CROSSLINKED HARD GELATIN CAPSULE AS A STRUCTURAL ASSEMBLY OF ELEMENTARY AND PUSH-PULL OSMOTIC PUMP DELIVERY SYSTEM

BY

CHAOWALIT MONTON

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACY

COLLEGE OF PHARMACY

GRADUATE SCHOOL, RANGSIT UNIVERSITY

ACADEMIC YEAR 2019

การใช้แคปซูลเจลาตินแข็งแบบเชื่อมขวางเป็นโครงสร้างของระบบนำส่งยาชนิดออสโมติกปั๊มแบบพื้นฐานและแบบผลัก-ดึง

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ดุษฎีนิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตาม
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12 กรกฎาคม 2562

Dissertation entitled

CROSSLINKED HARD GELATIN CAPSULE AS A STRUCTURAL ASSEMBLY OF ELEMENTARY AND PUSH-PULL OSMOTIC PUMP DELIVERY SYSTEM

by

CHAOWALIT MONTON

was submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Pharmacy

Rangsit University
Academic Year 2019

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July 12, 2019

ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor, Assoc. Prof. Dr. Poj Kulvanich, for his advice, encouragement, and guidance throughout this work. I am obliged to the thesis and proposal examining committees: Assoc. Prof. Dr. Sontaya Limmatvapirat, Assoc. Prof. Prasert Akkaramongkolporn, Assoc. Prof. Srisagul Sungthongjeen, Assoc. Prof. Dr. Pienkit Dangprasirt, Dr. Orawan Theanphong, and Dr. Sansanee Pongwai, for their valuable comments and suggestion. I also gratefully acknowledge Asst. Prof. Dr. Thanapat Songsak for his supports during the course of my study and Prof. Dr. Krisana Kraisintu for suggesting me to pursue the Ph.D. study program.

I would like to thank Siam Pharmaceutical Co., Ltd., Biolab Co., Ltd., Colorcon, Inc., Onimax Co., Ltd., and Sun Herb Thai Chinese Manufacturing for supporting some drug substances and materials; Capsule Products Co., Ltd. for supporting hard gelatin capsule shells; Faculty of Pharmaceutical Sciences, Ubon Ratchathani University and Asst. Prof. Dr. Sureewan Duangjit for making use of instrument to characterize some aspects of the drug substance properties; Asst. Prof. Dr. Jirapornchai Suksaeree for his assistance in mechanical testing of the capsule shells; Prof. J. E. Moreton, University of Maryland at Baltimore for his reading and editing English language of the published paper; Graduate School and College of Pharmacy, Rangsit University for research funding; Members of Drug and Herbal Product Research and Development Center and Department of Pharmaceutical Technology, Rangsit University for supporting scientific instruments and chemicals; Ajarn Chitradee Luprasong for her supporting some research funding and Rangsit University for giving the scholarship.

Above all, I would like to express my sincere and deepest gratefulness to my family for their support, suggestion, and encouragement throughout. Finally, I acknowledge the assistance from those whom I do not mention by names.

Chaowalit Monton

Researcher

กิตติกรรมประกาศ

ข้าพเจ้าขอขอบพระคุณ รศ.ดร.ภก. พจน์ กุลวานิช ที่ปรึกษาดุษฎีนิพนธ์ที่คอยให้คำปรึกษา ให้กำลังใจ และชี้แนะแนวทางการแก้ไขปัญหาตลอดระยะเวลาการศึกษาของข้าพเจ้า จนดุษฎีนิพนธ์ประสบผลสำเร็จด้วยดี ขอขอบพระคุณ รศ.ดร.ภก. สนทยา ลิ้มมัทวาภิรัติ์ รศ.ดร.ภก. ประเสริฐ อัครมงคลพร รศ.ดร.ภก.ศรีสกุล สังข์ทองจีน รศ.ดร.ภก. เพียรกิจ แดงประเสริฐ ดร.ภญ. อรวรรณ เทียรฆ์พงษ์ และ ดร.ภญ. ศันสนีย พงษ์วัย กรรมการสอบโครงร่างดุษฎีนิพนธ์และกรรมการสอบดุษฎีนิพนธ์ ที่ให้คำแนะนำที่ดีสำหรับการทำวิจัย ขอขอบพระคุณ ผศ.ดร.ภก.ธนภัทร ทรงศักดิ์ ที่ให้ความอนุเคราะห์ตลอดการวิจัย ขอขอบพระคุณ ศ.(พิเศษ) ดร.ภญ. กฤษณา ไกรสินธุ์ ที่สนับสนุนให้ข้าพเจ้าศึกษาต่อในระดับปริญญาเอก

ข้าพเจ้าขอขอบพระคุณบริษัท สยามฟาร์มาซูติคอล จำกัด บริษัท ไบโอแลป จำกัด บริษัท คัลเลอร์คอน จำกัด บริษัท โอนิแมกซ์ จำกัด และโรงงานซัน เฮิร์บ ไทย ไชนีส แมนูแฟคเจอริ่ง ที่อนุเคราะห์ตัวยาสำคัญและสารช่วยทางเภสัชกรรม บริษัท แคพซูลโปรดักส์ จำกัด ที่อนุเคราะห์เปลือกแคปซูลเจลาตินแข็ง คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานีและ ผศ.ดร.ภญ.สุรีวัลย์ ดวงจิตต์ ที่สนับสนุนเครื่องมือและบุคลากรสำหรับการวิเคราะห์คุณสมบัติของสาร ผศ.ดร.จิระพรชัย สุขเสรี ที่อนุเคราะห์การทดสอบคุณสมบัติเชิงกลของแคปซูล ขอบพระคุณ Prof. J. E. Moreton, School of Pharmacy, University of Maryland at Baltimore ที่ตรวจบทความวิจัยภาษาอังกฤษ ขอขอบพระคุณบัณฑิตวิทยาลัย และวิทยาลัยเภสัชศาสตร์ มหาวิทยาลัยรังสิต ที่สนับสนุนทุนวิจัยสำหรับดุษฎีนิพนธ์ ขอบพระคุณบุคลากรศูนย์วิจัยและพัฒนายาและผลิตภัณฑ์สมุนไพร และภาควิชาเทคโนโลยีเภสัชกรรม วิทยาลัยเภสัชศาสตร์ มหาวิทยาลัยรังสิต ที่สนับสนุนเครื่องมือและสารเคมีที่ใช้ในการวิจัย ขอบพระคุณ อ.ภญ.จิตรดี ลุประสงค์ ที่สนับสนุนงบประมาณสำหรับการวิจัย และขอขอบพระคุณมหาวิทยาลัยรังสิต ที่สนับสนุนทุนการศึกษาในระดับปริญญาเอกแก่ข้าพเจ้า

ข้าพเจ้าขอขอบพระคุณบิดามารดา ครอบครัว ญาติมิตร ตลอดจนครูบาอาจารย์ที่คอยสนับสนุน ให้คำแนะนำ และให้กำลังใจข้าพเจ้าด้วยดีเสมอมา ข้าพเจ้าจะไม่สามารถประสบความสำเร็จในการศึกษาได้เลยหากขาดการสนับสนุนจากทุกท่าน และข้าพเจ้าขออภัยหากไม่สามารถเอ่ยนามทุกท่านได้ครบ

เชาวลิต มณฑล

ผู้วิจัย

5608990 : MAJOR: PHARMACY; Ph.D. (PHARMACY)

KEYWORDS : CROSSLINKED HARD GELATIN CAPSULE, OSMOTIC PUMP, ELEMENTARY OSMOTIC PUMP, PUSH-PULL OSMOTIC PUMP

CHAOWALIT MONTON: CROSSLINKED HARD GELATIN CAPSULE AS A STRUCTURAL ASSEMBLY OF ELEMENTARY AND PUSH-PULL OSMOTIC PUMP DELIVERY SYSTEM. DISSERTATION ADVISOR: ASSOC. PROF. POJ KULVANICH, Ph.D., 144 p.

The objective of this study was to characterize the crosslinked hard gelatin capsules in order to use as a structural assembly of osmotic pump capsules for delivery of different water solubility model drugs including diltiazem hydrochloride, propranolol hydrochloride, ambroxol hydrochloride, and paracetamol. The hard gelatin capsules (HGCs) were crosslinked by exposure to formaldehyde vapor for 6, 12, and 24 hours. According to the results, the HGC shell crosslinked for 12 hours was selected for preparation of elementary osmotic pump (EOP) and push-pull osmotic pump (PPOP) due to its insoluble property, low formaldehyde residue, stability after storage, and providing reproducible drug release profiles. Drug release from EOP capsules was dependent of drug substance type and loading dose except diltiazem hydrochloride, a very highly water soluble drug. Drug release from PPOP capsules was independent of drug substance type, loading dose, and capsule size. But it was dependent of amount of polyethylene oxide in a pull layer. The osmolality of release medium affected drug release from PPOP capsules more than from EOP capsules. Drug release study using a medium with digestive enzymes did not alter drug release compared to medium without enzymes. EOP and PPOP capsules prepared using 12-month stored crosslinked HGCs gave consistent release profiles compared to those prepared using initial crosslinked HGCs. Almost all of the formulations gave drug release approaching Higuchi's release kinetic model. However, ambroxol hydrochloride could not deliver via these devices because of its high dense drug particle. In summary, the developed EOP and PPOP capsules were an alternative osmotic device that could be used for drug delivery systems and were applicable for several drugs with different water solubilities.

Student's Signature ..... Dissertation Advisor's Signature .....

5608990 : สาขาวิชาเอก: เภสัชศาสตร์; ภ.ด. (เภสัชศาสตร์)

คำสำคัญ : แคปซูลเจลาตินแข็งแบบเชื่อมขวาง, ออสโมติกปั๊ม, ออสโมติกปั๊มแบบพื้นฐาน, ออสโมติกปั๊มแบบผลัก-ดึง

เชาวลิต มณฑล: การใช้แคปซูลเจลาตินแข็งแบบเชื่อมขวางเป็นโครงสร้างของระบบนำส่งยาชนิดออสโมติกปั๊มแบบพื้นฐานและแบบผลัก-ดึง (CROSSLINKED HARD GELATIN CAPSULE AS A STRUCTURAL ASSEMBLY OF ELEMENTARY AND PUSH-PULL OSMOTIC PUMP DELIVERY SYSTEM) อาจารย์ที่ปรึกษา: รศ.ดร.ภก. พจน์ กุลวานิช, 144 หน้า.

วัตถุประสงค์ของการศึกษานี้เพื่อแสดงลักษณะเฉพาะของแคปซูลเจลาตินแข็งแบบเชื่อมขวางสำหรับใช้เป็นโครงสร้างของระบบออสโมติกปั๊มในการนำส่งยาที่มีค่าการละลายน้ำแตกต่างกัน ได้แก่ ดิลไทอะเซ็ม ไฮโดรคลอไรด์ โพรพราโนลอล ไฮโดรคลอไรด์ แอมบร็อกซอล ไฮโดรคลอไรด์ และพาราเซตามอล ทำการเตรียมแคปซูลเจลาตินแข็งชนิดเชื่อมขวางโดยการอังด้วยไอของฟอร์มัลดีไฮด์เป็นเวลา 6 12 และ 24 ชั่วโมง จากผลการศึกษาได้เลือกแคปซูลเจลาตินแข็งที่เชื่อมขวางเป็นเวลา 12 ชั่วโมง เพื่อเตรียมระบบออสโมติกปั๊มแบบพื้นฐาน (EOP) และแบบผลัก-ดึง (PPOP) เนื่องจากมีคุณสมบัติไม่ละลายน้ำ มีปริมาณฟอร์มัลดีไฮด์ตกค้างต่ำ มีความคงตัวดี และปลดปล่อยยาได้คงที่เมื่อเก็บไว้เป็นระยะเวลาหนึ่ง การปลดปล่อยยาจากออสโมติกปั๊มแบบพื้นฐานขึ้นอยู่กับชนิดของยาและขนาดยาที่บรรจุ ยกเว้นดิลไทอะเซ็ม ไฮโดรคลอไรด์ ซึ่งเป็นยาที่ละลายน้ำดีมาก การปลดปล่อยยาจากออสโมติกปั๊มแบบผลัก-ดึงไม่ขึ้นอยู่กับชนิดของยา ขนาดยาที่บรรจุ และขนาดของแคปซูล แต่ขึ้นอยู่กับปริมาณของพอลีเอธิลีนออกไซด์ในชั้นดึง ค่าออสโมแลลลิตี้ของตัวกลางการละลายมีผลต่อการปลดปล่อยยาจากออสโมติกปั๊มแบบผลัก-ดึงมากกว่าออสโมติกปั๊มแบบพื้นฐาน การใช้ตัวกลางการละลายที่มีส่วนผสมของเอนไซม์ย่อยโปรตีนไม่มีผลต่อการปลดปล่อยยาเมื่อเทียบกับการใช้ตัวกลางการละลายที่ไม่มีส่วนผสมของเอนไซม์ย่อยโปรตีน ออสโมติกปั๊มแบบพื้นฐานและแบบผลัก-ดึงที่เตรียมจากแคปซูลแบบเชื่อมขวางเมื่อเก็บไว้นาน 12 เดือน มีรูปแบบการปลดปล่อยยาคล้ายเดิมกับระบบที่เตรียมจากเปลือกแคปซูลชนิดเชื่อมขวางที่เวลาเริ่มต้นตำรับที่เตรียมได้ส่วนใหญ่มีจลนศาสตร์การปลดปล่อยแบบฮิกูชิ อย่างไรก็ตามระบบออสโมติกปั๊มที่พัฒนาขึ้นนี้ไม่สามารถใช้เป็นระบบนำส่งแอมบร็อกซอล ไฮโดรคลอไรด์ได้ เนื่องจากอนุภาคยามีความหนาแน่นสูง กล่าวโดยสรุป แคปซูลออสโมติกปั๊มแบบพื้นฐานและแบบผลัก-ดึงนี้เป็นระบบออสโมติกปั๊มทางเลือก และสามารถประยุกต์ใช้กับยาที่มีค่าการละลายน้ำที่แตกต่างกันได้

ลายมือชื่อนักศึกษา ..... ลายมือชื่ออาจารย์ที่ปรึกษา .....

