49±0.80	13.46±1.02	12.92±1.02	14.25±1.66
(score)	(3)	(5)	(2)	(1)	(4)
DAG content	1.95±0.03	1.84±0.05	1.81±0.09	1.80±0.06	1.32±0.24
(score)	(5)	(4)	(3)	(2)	(1)
% Core entrapment	96.64±7.23	107.38±4.79	93.24±5.74	90.67±6.64	96.04±11.62
(score)	(4)	(5)	(2)	(1)	(3)
Mean size (μm)±SD	42.4±20.2	46.4±21.6	65.4±34.9	56.6±29.2	60.1±35.7
(score)	(5)	(4)	(2)	(3)	(1)
P.I.	0.48	0.47	0.53	0.52	0.59
(score)	(4)	(5)	(2)	(3)	(1)
K Higuchi	1.9770	2.8669	1.8289	1.5910	1.6874
(pH 1.2)	±0.2263	±0.2813	±0.3937	±0.1001	±0.0629
(score)	(4)	(5)	(3)	(1)	(2)
K Higuchi	0.8304	0.9027	0.9738	0.8526	0.9458
(pH 6.8±0.1)	±0.1452	±0.1333	±0.0350	±0.0705	±0.0960
(score)	(1)	(3)	(5)	(2)	(4)
Total score	28	**32**	22	17	21

Table 3I Ranking score of parameters of microcapsules prepared with varied the core to wall ratios.

Core to wall	1:1	1:2	2:3	1:3
% Yield	99.823	88.137	92.031	82.981
(score)	(5)	(3)	(4)	(2)
AG content	22.06+1.08	16.04+0.91	16.53+0.20	11.43+0.34
(score)	(5)	(3)	(4)	(2)
DAG content	2.74± 0.04	1.90± 0.04	2.38± 0.18	1.59± 0.01
(score)	(5)	(3)	(4)	(2)
% Core entrapment	88.38± 3.73	95.62± 4.94	84.13± 1.03	92.13± 2.50
(score)	(3)	(5)	(2)	(4)
Mean size (μm)±SD	42.2± 23.9	53.1± 33.4	381.3± 316.1	120.1± 69.9
(score)	(5)	(4)	(2)	(3)
P.I.	0.57	0.63	0.83	0.58
(score)	(5)	(3)	(2)	(4)
K Higuchi (pH 1.2)	1.9857 ±0.3310	1.8298 ±0.1270	1.5938 ±0.05162	1.9384 ±0.0611
(score)	(5)	(2)	(4)	(3)
K Higuchi (pH 6.8±0.1)	0.4958 ±0.0265	0.5966 ±0.0450	0.7784 ±0.0234	1.0469 ±0.1211
(score)	(2)	(3)	(4)	(5)
Total score	**35**	26	26	25

VITA

Mr.Saroch Onlaor was born on December 9, 1971 in Ubonrachathanee, Thailand. He received the Bachelor degree of Pharmacy from the Faculty of Pharmacy, Chiangmai University, Chiangmai in 1996. He has worked at Yasothorn Hospital. He entered the Master's Degree Program in Pharmacy at Chulalongkorn University in 1999.