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ABBREVIATIONS

Abbreviations	Meaning
%v/v	percent volume by volume
%w/v	percent weight by volume
%w/w	percent weight by weight
°C	degree celsius
µg/mL	microgram per milliliter
µL	microliter
µm	micrometer
AMB HCl	ambroxol hydrochloride
atm	atmosphere
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared
BCA	bicinchoninic acid
CA	cellulose acetate
cm^{-1}	reciprocal centimeter
CPOP	controlled-porosity osmotic pump
CR	controlled release
D10	10 volume percent less than or equal to diameter
D50	50 volume percent less than or equal to diameter
D90	90 volume percent less than or equal to diameter
DIL HCl	diltiazem hydrochloride
EOP	elementary osmotic pump
Eq.	equation
f_2	similarity factor
g	gram
GI	gastrointestinal
h	hour/ hours
GITS	gastrointestinal therapeutic systems

ABBREVIATIONS (CONT.)

Abbreviations	Meaning
HCl	hydrochloric acid
HGC	hard gelatin capsule
HGCs	hard gelatin capsules
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose/ hypromellose
i.d.	internal diameter
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
kg	kilogram
LOD	limit of detection
LOQ	limit of quantitation
L-OROS	liquid oral osmotic pump
m^3	cubic meter
MBTH	3-methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mPa s	milliPascal second
Mw	molecular weight
N	Newton
NaCl	sodium chloride
NF	National Formulary
ng	nanogram
nm	nanometer
One-Way ANOVA	One-way analysis of variance

ABBREVIATIONS (CONT.)

Abbreviations	Meaning
p-value	probability value
PAR	paracetamol
PBS	phosphate buffer solution
PEO	polyethylene oxide
PEOs	polyethylene oxides
pH	potential of hydrogen ion
pKa	logarithm of the acid dissociation constant
PPOP	push-pull osmotic pump
PRO HCl	propranolol hydrochloride
R^2	coefficient of determination
RSD	relative standard deviation
SD	standard deviation
SGF	simulated gastric fluid
SIF	simulated intestinal fluid
SOT	sandwiched osmotic tablets
US	The United States
USP	The United States Pharmacopeia
UV	ultraviolet
WSR	water-soluble resins

CHAPTER 1

INTRODUCTION

1.1 Background and rationale

Tablet-based osmotic pumps are commonly prepared and commercially available, while capsule-based osmotic pump is found with a limit number of publications and there is no marketed products. Capsule dosage form is selected in this study because it has several advantages over tablet dosage form such as fewer additives, faster development, fewer production steps, flexibility in formulation, easier to swallow, etc. (Cole, 1998). Recently, cellulose acetate (CA) capsule shell has been prepared as a structural assembly of capsule-based osmotic pump. These CA capsules play important role on controlling of water imbibing into the internal structure of osmotic pump. Waterman et al. (2011) produced CA capsule with a multistage procedure. Initially, high-density polyethylene mold is prepared. After that, Tween® 80 solution is sprayed onto the mold to make removal of CA capsule shell from molds easier with a fewer defect. Then, CA solution containing other additives is coated onto the mold and then dried in a hot air oven. The obtained CA capsule shell is trimmed using a razor blade and removed from the mold using positive air pressure. Finally, the capsule shell is drilled to have the delivery orifice at the top of capsule cap. The other reported procedure, Liu et al. (2014) use a Coni-Snap® hard gelatin capsule as a mold of CA capsule production. Coating solution containing CA and plasticizer dissolved in acetone is filled into Coni-Snap® hard gelatin capsule, then, the filled shell is dried to obtain CA semipermeable membrane capsule shell. Finally, the shell is peeled off from hard gelatin capsule shell. Sun et al. (2019) prepare enteric positioning osmotic pump capsule by dipping method. Stainless steel molds are dipped into coating solution containing CA and hydroxypropyl methylcellulose phthalate HP50 for three times, then, they are dried. The obtained enteric positioning osmotic pump capsules are

removed from the mold by capsule pliers and cut into sizes. They are drilled to produce delivery orifice by laser followed by sealing the hold with hydroxypropyl methylcellulose phthalate HP50. Unfortunately, CA capsule shell preparation might be complicated for industrial scale production. In addition, ethylcellulose-coated capsule was also applied as a structural assembly of osmotic pump capsule. However, it lacked of sealing band, so capsule body and cap could be dismantled due to high osmotic pressure inside the device (Shaikh, Deshmukh, Patil, Chatap, & Bari, 2013).

Hard gelatin capsules (HGCs) are commercially available material. The usage of non-crosslinked HGCs for osmotic pump preparation may cause difficulty during manufacturing process due to their water soluble manner. However, HGCs will be cheaper than the specific osmotic capsule shells i.e., CA capsules. The crosslinking of HGCs with formaldehyde vapor can be an interesting alternative method for solving the drawback of using plain HGCs; complexity and cost of capsule shell production are reduced. Wichianprasit and Kulvanich (2009) succeeded in using the crosslinked HGCs as a structural assembly of elementary osmotic pump (EOP) for delivery of diltiazem hydrochloride and propranolol hydrochloride, a freely water soluble and water soluble model drugs, respectively. Using of crosslinked HGCs provided less fluctuation of propranolol hydrochloride release rate compared to non-crosslinked HGCs. While, the release rate of diltiazem hydrochloride is apparently similar for both crosslinked and non-crosslinked HGCs due to highly water soluble property of diltiazem hydrochloride. However, physicochemical and mechanical properties of osmotic pump capsule are required to fulfill the feasibility of crosslinked HGCs in osmotic drug delivery application. A type of osmotic pump such as push-pull osmotic pump (PPOP) can be prepared using crosslinked HGCs as a structural assembly. The researchers expected that this crosslinked HGCs can be used as a structural assembly of both EOP and PPOP. Furthermore, this developing osmotic pump system can be applied to the delivery of various types of drug.

1.2 Objectives

The aims of the work are:

1) to prepare and evaluate the physicochemical and mechanical properties of crosslinked hard gelatin capsule.

2) to prepare and evaluate the PPOP prepared using crosslinked HGC as a structural assembly for delivery of model drugs with different water solubilities; diltiazem hydrochloride, propranolol hydrochloride, ambroxol hydrochloride, and paracetamol. In addition, factors affecting drug release are also investigated.

3) to compare the PPOP capsule with EOP capsule.

1.3 Expected outcomes

1.3.1 Osmotic pump drug delivery capsule is developed and to be an alternative system instead of tablet form.

1.3.2 Understand the preparation condition and physicochemical and mechanical properties of crosslinked HGC that suitable for using as a structural assembly for the osmotic pump system.

1.3.3 Obtain the formulation of the EOP and PPOP capsule for delivery of different water solubility model drugs.

1.3.4 Obtain the osmotic pump capsule system with clarified release mechanism.

CHAPTER 2

LITERATURE REVIEWS

Oral drug administration is the most widely used, due to their convenient way compared to other routes. However, the rate and amount of drug absorption from conventional preparation may vary and unpredictable, depending on physicochemical properties of drug, presence of pharmaceutical additives, food consumption, pH of gastrointestinal (GI) tract, GI motility, etc. Sub-therapeutic and supratherapeutic plasma drug levels are usually found in conventional preparations, cause to marked side effects in some patients (Verma, Mishra, & Garg, 2000).

Controlled release (CR) dosage form provides a uniform concentration or extent of the drug at the absorption site. It can maintain of plasma drug concentrations within a therapeutic range. CR dosage form minimizes side effects of the drug and also decreases the dosing frequency (Verma et al., 2000). Oral CR dosage form can be divided into 5 categories including dissolution-controlled release (i.e. encapsulation dissolution control and matrix dissolution control), diffusion-controlled release (i.e. reservoir devices and matrix devices), ion exchange resins, gastroretentive systems, and osmotic controlled release (Venkatraman, Davar, Chester, & Kleiner, 2000). However, it can be affected by pH of GI tract, GI motility, and concomitant food intake, except osmotic controlled release preparations. A suitably designed osmotically controlled oral drug delivery systems or osmotic pump can solve these problems (Verma et al., 2000).

2.1 Osmotic pump

Osmotic pump is developed for both oral extended-release products called gastrointestinal therapeutic systems (GITS) and for implantable drug delivery such as osmotic minipump (Ding, 2016). Osmotic pump is the drug delivery that based on principles of osmotic pressure for controlling the delivery of active pharmaceutical ingredients. The osmotic pressure is an energy source of osmotic pump. Water influx is controlled by the semipermeable membrane. When hydrodynamic pressure is generated inside the osmotic device, thus it can control drug delivery (Gerk, Yu, & Shargel, 2016; Malaterre, Ogorka, Loggia, & Gurny, 2009c). It has many advantages over other oral dosage forms such as it had a constant delivery rate with approximately zero-order release kinetics. It has a good *in vivo-in vitro* correlation manner. Thus, it can predict *in vivo* release rate based on *in vitro* data, decrease dosing frequency with long action period and thereby improve patient compliance (Conley, Gupta, & Sathyan, 2006; Gerk et al., 2016; Liu et al., 2000; Marroum, 2008). Furthermore, it is independent of chemical properties of drug, physiological factors of the patient, and food intake (Fara & Ray, 1988; Malaterre et al., 2009c).

Integrity and consistency of coating are important. There is a risk of membrane defects or ruptures if the coating procedure is not well organized, which can result in dose dumping (Collett & Moreton, 2002). Some types of drugs may cause severe side effect such as GI irritation and perforation of the intestinal wall that found in controlled-release indomethacin, Osmosin®. Pre-existing GI injury or GI occlusion, due to non-degradable nature of the osmotic pump, can be found in patient with GI narrowing. In addition, the physician and pharmacist must notify the patient that the empty shell is excreted in the feces, which can disturb some fragile patients (Malaterre et al., 2009c). Initially, osmotic pump is developed for veterinary application such as Rose-Nelson pump, Higuchi-Leeper pump, and Higuchi-Theeuwes pump. After that, the elementary osmotic pump is developed as the first oral osmotic pump for a human. Moreover, other types of osmotic pump are continuously developed including push-pull osmotic pump,

controlled-porosity osmotic pump, liquid oral osmotic pump, sandwiched osmotic pump, etc.

2.1.1 Rose-Nelson pump

The first developed device used the principle of osmotic pressure for drug delivery; Rose-Nelson pump, was developed in 1955. The device consists of three chambers i.e. drug chamber, salt chamber, and water chamber. A semipermeable membrane locates between the salt chamber and the water chamber. The salt chamber and the drug chamber are separated by an elastic diaphragm. The basic compositions of Rose-Nelson pump are shown in Figure 2.1. During the operation, water moves from the water chamber into the salt chamber across the semipermeable membrane. The volume of the salt chamber rises due to water entry, which distends the elastic diaphragm, thus pumping the drug out of the device. One of the major difficulties of this pump is the osmotic pressure initiate immediately after water contact with the semipermeable membrane. Thus, pump should be empty during storage and water is loaded prior to use (Santus & Baker, 1995).

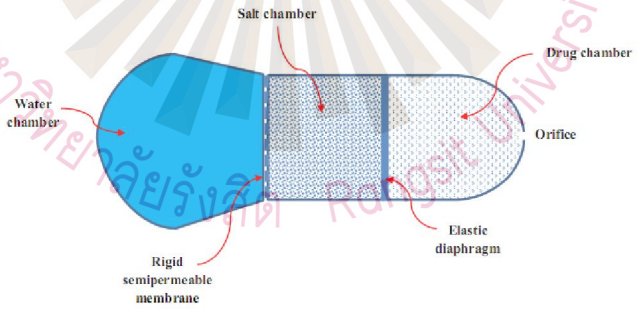


Figure 2.1 Compositions of Rose-Nelson pump.
Source: modified from Rose & Nelson, 1955

2.1.2 Higuchi-Leeper pump

Higuchi-Leeper pump is developed by Alza Corporation in 1971 and patented in 1973. Higuchi-Leeper pump has no water chamber. The device is driven by water imbibe from the surrounding environment. Thus, the pump can be loaded with drug and stored for months prior to use. The pump is driven when it is swallowed or implanted in the body. This pump consisted of a rigid housing and the semipermeable membrane is supported on a perforated frame. Furthermore, salt chamber usually contains a salt solution with excess solid salt (Figure 2.2). The operation starts when water from surrounding environment is passed through the semipermeable membrane into the salt chamber, distending of the movable separator, thus pumping the drug out of the device (Higuchi & Leeper, 1973; Santus & Baker, 1995).

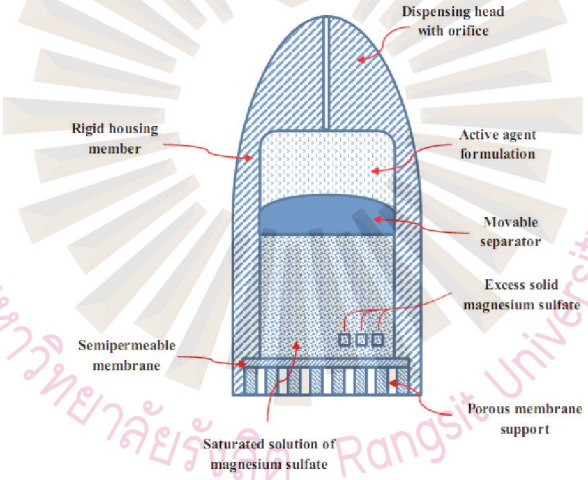


Figure 2.2 Compositions of Higuchi-Leeper pump (US patent 3760804).
Source: modified from Higuchi & Leeper, 1973

2.1.3 Higuchi-Theeuwes pump

Higuchi-Theeuwes pump is developed in 1973. One of this device is illustrated in Figure 2.3. This design and operation are similar to the Higuchi-Leeper pump. Water to motivate the device is obtained from surrounding environment similar to Higuchi-Leeper pump. Higuchi-Theeuwes pump is loaded with the desired drug prior

to use. In addition, it can also deliver semisolid preparation. When the device is placed in the aqueous environment, the release of drug is motivated by the salt consisted in the salt chamber and water permeability of the semipermeable membrane (Santus & Baker, 1995).

Higuchi-Theeuwes mini-pump is a model of ALZET® osmotic pump produced by Alza Corporation. ALZET® pump are miniature, implantable pumps for research in laboratory animals. ALZET® osmotic pump have three major layers; semipermeable membrane, osmotic layer, impermeable drug reservoir (Figure 2.4 and Figure 2.5). In this design, the semipermeable membrane acts as the outer casting of the pump. During the operation, water enters the pump across the semipermeable membrane. The influx of water causes the osmotic layer to expand, thus compressing of the collapsible reservoir and pumping drug solution through the delivery orifice (Higuchi & Leeper, 1976; Theeuwes & Yum, 1976).

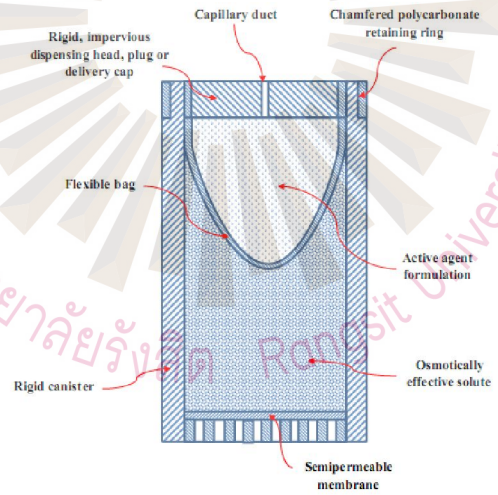


Figure 2.3 Compositions of Higuchi-Theeuwes pump (US patent 3995631).
Source: modified from Higuchi & Leeper, 1976

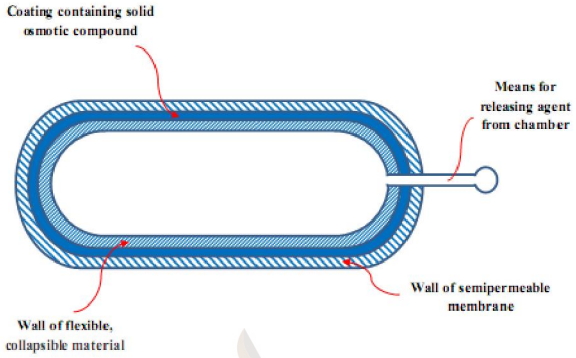


Figure 2.4 Compositions of Higuchi-Theeuwes mini-pump (US patent 3995631).
Source: modified from Higuchi & Leeper, 1976

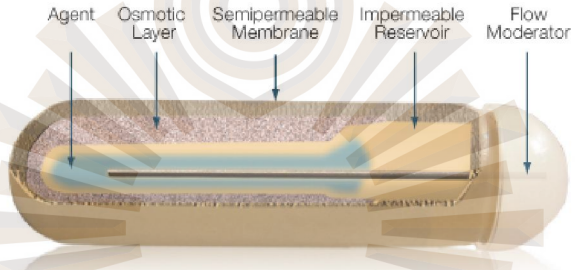


Figure 2.5 Compositions of ALZET® implantable osmotic pump.
Source: Durect Corporation, 2015

2.1.4 Elementary osmotic pump

Elementary osmotic pump (EOP) is developed in 1974. It is a first oral osmotic pump that uses in human. This device is developed to simplify the Higuchi-Theeuwes pump. The drug with suitable osmotic inducing agent (osmogen) is compressed into a tablet. Then, the core tablets are coated with semipermeable membrane polymer and tablets are drilled the delivery orifice in the outer membrane. The system can contain in solid form of the agent at loading higher than 90% of the total volume. When EOP contact with the aqueous environment in GI tract, the additive inside core tablet draws water through the semipermeable membrane. A saturated solution is formed inside the device. The membrane is non-extensible, the increasing in volume caused by the imbibition of water leads to the development of hydrostatic

pressure inside the core tablets. The pressure is released out of the device by the pump out of saturated solution through the delivery orifice. The principle of the EOP is shown in Figure 2.6 (Santus & Baker, 1995; Theeuwes, 1975; Verma et al., 2000; Wong, Gupta, & Stewart, 2003). The EOP can be prepared in a simple step and it is suitable for delivery of water-soluble drugs (Srikonda, Kotamraj, & Barclay, 2006) with solubility in the range of 50-400 mg/mL (Davar, Barcley, & Gupta, 2008). But, the EOP is inappropriate for delivery of water insoluble drug (Liu et al., 2000).

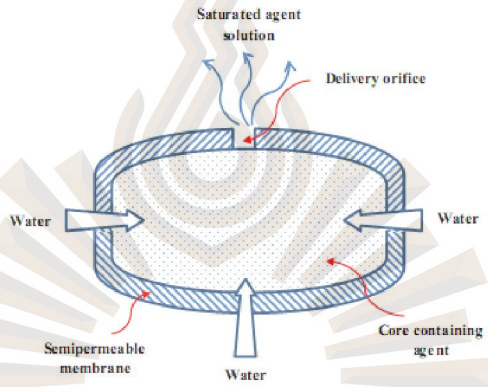


Figure 2.6 The principle of the elementary osmotic pump.
Source: modified from Santus & Baker, 1995

The EOP is also developed by Alza Corporation under the name of OROS®. The first product that launched to the market in the 1980s is controlled release indomethacin, Osmosin®. Unfortunately, this product is withdrawn from the market due to their severe side effect i.e. GI irritation and perforation of the intestinal wall. This event causes 18 patients had died and at least 400 patients suffered from severe intestinal ulceration (Laidler, Maslin, & Gilhome, 1985).

2.1.5 Push-pull osmotic pump

Push-pull osmotic pump (PPOP) is developed by Alza Corporation in 1982. PPOP is two compartments device; first compartment is push layer which contains swellable polymer (with osmogen). The other compartment is drug layer (sometime calls pull layer). The tablet core is coated with a semipermeable membrane. Finally, the

tablet is drilled for the delivery orifice on drug layer side (Wong et al., 2003). Inorganic pigment usually mixed in push layer for identification of the side during laser drilling. During operation, water is drawn into both compartments simultaneously, so the drug is dissolved or suspended. As polymer in push layer expanded, it pumps the drug solution or suspension out via delivery orifice (Allen, Popovich, & Ansel, 2010; Srikonda et al., 2006). This allowed both water soluble and water insoluble drugs can be delivered using PPOP. However, PPOP is more suitable for water insoluble drugs, because water soluble drugs can be delivered by the simple manner of EOP. The compositions and the basic principle of PPOP are shown in Figure 2.7.

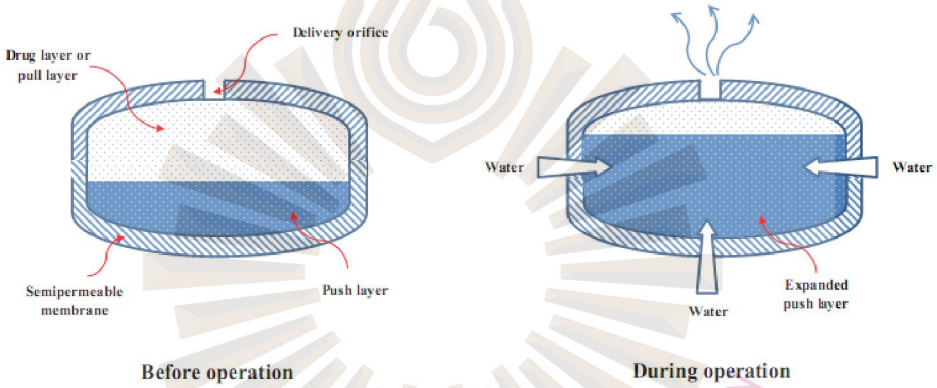


Figure 2.7 The compositions and the basic principle of PPOP.
Source: modified from Cortese & Theeuwes, 1982

2.1.6 Controlled-porosity osmotic pump

Controlled-porosity osmotic pump (CPOP) is developed in 1980 and patented in 1987 (Baker & Brooke, 1987). The side identification during laser drilling process is required in PPOP. However, laser drilling can be negligible in CPOP. The microporous wall can be formed *in situ* using the pore forming agent which can dissolve when contact to water, thus the microporous wall is formed (Figure 2.8). Examples of pore forming agents that can be used to form a microporous wall were water-soluble sugars (lactose, mannitol, sorbitol, and sucrose) and water-soluble salts (calcium chloride, potassium chloride, sodium carbonate, sodium chloride, sodium sulfate, etc). The drug

is delivered from the entire surface of the osmotic pump rather than single hole, therefore it can lessen local irritation in GI tract. Finally, the tablets can be made very small size by coating with a suitable membrane (Santus & Baker, 1995).

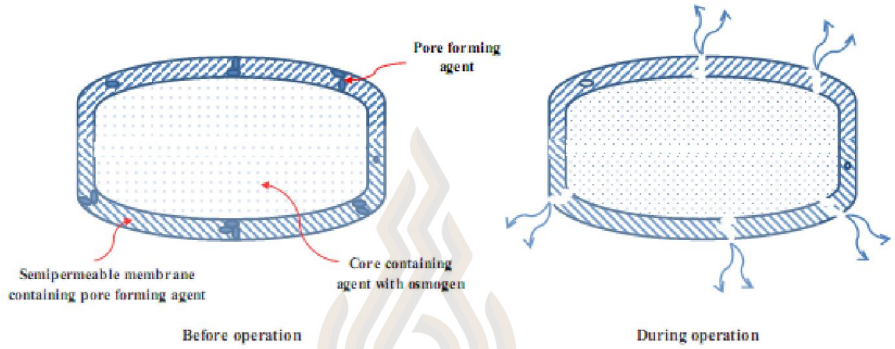


Figure 2.8 The compositions and the basic principle of CPOP.
Source: modified from Baker & Brooke, 1987

2.1.7 Liquid oral osmotic pump

Liquid oral osmotic pump (L-OROS) is developed by Alza Corporation in 1991 (Wong, Theeuwes, Barclay, & Dealey, 1991). It is designed to deliver non-aqueous liquid formulations and is appropriate for delivery of low water soluble drugs. The liquid formulation is filled in soft gelatin capsule surrounded by the three layers; the barrier layer, the osmotic layer, and the release rate-controlling membrane. When L-OROS SOFTCAP contacts with the aqueous solution, water permeates through the rate-controlling membrane and the osmotic layer is activated. The osmotic pressure produced by osmotic layer and liquid formulation is pumped out via a delivery orifice (Verma, Krishna, & Garg, 2002). The compositions and basic principle of L-OROS SOFTCAP is shown in Figure 2.9. L-OROS HARDCAP is similar to L-OROS SOFTCAP. All compositions are filled in a hard gelatin capsule and coated with a semipermeable membrane. The invention of L-OROS HARDCAP is described in US patent no. 5324280 (Wong, Theeuwes, Barclay, & Dealey, 1994) and no. 5413572 (Wong, Theeuwes, Barclay, & Dealey, 1995).

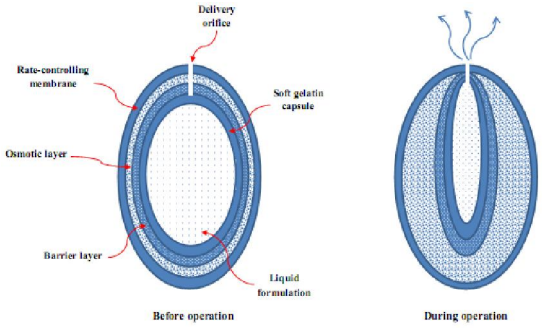


Figure 2.9 The compositions and the basic principle of L-OROS SOFTCAP.
Source: modified from Verma et al., 2002

2.1.8 Sandwiched osmotic tablets

Sandwiched osmotic tablets (SOT) are multichamber osmotic tablets. It consists of a middle push layer and two attached drug layers. After coating with a semipermeable membrane, tablet is drilled to have the delivery orifices on both side surface of drug layer. The composition is shown in Figure 2.10. This system can avoid side identification in drilling process compared to PPOP. Moreover, it perhaps decreases the potential local drug irritation due to their delivering drug from two opposite orifices (Liu et al., 2000).

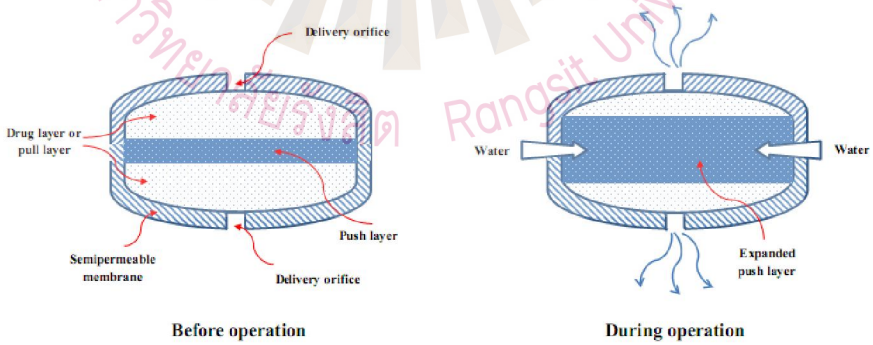


Figure 2.10 The compositions and the basic principle of SOT.
Source: modified from Liu et al., 2000

Nowadays, osmotic pump products are launched in the market. Examples of the products are shown in Table 2.1. In Thailand, two products are available i.e. Concerta® and Invega®.

Table 2.1 Examples of marketed osmotic pump products.

Approval year	Trade name	Generic name	Form
1987	Volmax	Albuterol	EOP
1989	Procardia XL	Nifedipine	PPOP
1992	Minipress XL or Alpress LP	Prazosin HCl	PPOP
1992	Sudafed 24 hour	Pseudoephedrine HCl	EOP
1993	Efidac 24	Pseudoephedrine HCl	EOP
1994	DynaCirc CR	Isradipine	PPOP
1994	Glucotrol XL	Glipizide	PPOP
1994	Efidac 24 chlorpheniramine	Pseudoephedrine HCl/chlorpheniramine	EOP
1996	Teczem	Enalapril/diltiazem	CPOP
1996	Tiamate	Diltiazem HCl	CPOP
1996	Tegretol XL	Carbamazepine	EOP
1996	Efidac 24 brompheniramine	Pseudoephedrine HCl/brompheniramine	EOP
1997	Teosona Sol	Theophylline	EOP
1997	Loremex	Pseudoephedrine HCl/loratadine	EOP
1998	Diutropan XL	Oxybutynin HCl	PPOP
1999	Elafax XR	Venlafaxine HCl	EOP
1999	Osmoran 300	Ranitidine HCl	EOP
2000	Concerta*	Methylphenidate HCl	PPOP
2002	Altoprev	Lovastatin	EOP
2004	Fortamet	Metformin HCl	EOP
2004	Allegra-D 24 HR	Pseudoephedrine HCl/fexofenadine HCl	EOP
2005	Cardura XL	Doxazosin mesylate	PPOP
2007	Invega*	Paliperidone	PPOP

* Available in Thailand.

Source: Malaterre et al., 2009c

2.2 Push-pull osmotic pump excipients

2.2.1 Osmogen

Osmogen is used for generating the osmotic pressure to deliver the drug from the osmotic device. Sodium chloride and potassium chloride are widely used as osmogen. A compound or mixtures of a compound that can be used as osmogen are shown with their osmotic pressure in Table 2.2.

Table 2.2 Osmotic pressure of saturated solutions of common pharmaceutical solutes.

Compound or mixture	Osmotic pressure (atm)
Lactose-fructose	500
Dextrose-fructose	450
Sucrose-fructose	430
Mannitol-fructose	415
Sodium chloride	356
Fructose	335
Lactose-sucrose	250
Potassium chloride	245
Lactose-dextrose	225
Mannitol-dextrose	225
Dextrose-sucrose	190
Mannitol-sucrose	170
Sucrose	150
Mannitol-lactose	130
Dextrose	82
Potassium sulfate	39
Mannitol	38
Sodium phosphate tribasic·12H_2O	36
Sodium phosphate dibasic·12H_2O	31
Sodium phosphate dibasic·7H_2O	31
Sodium phosphate dibasic anhydrous	29
Sodium phosphate monobasic·H_2O	28

Source: Zentner, Rork, & Himmelstein, 1990

2.2.2 Swelling agent

PPOP used a swelling agent to generate a driving force to pumping drug through the delivery orifice. Polyethylene oxide (PEO) is the most popular swelling agent for the osmotic pump. PEO have a good swelling capacity and hydration kinetics (Missaghi, Patel, Farrell, Huatan, & Rajabi-Siahboomi, 2014). High molecular weight (Mw) PEO is usually used as the swelling agent. While low Mw PEO has the low swelling capacity, thus it is used as a thickening agent or suspending agent in pull layer of PPOP. Hydroxypropyl methylcellulose (HPMC) K15M is also used as the swelling agent. However, it dramatically decreases the drug delivery rate compared to PEO (Emara, Taha, Badr, & Mursi, 2012), thus it is less popular than PEO. While, Wang, Xie, Yang, and Chen (2009) suggested that the mixture of HPMC K15M and carboxymethylcellulose sodium (1:1) is the choice instead of PEO for PPOP. In some cases, crosslinked polyacrylic acid is used as swelling agent (Davar et al., 2008).

2.2.3 Semipermeable membrane

Various types of polymer that is impermeable to solute but permeable to water can be used as a coating material in osmotic pump i.e. cellulose esters such as cellulose acetate, cellulose acetate butyrate, cellulose diacetate, cellulose propionate, cellulose triacetate, etc. Among these polymers, cellulose acetate (CA) is mostly used in this application because of its relatively high water permeability (Verma et al., 2002) as well as sufficient film mechanical strength (Davar et al., 2008). In addition, it can be adjusted easily by varying the degree of acetylation of the polymer. When the acetyl content increases, the CA film permeability decreases while solvent resistance increases (Verma et al., 2002). CA is incompatible with strongly acidic or alkaline substances while it is compatible with plasticizers such as diethyl phthalate, polyethylene glycol, triacetin, and triethyl citrate (Rowe, Sheskey, & Quinn, 2009). The chemical structure of CA is shown in Figure 2.11. The mixture of ethyl cellulose and HPMC (4:1) is compared with CA membrane. The ethyl cellulose-HPMC membrane gave drug release rate about the half of the CA membrane for the same membrane thickness (Ramakrishna & Mishra, 2002). This effect can be described by the lower water permeability of

ethylcellulose than CA (Bindschaedler, Gurny, & Doelker, 1986). In addition, Eudragit® RL30D and Eudragit® RS30D are also used as a semipermeable membrane (Jensen, Appel, Clair, & Zentner, 1995; Zhang Y., Zhang Z., & Wu, 2003). Regularly, the plasticizer is added to the semipermeable membrane during the coating process to modify water permeability of semipermeable membrane and drug delivery rate.

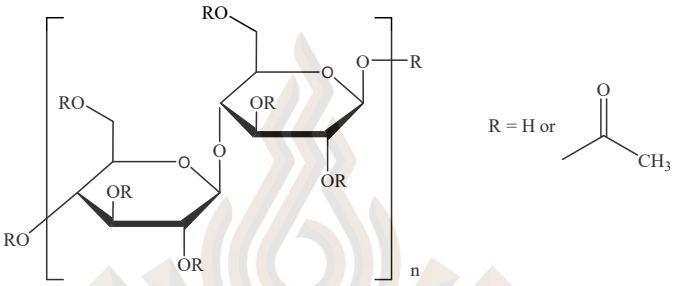


Figure 2.11 The chemical structure of CA, where n = a number of repeat units.

2.3 Polyethylene oxide

Polyethylene oxide (PEO) is a nonionic synthetic homopolymer of ethylene oxide. It is represented by the formula $(CH_2CH_2O)_n$, where n is the average number of oxyethylene groups. PEO has the same chemical structure as polyethylene glycol but higher Mw. The chemical structure of PEO is shown in Figure 2.12. Different grades of PEO differ with the length of molecular chains. Materials with Mw less than 100000 are usually called polyethylene glycols, while higher Mw polymers are classified as PEOs. Commercial PEO (marketed as Polyox™) is dry, white to off-white, free-flowing powder, slight ammoniacal odor. It is soluble in water and many organic solvents such as dichloromethane, chloroform, acetonitrile. But, it is insoluble in aliphatic hydrocarbons, ethylene glycol, and most alcohols. PEOs have the melting point range from 65-70 °C (Ma, Deng, & Chen, 2014; Rowe et al., 2009). The grades, approximate Mw, and a viscosity range of Polyox™ are shown in Table 2.3.

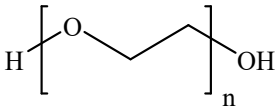


Figure 2.12 The chemical structure of PEO, where n = an average number of oxyethylene groups.

PEO can be completely dissolved in both cold and hot water. But, it is precipitated out when the temperature of the solution is close to 100 °C, called cloud point. The PEO concentration, the Mw of PEO, the concentration of salts, and the pH value affected the cloud point. PEOs have a very slow dissolution rate. However, the dry powder is easily wetted by water. If it is not dispersed in water appropriately, it tends to form agglomeration and gel. The glass transition temperature of PEOs ranges from -50 to -57 °C. The Mw of PEOs does not have a significant influence on the glass transition temperature (Ma et al., 2014).

Table 2.3 Grades, approximate molecular weight, and viscosity ranges of Polyox™ water-soluble resin (WSR) NF products.

Polyox™ grade	Approximate Mw	Viscosity at 25 °C (mPa s)		
		5% solution	2% solution	1% solution
WSR N-10	100,000	30-50	-	-
WSR N-80	200,000	55-90	-	-
WSR N-750	300,000	600-1,200	-	-
WSR N-3000	400,000	2,250-4,500	-	-
WSR 205	600,000	4,500-8,800	-	-
WSR 1105	900,000	8,800-17,600	-	-
WSR N-12K	1,000,000	-	400-800	-
WSR N-60K	2,000,000	-	2,000-4,000	-
WSR 301	4,000,000	-	-	1,650-5,500
WSR coagulant	5,000,000	-	-	5,500-7,500
WSR 303	7,000,000	-	-	7,500-10,000

Source: Rowe et al., 2009

Note all solutions are based on the hydro-alcoholic solutions.

2.4 Application of PEO in PPOP

PEO is a water-soluble polymer and can be used to generate a very high osmotic pressure. Uniformity of swelling rate of PEO ensures that the release rate of the drug from delivery device is relatively constant. The pressure created during swelling process does not rupture the osmotic device. These properties make PEO to be one of the most popular materials for the osmotic pump (Ma et al., 2014; Verma et al., 2002). The low Mw PEO is usually used as a thickening agent (40-90%) or suspending agent in drug layer. Conversely, the high Mw PEO is used as swelling agent (30-70%) in push layer of PPOP due to their good swelling capacity (Shamblin, 2010).

The Mw and amount of PEO in drug layer play an important role on drug releasing from the osmotic device. Increasing of PEO Mw in drug layer, the viscosity is increased and longer lag time is found. Malaterre, Ogorka, Loggia, and Gurny (2009a) reported that increasing of PEO Mw from 100K to 600K, the lag time of the release of isradipine and chlorpheniramine maleate is increased from 1 to 2.5 h. Furthermore, the drug property does not affect lag time and the release rate is constant for all PEO Mw. Glipizide release from the push-pull osmotic device is not significantly affected by PEO Mw in push layer while the use of higher Mw PEO in drug layer leads to longer lag time (Missaghi et al., 2014). The effect of PEO Mw in drug layer on glipizide release is similar to the other study that the higher PEO Mw in drug layer led to longer lag time and slower drug release rate (Liu et al., 2014b). Release profile and lag time are similar when to use PEO with Mw 100K and 200K in drug layer while PEO with Mw 300K shows longer lag time. In addition, release rate and lag time are not affected by PEO Mw in push layer (Zhang et al., 2009). In case of PEO amount, the higher amount of PEO in drug layer makes more viscosity that inhibit drug aggregation and precipitation. PEO amount in drug layer has little effect on drug release from an osmotic device. However, high amount of PEO may reduce drug release rate (Nie, Li, Luan, Pan, & Wang, 2007; Wu et al., 2014). Varying of PEO amount in push layer, similar release profile is found, except when a very low amount of PEO is used. A negative role on drug release is observed, due to it is lack of swelling capacity compared to the high amount of PEO (Wu et al., 2014).

Conventional PPOP requires side identification during laser drilling, thus inorganic pigment in push layer is needed. Li et al. (2008) developed PPOP with orifices on both side surfaces to avoid side identification, drug layer and push layer surfaces have drilled a hole. Gliclazide is used as a model drug in this study. The results indicated that PPOP with orifices on both side surfaces has similar release profile compare to PPOP with an orifice on one side surface, except when the low Mw PEO is used as a swelling agent in push layer. Low Mw PEO is inappropriate for use as swelling agent due to their low swelling capacity, pumped out process is incomplete manner. These results are similar to the study of Zhang et al. that use antiplatelet dipyridamole as a model drug (Zhang et al., 2009).

2.5 Drug delivery rate of EOP and PPOP

The osmotic flow of liquid depends on the difference of osmotic pressure and hydrostatic pressure across the semipermeable membrane. This phenomenon is the basic feature of nonequilibrium thermodynamics. Volume flux (dV/dt) across the semipermeable membrane is described by Felix Theeuwes (Theeuwes, 1975) and shown in Equation 2-1.

The relationship between volume flux and solute delivery rate (dm/dt) is shown in Equation 2-2.

$$\frac{dV}{dt} = \frac{A}{h} L_p(\sigma\Delta\pi - \Delta P) \qquad (2\text{-}1)$$

$$\frac{dm}{dt} = \frac{dV}{dt} \times C \qquad (2\text{-}2)$$

Where A = the surface area of membrane

h = the membrane thickness

L_p = the water permeability

σ = the reflection coefficient (the leakage of solute through the membrane)

$\Delta\pi$ = the osmotic pressure difference across the membrane

ΔP = the hydraulic pressure difference across the membrane

C = the solute concentration in the delivered fluid.

Thus, the corresponding solute delivery rate can be expressed as Equation 2-3.

$$\frac{dm}{dt} = \frac{A}{h} L_p(\sigma\Delta\pi - \Delta P)\times C \qquad (2\text{-}3)$$

As the size of delivery orifice rises, the osmotic pressure of the system reduces, and $\Delta\pi$ >>>ΔP. Thus, $\Delta\pi$-ΔP approximately $\Delta\pi$. The osmotic pressure of the formulation, π, can be substituted for $\Delta\pi$ when the environmental osmotic pressure is small. Consequently, the equation can be simplified as Equation 2-4.

$$\frac{dm}{dt} = \frac{A}{h} L_p \sigma\pi C \qquad (2\text{-}4)$$

A constant, k, may replace $L_p\sigma$. So, the equation can be further reduced to Equation 2-5.

$$\frac{dm}{dt} = \frac{A}{h} k\pi C \qquad (2\text{-}5)$$

The release rate of EOP, when all solid dissolved and the solute concentration begins to drop below saturation, can be defined as Equation 2-6.

$$\frac{dm}{dt} = \frac{A}{h} k\pi_S S \qquad (2\text{-}6)$$

Where S = the solubility at saturation

π_s = the osmotic pressure at saturation

When the dissolution rate is not limiting relative to the delivery rate through the aperture, the concentration (C) can be replaced with solubility (S) (Srikonda et al., 2006).

The equation that can be used for describing drug delivery from PEO-contained osmotic pump system both monolithic osmotic tablet (Lu, Jiang Z., Zhang, & Jiang X., 2003) and PPOP (Malaterre et al., 2009a; Xu et al., 2013; Zhang et al., 2009) is called Poiseuille's law of laminar flow. The Poiseuille's law can be expressed as Equation 2-7 and the schematic of drug release is shown in Figure 2.13.

$$\left(\frac{dV}{dt}\right)_{outlet} = \frac{\pi R^4 \Delta P}{8\eta L} \qquad (2\text{-}7)$$

Where $\pi = 3.14159$

R = the radius of the tube = the radius of the orifice

ΔP = the pressure difference between two end of the tube = the pressure difference between inside and outside the membrane

η = the dynamic viscosity of flow

L = the length of the tube = the thickness of the membrane

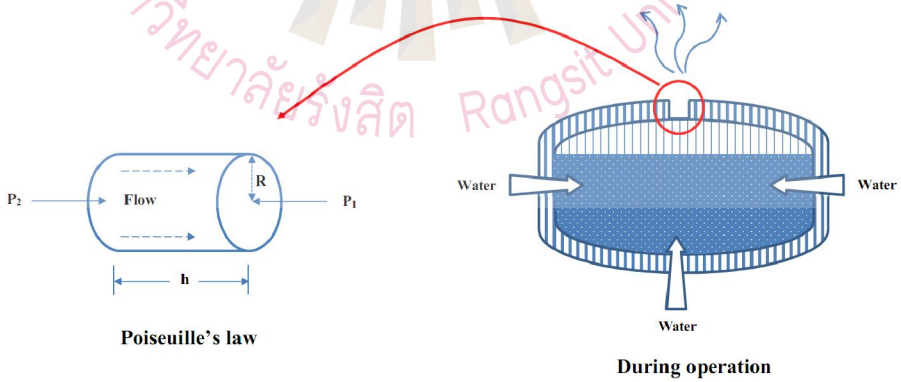


Figure 2.13 Schematic of drug release mechanism from PPOP.

Source: modified from Zhang et al., 2009

As a definite time, the relationship between swelling pressure (P), the polymer concentration (W), and the volume (V) of the hydrated polymer can be described as Equation 2-8.

$$P = \Delta P \left(\frac{W}{V}\right)^{k} \quad (2\text{-}8)$$

Where ΔP is the pressure difference between inside and outside the membrane when the polymer concentration is 1 kg/m^3 and it is constant. The k value is a constant in the range (k>1). According to PPOP, drug release approaches the zero-order release kinetic, the P_0 is constant. Thus, Equation 2-7 and 2-8 can be combined to describe the swelling rate of the polymer $(dV/dt)_{sw}$ as Equation 2-9.

$$\left(\frac{dV}{dt}\right)_{sw} = \frac{dW}{dt}\left(\frac{\Delta P}{P}\right)^{1/k} \quad (2\text{-}9)$$

The drug suspension release rate from PPOP, $(dV/dt)_{sw}$ is equal to swelling rate, $(dV/dt)_{outlet}$ for a zero-order release. It can be described as Equation 2-10.

$$\left(\frac{dV}{dt}\right)_{sw} = \left(\frac{dV}{dt}\right)_{outlet} \quad (2\text{-}10)$$

However, the suspension release rate is quite slower than the swelling rate until the polymer in the pull layer hydrates consistently. In addition, a rapid hydration rate of the polymer in the pull layer is necessary for the zero-order release (Xu et al., 2013).

2.6 Hard gelatin capsules

Gelatin is a mixture of proteins derived from animal collagen. The most abundant sources of gelatin manufacture obtained from pig skin (46.0%), cattle skin (hide) (29.4%), cattle and pig bone (23.1%), and fish skin (<1.5%) (Duconseille, Astruc, Quintana, Meersman, & Sante-Lhoutellier, 2015). Eastoe (1955) and Farris, Song, and Huang (2010) reported various amino acids composed in pig skin and cattle

hide gelatin, furthermore, the major amino acid is glycine which are shown in Table 2.4. Gelatin is widely used in the pharmaceutical field. It is used as a binding agent, coating agent, film-forming agent, gelling agent, and suspending agent. It is usually used to prepare hard gelatin capsules and soft gelatin capsules (Rowe et al., 2009).

Table 2.4 Composition of amino acid and their content in gelatin.

Amino acid	Percentage* (Eastoe, 1955)	Percentage** (Farris et al., 2010)
Glycine	26.40	32.20
Proline	16.50	13.10
Alanine	10.70	11.05
Hydroxyproline	13.50	9.80
Glutamic acid	11.30	7.10
Arginine	9.10	4.96
Aspartic acid	6.70	4.42
Serine	4.13	3.4
Lysine	4.14	2.65
Leucine	3.34	2.35
Valine	2.77	1.90
Threonine	2.19	1.80
Phenylalanine	2.56	1.38
Isoleucine	1.36	1.02
Hydroxylysine	1.04	0.75
Asparagine	0	0.60
Histidine	1.01	0.45
Tyrosine	0.60	0.35
Methionine	0.88	0.32
Tryptophan	0	0
Cysteine	0	0

* Percent of dry ash-free protein, ** mol%

Source: Eastoe, 1995; Farris et al., 2010

Under high humidity and elevated temperature or exposition of trace amounts of aldehyde, gelatin capsule can become crosslinked (Ofner, Zhang, Jobeck, & Bowman, 2001). Crosslinked gelatin capsule can interfere *in vitro* drug dissolution. However, reversibility of crosslinking by an enzyme in GI tract is observed (Brown, Madit, Cole, Wilding, & Cade, 1998; Digenis, Gold, & Shah, 1994; Marchais,

Cayzeele, Legendre, Skiba, & Arnaud, 2003; Meyer et al., 2000; Singh, Rao, Venugopal, & Manikandan, 2002).

Salsa, Pina, and Teixeira-Dias (1996) studied the reaction of a formaldehyde aqueous solution with gelatin dispersed in potassium bromide disc and monitored the real-time reaction by FTIR spectroscopy. Results showed that the crosslinking of HGCs occurred between lysine and arginine or arginine and arginine. The reaction was started by the lysine methylol formation and followed by arginine methylol, which reacted with lysine methylol to form lysine/arginine crosslink. Study of Tengroth, Gasslander, Andersson, and Jacobsson (2005) supported this crosslink mechanism. Gelatin capsules are crosslinked by the inducing of aldehydes such as formaldehyde, acetaldehyde, and propionaldehyde. Crosslink mechanism is shown in Figure 2.14 and Figure 2.15.

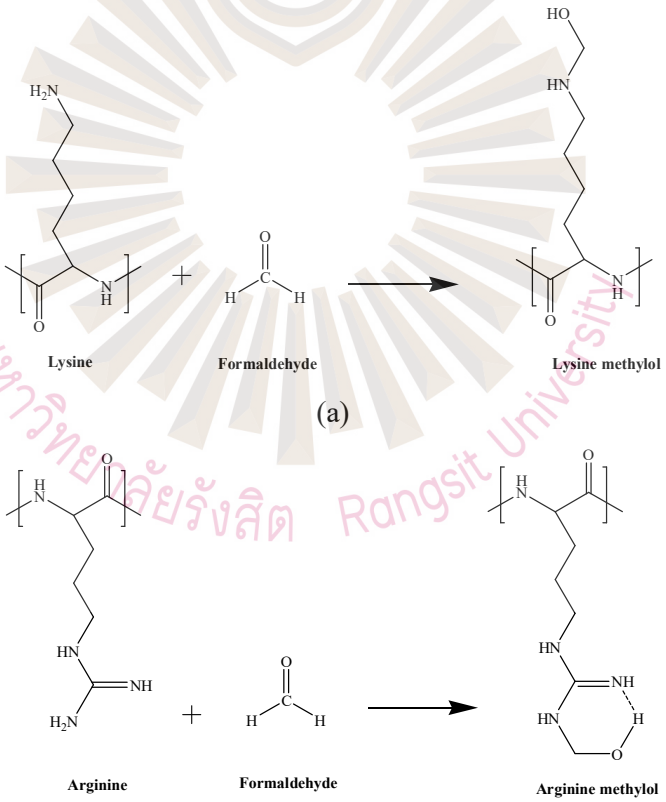
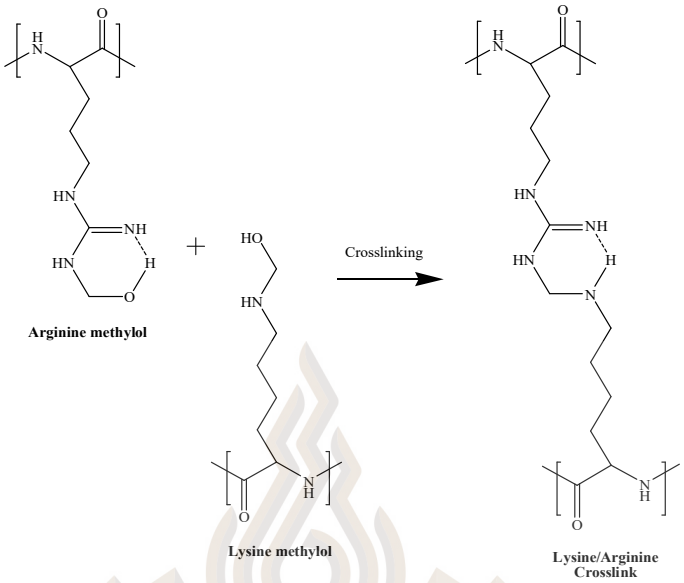
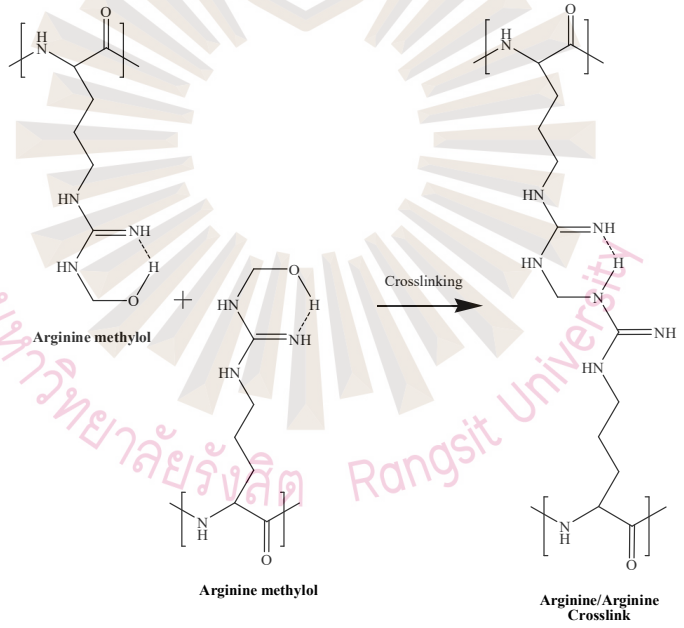


Figure 2.14 Formation of lysine methylol and arginine methylol induced by formaldehyde.

Source: Digenis et al., 1994



(a)



(b)

Figure 2.15 Crosslinking between (a) arginine methylol and lysine methylol and (b) arginine methylol and arginine methylol.

Source: Tengroth et al., 2005

2.7 Model drugs

2.7.1 Diltiazem hydrochloride (DIL HCl)

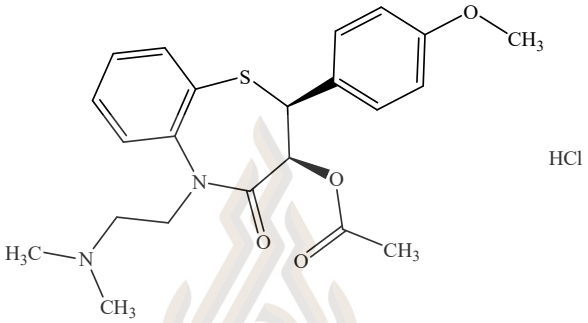


Figure 2.16 Chemical structure of DIL HCl.

Empirical name:	$C_{22}H_{26}N_2O_4S$, HCl
Chemical structure:	Figure 2.16
Molecular weight:	451.0 g/mol
Melting point:	207.5-212 °C
Appearance:	A white, odorless, crystalline powder, or small crystals.
BCS class:	I (Prabhu et al., 2008).
Solubility:	Freely soluble in water, chloroform, formic acid, and methanol; sparingly soluble in dehydrated alcohol; insoluble in ether. Additional data are shown in Table 2.5.
Stability:	Desacetyl diltiazem hydrochloride is a major degradation product from hydrolysis of DIL HCl with pseudo-first order kinetic degradation. The drug is stable over pH ranged 3-6, pH 5 is the optimum stable pH (Suleiman, Abdulhameed, Najib, & Muti, 1990). The drug has extreme degradation in acidic, basic, and photolytic stress condition. In basic and acidic stress, approximately 11% and 17% drug remaining, respectively (Sadeghi, Navidpour, Bayat, & Afshar, 2013).

Table 2.5 Solubility of DIL HCl.

Mediums	Solubility
Water	611.16±2.96 mg/mL (Prabakaran, Singh, Kanaujia, & Vyas, 2003)
Simulated gastric fluid (pH 1.2)	636.63±3.41 mg/mL (Prabakaran et al., 2003)
Simulated intestinal fluid (pH 7.4)	606.38±1.68 mg/mL (Prabakaran et al., 2003)
0.25 M NaCl (1.5% NaCl)	545 mg/mL (Zentner, McClelland, & Sutton, 1991)
0.50 M NaCl (2.9% NaCl)	395 mg/mL (Zentner et al., 1991)
0.75 M NaCl (4.4% NaCl)	278 mg/mL (Zentner et al., 1991)
1.00 M NaCl (5.8% NaCl)	155 mg/mL (Zentner et al., 1991)
1.20 M NaCl (7.0% NaCl)	40 mg/mL (Zentner et al., 1991)

2.7.2 Propranolol hydrochloride (PRO HCl)

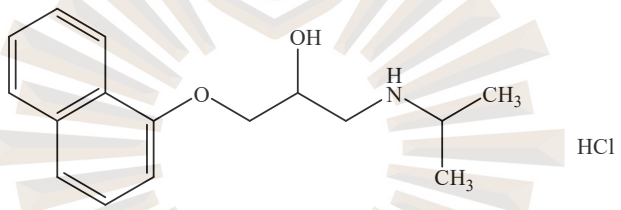


Figure 2.17 Chemical structure of PRO HCl.

Empirical name: $C_{16}H_{21}NO_2$, HCl

Chemical structure: Figure 2.17

Molecular weight: 295.8 g/mol.

Melting point: 162-165 °C.

Appearance: White, crystalline powder.

BCS class: I (Vogelpoel et al., 2004).

Solubility: The pKa value is about 9.05 at pH 7.2, thus it is soluble in water (Vogelpoel et al., 2004). Additional data are shown in Table 2.6.

Stability: Due to its naphthalene skeleton, this drug may be light unstable. Photodegradation products of PRO HCl are 1-naphthol, *N*-acetylpropranolol, and *N*-formylpropranolol (Uwai et al., 2005). Solutions of PRO HCl have maximum stability at pH 3 and decompose rapidly at alkaline pH (McEvoy, 2014).

Table 2.6 Solubility of PRO HCl.

Mediums	Solubility
Water	33.3-100 mg/mL (Vogelpoel et al., 2004)
Simulated gastric fluid (pH 1.2)	33.79±0.09 mg/mL (Garg, Gupta, & Bhargava, 2007)
Simulated intestinal fluid (pH 6.8)	57.23±1.08 mg/mL (Garg et al., 2007)

2.7.3 Ambroxol hydrochloride (AMB HCl)

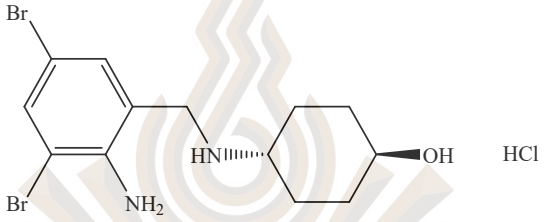


Figure 2.18 Chemical structure of AMB HCl.

Empirical name: $C_{13}H_{18}Br_2N_2O$, HCl

Chemical structure: Figure 2.18

Molecular weight: 414.6 g/mol

Melting point: 233-234.5 °C

Appearance: White or yellowish crystalline powder.

BCS class: I (Stetinova, Smetanova, Kholova, Svoboda, & Kvetina, 2009).

Solubility: Sparingly soluble in water (approximately 30 mg/mL) (Passetti, 2012), soluble in methanol, practically insoluble in methylene chloride.

Stability: Acidic medium may decrease the solubility of AMB HCl (Jagdale, Padekar, Bhadoriya, Ghorpade, & Kuchekar, 2010). Moreover, AMB HCl is degraded under oxidation and heat condition, approximately 20% drug degraded (Jain, 2010).

2.7.4 Paracetamol (PAR)

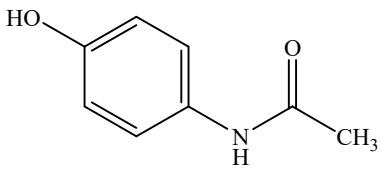


Figure 2.19 Chemical structure of PAR.

Empirical name:	$C_8H_9NO_2$
Chemical structure:	Figure 2.19
Molecular weight:	151.16 g/mol
Melting point:	168-172 °C
Appearance:	Odorless white crystalline powder.
BCS class:	III (Kalantzi et al., 2006).
Solubility:	One part of paracetamol is soluble in 70 parts of water at room temperature and soluble in 20 parts of boiling water. Some reports showed an aqueous solubility of 14.7, 14.3, and 27.3 mg/mL at 20, 25, and 37 °C, respectively (Kalantzi et al., 2006).
Stability:	PAR is stable to 45 °C. Humid condition causes hydrolysis to *p*-aminophenol that contaminated *p*-aminophenol leads to degradation and discoloration. Slightly-light sensitive in solution, and degradation is catalyzed by acids or bases (The International Agency for Research on Cancer, 2016).

CHAPTER 3

EXPERIMENTAL

3.1 Materials

3.1.1 Model drugs

1) Ambroxol hydrochloride (lot no. VBN0610814) was obtained from Biolab Co., Ltd., Thailand.

2) Diltiazem hydrochloride (lot no. DIL/M-20414) was obtained from Siam Pharmaceutical Co., Ltd., Thailand.

3) Propranolol hydrochloride (lot no. M09115) was purchased from Changzhou Yabang Pharmaceutical Co., Ltd., China.

4) Paracetamol (lot no.637512A036) was purchased from Srichand United Dispensary Co., Ltd., Thailand.

3.1.2 Pharmaceutical excipients

1) Gelatin powder (lot no. 5131710347) was obtained from Capsule Products Co., Ltd., Thailand.

2) Hydroxypropyl methylcellulose (HPMC E5) (lot no. AFN005-440034, Samsung) was obtained from Onimax Co., Ltd., Thailand.

3) Magnesium stearate (lot no. F20120001) was obtained from Sun Herb Thai Chinese Manufacturing, Thailand.

4) Opadry® CA, a mixture of cellulose acetate 398-10 and polyethylene glycol 3350 (9:1) (lot no. SH574204) was obtained from Colorcon, Inc., USA.

5) Polyethylene glycol (PEG) 4000 (lot no. PT 06101001) was obtained from Onimax Co., Ltd., Thailand.

6) Polyox™ Coagulant (PEO, Mw 5,000K) (lot no. GA415952) was obtained from Colorcon Inc., USA.

7) Polyox™ N-80 (PEO, Mw 200K) (lot no. GA416882) was obtained from Colorcon Inc., USA.

8) Sodium chloride (lot no. V3M716173M) was purchased from Carlo Erba, France.

9) Spray dried lactose (FlowLac® 100, lot no. L1509 A4969) was purchased from Molkerei Meggle Wesserburg GmbH & Co., Germany.

3.1.3 Capsule shell and crosslinking agent

1) Hard gelatin capsules (clear, no.1 and no. 2) were obtained from Capsule Products Co., Ltd., Thailand.

2) Formaldehyde (40 %v/v) (lot no. V4A492094A) was purchased from Carlo Erba, France.

3.1.4 Chemicals

1) 3-methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate (lot no. A0357243) was purchased from Acros, USA.

2) Adilake Carmoisine® (alumina 78% + carmoisine 22%) (lot no. 008038) was purchased from Adinop Co., Ltd., Thailand.

3) BCA protein assay kit (albumin standard (lot no. QB212308), Reagent A (lot no. QC213373), and Reagent B (lot no. QB212278)) was purchased from Pierce, Thermo Scientific, USA.

4) Ferric chloride (lot no. V1N920282H) was purchased from Carlo Erba, France.

5) Pancreatin (lot no. SLBT2247) was purchased from Sigma-Aldrich, USA.

6) Pepsin from porcine stomach mucosa (lot no. SLBT7899) was purchased from Sigma-Aldrich, USA.

7) Potassium dihydrogen phosphate (monobasic potassium phosphate) (lot no. V1L877102B) was purchased from Carlo Erba, France.

8) Sodium acetate trihydrate (lot no. 2L100752L) was purchased from Carlo Erba, France.

9) Sodium hydroxide (lot no. 3C072693C) was purchased from Carlo Erba, France.

10) Sulphamic acid (lot no. 0000218454) was purchased from Panreac, Spain.

3.1.5 Solvents

1) Acetone (AR grade) was purchased from Honeywell-Burdick & Jackson, USA.

2) Ethanol (AR grade) was purchased from Honeywell-Burdick & Jackson, USA.

3) Glacial acetic acid (AR grade) was purchased from Carlo Erba, France.

4) Hydrochloric acid (37%) was purchased from Merck KGaA, Germany.

5) Methanol (HPLC grade) was purchased from Honeywell-Burdick & Jackson, USA.

6) Water (reverse osmosis) was produced by Puris-Expe RO water system, Korea.

7) Water (ultrapure) was produced by Puris-Expe UP water system, Korea.

3.2 Instrument

1) Balance (analytical) (model: Entris224i-1S, Sartorius, Germany)
2) Balance (top loading) (model: PAJ3102, Ohaus Corp., USA)
3) Digital orbital shaker (Wiseshake, Daihan Scientific, Korea)

4) Dissolution tester (model: 72-600-400, Hanson Research Corp., USA)

5) Fourier transform infrared (FTIR) spectrometer (model: Nicolet 6700, Thermo Scientific, USA)

6) Gas pycnometer (Ultrapyc 1200e, Quantachrome Instruments, USA)

7) Hot air oven (model: JSOF-100, JS Research Inc., Korea)

8) High performance liquid chromatography (HPLC) instrument (Agilent 1260 infinity, Agilent, USA)

9) Magnetic stirrer (CMAG HS7, Ika, Germany)

10) Moisture balance (MAC 50/NH, Radwag, Poland)

11) Osmometer (Fiske® Micro Osmometer, model: 210, Fiske® Associates, USA)

12) Particle size analyzer (Mastersizer-2000, Malvern Instruments Ltd., UK)

13) pH meter (model: SevenCompact S220, Mettler Toledo, Switzerland)

14) Puris-Expe RO water system (model: Expe-RO Ele10-M, Mirae ST Co., Ltd., Korea)

15) Puris-Expe UP water system (model: Expe-UP Ele-M, Mirae ST Co., Ltd., Korea)

16) Scanning electron microscope (Hitachi S-3400N, Hitachi High-Technologies, Japan)

17) Texture analyzer (TA.XTplus texture analyzer, Stable Micro Systems Ltd., UK)

18) Ultrasonic bath (model: SB 25-12 DTDN, Laboratory Sky Shanghai, China)

19) UV-visible spectrophotometer (Spectronic Genesys 5, Milton Roy Company, USA)

20) Vortex mixer (model: G560E, Scientific Industries, Inc., USA)

21) Waterbath (model: WNB 14, Memmert, Germany) equipped with shaker (model: SV 1422, Memmert, Germany)

3.3 Methods

3.3.1 Preparation of crosslinked HGCs

Body and cap of clear HGCs (no. 1) were separated, spread on Petri dish, and placed in desiccator that equilibrated overnight with vapor of formaldehyde solution (40%v/v). Capsules were exposed to formaldehyde vapor for 6, 12, and 24 h. At a predetermined time, the capsules were removed from the desiccator and dried in hot air oven at 40 °C overnight. The crosslinked HGCs were kept at ambient condition and protected from excess moisture until use.

3.3.2 Evaluation of crosslinked HGCs

3.3.2.1 Physical evaluation of weight loss after immersing in mediums

Ten crosslinked HGCs were dried overnight in a hot air oven at 40 °C, weighed (W_1), and immersed in 100 mL water, hydrochloric acid (HCl) pH 1.2, and phosphate buffer solution (PBS) pH 6.8. The immersed capsules were shaken by orbital shaker at 100 rpm for 24 h. Then, capsules were dried overnight in the hot air oven at 40 °C, weighed (W_2), and the percent weight loss was calculated (Equation 3-1).

$$\text{Weight loss (\%)} = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \qquad (3\text{-}1)$$

3.3.2.2 Chemical evaluation of water soluble protein fraction

A crosslinked HGC was added into the 10-mL volumetric flask (n=3). Water was added, adjusted to the volume and shaken for 24 h at ambient temperature. The water soluble protein was assayed by Bicinchoninic acid (BCA) method using Pierce™ BCA protein assay kit (Walker, 2002). Working reagent was prepared by mixing BCA reagent A and BCA reagent B with a ratio of 50:1. The 0.1 mL sample

and 2 mL working reagent were added into a test tube and mixed well by a vortex mixer. The mixture was incubated at 37 °C for 30 min. Absorbance was measured using UV-visible spectrophotometer at 562 nm. The amount of water soluble protein was determined from a calibration curve of BCA protein assay of gelatin powder, which was the same as that used for making HGC shells. Water soluble protein fraction was calculated from Equation 3-2.

$$\text{Water soluble protein fraction (\%)} = \left(\frac{\text{Amount of water soluble protein}}{\text{Initial weight of capsule}}\right) \times 100 \qquad (3\text{-}2)$$

This method was validated according to ICH Harmonised Tripartite Guideline (ICH Expert Working Group, 2005) in following topics;

Linearity and range

Gelatin powder was dissolved in ultrapure water to obtain five concentrations of 91, 226, 453, 905, and 1358 µg/mL. Each concentration was assayed by BCA protein assay kit, analyzed by UV-visible spectrophotometer at 562 nm, and performed in triplicate. The calibration curve was constructed. The linear equation, the coefficient of determination (R^2), and range were reported.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated based on the calibration curve according to Equation 3-3 and 3-4.

$$\text{LOD} = \frac{3.3 \times \sigma}{S} \qquad (3\text{-}3)$$

$$\text{LOQ} = \frac{10 \times \sigma}{S} \qquad (3\text{-}4)$$

Where σ = the SD of y-intercepts of regression lines

S = the slope of the calibration curve

Precision

The three concentration levels of gelatin, 226, 453, and 905 µg/mL were prepared. Each concentration was assayed by BCA protein assay kit and analyzed in triplicate. The percent relative standard deviation (%RSD) of the analysis on the same day was reported as intraday precision and %RSD of the analysis in different three days was reported as inter-day precision. The %RSD should be not more than 2% and 5% for intraday and inter-day precision, respectively.

Accuracy

The accuracy was evaluated by the addition of three gelatin concentration levels (226, 453, and 905 µg/mL) to the sample solutions (HGCs dissolved in water). Each concentration was assayed by BCA protein assay kit. The percent recovery of gelatin was calculated. Percent recovery should be close to 100%. Each concentration was analyzed in triplicate.

3.3.2.3 Determination of loss on drying

Loss on drying was determined using moisture balance. Five capsules were placed on the pan. The temperature was increased to 120 °C and maintained throughout the test. The test progressed until a constant weight of capsules was obtained (i.e., weight change less than 1 mg within 2 minutes). The loss on drying was reported by the moisture balance following Equation 3-5. The test was run in triplicate.

$$\text{Loss on drying (\%)} = \left(\frac{W_1 - W_2}{W_1}\right) \times 100 \qquad (3\text{-}5)$$

Where W_1 = the weight of capsules before test

W_2 = the weight of capsules after test

3.3.2.4 Evaluation of formaldehyde residue in crosslinked HGCs

The 3-methyl-2-benzothiazolinone hydrazine hydrochloride monohydrate (MBTH) colorimetric method was employed for the determination of formaldehyde residue (Council of Europe, 2005). Three crosslinked HGCs were placed in a 10-mL volumetric flask, adjusted with water to the volume, and sonicated for 1 h. The supernatant was collected to measure the formaldehyde residue.

According to MBTH method, 0.5 mL sample and 5 mL of freshly prepared 0.5 mg/mL MBTH were added into a test tube, mixed together, and let to stand for 1 h. One mL of a mixture of 1.0% w/v ferric chloride and 1.6% w/v sulphamic acid aqueous solution was added, let to stand for 15 min before measuring the absorbance using UV-visible spectrophotometer at 628 nm. The content of formaldehyde residue in crosslinked HGCs was calculated from the calibration curve. Formaldehyde residue was calculated according to Equation 3-6.

$$\text{Formaldehyde residue (\%)} = \left(\frac{\text{Formaldehyde amount}}{\text{Weight of capsule}}\right) \times 100 \qquad (3\text{-}6)$$

This method was validated based on ICH Harmonised Tripartite Guideline (ICH Expert Working Group, 2005) in following topics;

Linearity and range

Formaldehyde solution was diluted in ultrapure water to obtain five concentrations of 0.5, 1.25, 2.50, 3.75, and 5.00 µg/mL. Each concentration was assayed by the MBTH method (n=3). The calibration curve was constructed. The linear equation, R^2, and range were reported.

LOD and LOQ

LOD and LOQ are calculated based on the calibration curve according to Equation 3-3 and 3-4.

Precision

The three concentration levels of formaldehyde solution, 1.25, 2.50, and 3.75 µg/mL, were prepared. Each concentration was assayed by the MBTH method (n=3). The percent relative standard deviation (%RSD) of the analysis on the same day was reported as intraday precision and %RSD of the analysis in different three days was reported as inter-day precision. %RSD should be not more than 2% and 5% for intraday and inter-day precision, respectively.

Accuracy

The accuracy was evaluated by the addition of three formaldehyde concentration levels (1.25, 2.50, and 3.75 µg/mL) to sample solutions (crosslinked HGCs immersed in water). The percent recovery of formaldehyde was calculated. Percent recovery should be close to 100%. Each concentration was analyzed in triplicate.

3.3.2.5 ATR-FTIR spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrometer was used for determination of crosslinking process. The spectra of capsule shells exposed to formaldehyde vapor for different lengths of time were collected by the OMNIC 8.0 software at wavenumber 400 to 4000 cm^{-1}, accumulating 32 scans with the resolution of 4 cm^{-1}. FTIR spectra at wavenumber 700-1400 cm^{-1} of each sample were compared.

3.3.2.6 Determination of apparent density

The apparent density of non-crosslinked and crosslinked HGCs was determined using gas displacement technique to evaluate the condensation of HGCs structure. Five capsules were accurately weighed and transferred to the small cell. The determination was performed in five replicates. Mean and SD of the apparent density of capsules were reported.

3.3.2.7 Texture analysis

The mechanical properties of capsules were performed using texture analyzer. A 50 kg load cell fitted with the probe (A/CLT; Capsule-loop tensile RIG) was used. Capsule body was mounted onto the pair of rods (n=10). The probe travels upward until capsule broken. The applied force was recorded as a function of distance. Tensile force (N), elongation at break (mm), and elastic stiffness (N/mm) were reported. Which the tensile force was the force applied at rupture point, elongation at break was the deformation when the capsule was broken, and elastic stiffness was the slope of linear region of the force-distance profile.

3.3.2.8 Stability of crosslinked HGC shell during storage

Crosslinked HGC shells were stored for 90 days at ambient temperature and relative humidity. Crosslinked HGC shells were taken at the predetermined time of 30, 60, and 90 days to evaluate weight loss after immersing in mediums, water soluble protein fraction, loss on drying, and formaldehyde residue. ATR-FTIR spectra of stored capsule shells were also investigated. Stored capsule shells were collected to prepare EOP capsules. Drug release studies of those osmotic pump capsules were conducted to demonstrate that crosslinked HGC shells after storage would maintain the same release properties.

3.3.3 Evaluation of drug property

3.3.3.1 Solubility

The 0.5 mL solvent; water, isoosmolality adjusted HCl pH 1.2, isoosmolality adjusted PBS pH 6.8, SGF, SIF, 0.45% NaCl, 0.9% NaCl, or 3% NaCl, was added to a microcentrifuge tube (n=3). The excess amount of each model drug was then added. The mixture was shaken at 37 °C for 24 h in water bath. The obtained mixture was filtered using a syringe filter with pore size of 0.45 µm. The supernatant was diluted into proper concentration and analyzed for drug content by HPLC.

3.3.3.2 Apparent density

The apparent density of drug powder was determined using the helium gas displacement technique. Drug powder was passed through a 60-mesh sieve to prevent drug agglomeration. The drug powder was accurately weighed and transferred to the micro cell with lid cover. The determination was performed in five replicates using a gas pycnometer. The mean and SD of the apparent density of drug powder were reported.

3.3.3.3 Particle size and shape

The drug powder was passed through a 60-mesh sieve to break up powder agglomeration. Each model drug powder was analyzed for particle size using a laser diffraction particle size analyzer. The mean diameter, 10, 50, 90 volume percent less than or equal to diameter (D10, D50, D90, respectively) and span were reported. In addition, a scanning electron microscope was used to define approximate particle size and shape of drug powder.

3.3.4 Preparation of EOP capsule

The 400 mg powder mixture formulation composed of 100 mg model drug (DIL HCl, PRO HCl, AMB HCl, or PAR), 100 mg sodium chloride, 196 mg spray dried lactose, and 4 mg magnesium stearate was filled into individual non-crosslinked and crosslinked HGC. Capsules were dipped once in a subcoating polymer solution composed of 3%w/w HPMC E5 and 2% w/w PEG 4000 dissolved in the mixture of 95% ethanol and water of 1:1 volume ratio (Wichianprasit & Kulvanich, 2009). The capsules were dried. Subcoated capsules were dipped two times in semipermeable membrane polymer solution containing 6% w/w Opadry® CA dissolved in the 90% acetone aqueous solution (Colorcon, 2012). Coated capsules were dried in hot air oven overnight at 40 °C. The coated capsules were drilled with 0.6 mm diameter needle at the top of capsule cap. The schematic of EOP capsule developing in this work is shown in Figure 3.1. Formulation factors were varied i.e. the amount and type of model drugs,

particle size of AMB HCl in order to modify drug release characteristic. In addition, powder mixture-filled crosslinked HGCs without any subcoated and semipermeable coated layer were also prepared to investigate factors affecting the rate limiting step of drug release from EOP capsule.

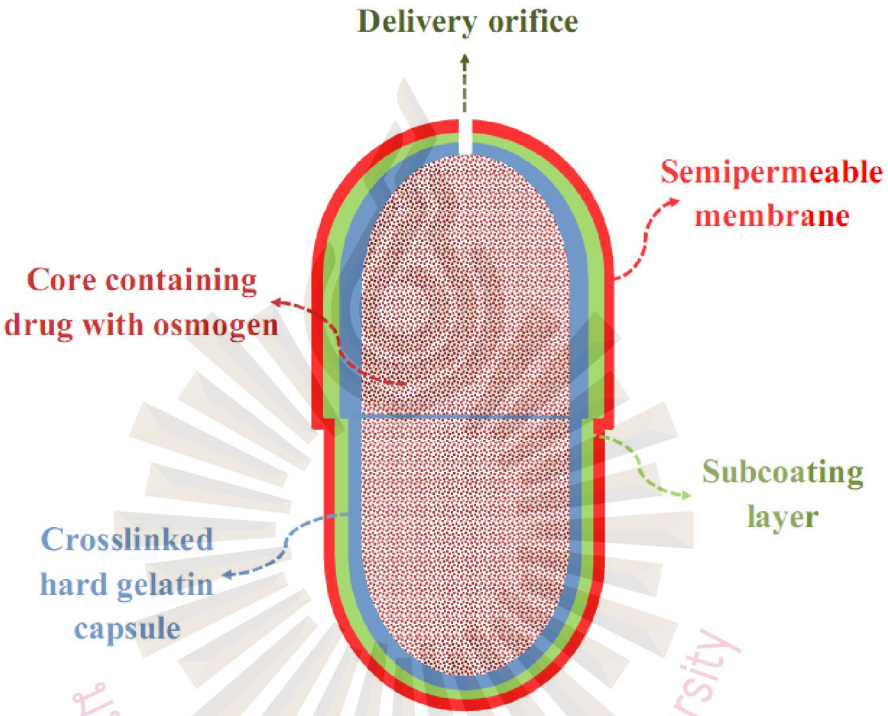


Figure 3.1 Structure of developed EOP capsule.

3.3.5 Evaluation of EOP capsule

3.3.5.1 Drug release

Three osmotic capsules were individually tested for drug release. Dissolution apparatus 2 (paddle apparatus) was used. The paddle speed was set at 100±1 rpm. The dissolution medium was 900 mL water (or HCl pH 1.2, PBS pH 6.8, SGF, SIF, 0.45% NaCl, 0.9% NaCl, and 3% NaCl). The medium temperature was controlled at 37±0.5 °C. EOP capsules (n=3) were sunk at the bottom of vessel throughout the study using a sinker. The dissolution medium of 3 mL was sampled at predetermined times for 12 h. The same volume of fresh medium was replenished. The

sampled medium was filtered and injected into HPLC instrument. The amount of drug release was calculated from the calibration curve. Then, drug release profile was constructed. The R^2 was determined using different release kinetic models including zero-order, first-order, and Higuchi's model (Dash, Murthy, Nath, & Chowdhury, 2010). The R^2, drug release rate, and lag time were calculated using DDSolver, an add-in program of Microsoft Excel developed by Zhang's research group (except the preliminary study data in Table 4.1, which using Microsoft Excel without add-in program). According to drug release analysis, it was performed on HPLC instrument equipped with photodiode array detector and autosampler. Separation was achieved on a Luna C18(2) column (250×4.6 mm i.d., 5 µm). Column temperature was controlled at 25 ºC. The injection volume was 10 µL. The isocratic system comprises of methanol (A) and acetate buffer pH 4.5 (B) with flow rate of 1 mL/min. The mobile phase ratio, quantitation wavelength, and total analysis time for each model drug are shown in Table 3.1.

Table 3.1 Mobile phase ratio, quantitation wavelength, and total analysis time of HPLC for each model drug.

Model drugs	A:B ratio	Wavelength (nm)	Total analysis time (min)
DIL HCl	66:34	240	7
PRO HCl	75:25	290	5
AMB HCl	55:45	248	9
PAR	45:55	246	5

Drug analysis method is validated based on ICH Harmonised Tripartite Guideline (ICH Expert Working Group, 2005) in following topics;

Linearity and range

A stock standard solution of model drugs was diluted in ultrapure water (or HCl pH 1.2, PBS pH 6.8, SGF, SIF, 0.45% NaCl, 0.9% NaCl, and 3% NaCl) to obtain six concentrations of 5, 10, 20, 30, 40, and 50 µg/mL, filtered, and injected into HPLC instrument. Each concentration was performed in triplicate. The calibration curve was constructed. The linear equation, R^2, and range were reported.

Specificity

The specificity was tested by observing UV spectrum of sample in the upslope, apex, and downslope of the peak. The same spectrum of three peak regions should be similar to UV spectrum of standard. Furthermore, the blank sample of EOP in each medium was injected into HPLC instrument. The specificity was recognized when chromatogram of the blank sample does not interfere the chromatogram of a drug sample.

LOD and LOQ

LOD and LOQ were calculated based on the calibration curve according to Equation 3-3 and 3-4.

Precision

The three concentration levels of a standard solution of each model drug; 10, 20, and 40 µg/mL were prepared. The solution was filtered and injected into HPLC instrument. Each concentration was analyzed in triplicate. The percent relative standard deviation (%RSD) of the analysis on the same day was reported as intraday precision and %RSD of the analysis in different three days was reported as inter-day precision. %RSD should be not more than 2% and 5% for intraday and inter-day precision, respectively.

Accuracy

The accuracy was evaluated by the addition of three drug concentration levels (10, 20, and 40 µg/mL) to sample solutions. The percent recovery of the drug was calculated. Percent recovery should be close to 100%. Each concentration was analyzed in triplicate.

System suitability

Standard solution of model drugs was injected into HPLC instrument in six replicates. Retention time, peak area, number of theoretical plates (USP), and asymmetry were recorded. The mean, SD, and %RSD were reported.

3.3.5.2 Morphology of EOP capsule structure

The morphology of EOP capsule structure was evaluated using scanning electron microscope. The delivery orifice, surface, and cross section of EOP capsule were investigated with proper magnification. Furthermore, the hole of crosslinked HGCs made by the needle before and after coated with CA was also studied.

3.3.6 Preparation of PPOP capsule

All ingredients of the pull and push layers were individually mixed by the geometric dilution technique. The composition of pull and push layers are shown in Table 3.2 and Table 3.3, respectively. The 90 mg of push layer mixture was added into the body of crosslinked HGCs. The 180 mg of pull layer mixture was then added. A capsule body was snapped with a capsule cap. The obtained capsule was subcoated and coated using manual dipping method. The capsule once dipped in the subcoating polymer solution (3%w/w HPMC E5 and 2%w/w PEG 4000 was dissolved in a mixture of 95% ethanol and water, 1:1 volume ratio) and dried. Then, the subcoated capsule was dipped in semipermeable membrane polymer solution (6%w/w Opadry® CA dissolved in the mixture of acetone and water, 9:1 volume ratio) and dried. CA coating process was repeated till eight coating layers were obtained. Then, the coated capsule was dried overnight in a hot air oven at 40 °C. Finally, the capsule was drilled with 0.6 mm diameter needle at the top of capsule cap to make the delivery orifice. The schematic structure of the developed PPOP capsule is shown in Figure 3.2. Formulation factors were varied from the standard composition as presented in Table 3.2 and Table 3.3, i.e. the amount (10, 30, and 50 mg) and type of model drugs (DIL HCl, PRO HCl, AMB HCl, and PAR), the amount of PEO Mw 200K (90, 130, 170 mg), and size of capsule (No.1 vs No.2) in order to observe drug release characteristic.

Table 3.2 Composition of pull layer.

Ingredients	Amount per capsule (mg)	Function
Model drug	10	Active ingredient
PEO Mw 200K	130	Entraining agent
Spray dried lactose	40	Diluent
Total	180	

Table 3.3 Composition of push layer.

Ingredients	Amount per capsule (mg)	Function
PEO Mw 5,000K	55	Swelling agent
Sodium chloride	34.5	Osmogen
Pigment (Adilake Carmoisine®)	0.5	Coloring agent
Total	90	

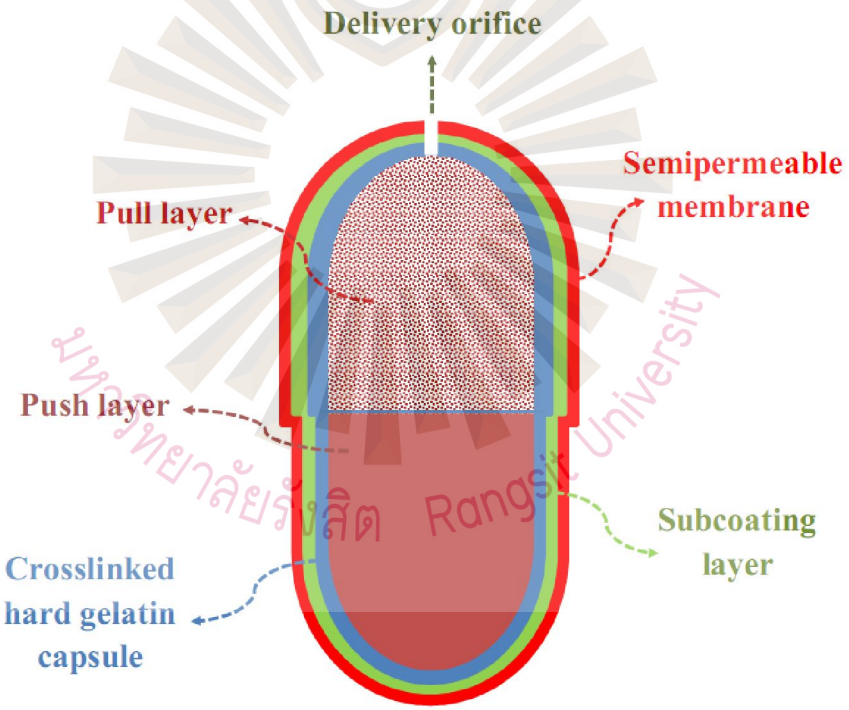


Figure 3.2 Structure of developed PPOP capsule.

3.3.7 Evaluation of PPOP capsule

3.3.7.1 Drug release

Drug release was determined using the same method as described for EOP capsule evaluation. Various types of dissolution medium were used including water, HCl pH 1.2, PBS pH 6.8, SGF, SIF, 0.45% NaCl, 0.9% NaCl, and 3% NaCl. Release kinetic models were investigated. Furthermore, method validation was also performed using several mediums.

3.3.7.2 Morphology of PPOP capsule structure

The morphology was determined using scanning electron microscope. The delivery orifice, surface, and cross section of PPOP capsule were investigated with proper magnification.

3.3.8 Statistical analysis

All experimental data were presented as mean±SD. The independent-samples t-test was used for comparison of any two data sets. One-way analysis of variance (One-Way ANOVA) follow by post hoc analysis was used for comparison of more than two data sets. Data was significantly different when p-value was less than 0.05 at 95% confident interval. Goodness of fit test used Pearson correlation to compare the correlation of two factors. Data was significantly correlated when p-value was less than 0.05 at 95% confident interval.

CHAPTER 4

RESULTS AND DISCUSSION

The results of the work are presented in accordance with the sequential order of the experimental work done as follow:

1) the property and stability under storage of crosslinked HGCs and preliminary investigation on its application for osmotic pump system.

2) the property of four model drugs

3) appearance of EOP and PPOP capsules by visual observation and SEM photomicrography

4) comparative drug release from EOP and PPOP capsules prepared using non-crosslinked and crosslinked HGCs and reproducibility of HGCs osmotic pump preparation

5) influencing factors on drug release from EOP and/or PPOP capsules i.e. drug type, loading dose, amount of PEO Mw 200K in pull layer of PPOP, sodium chloride in push layer of PPOP, capsule size, CA coating, osmolality of release medium, pH of release medium (with or without enzyme), and storage time of crosslinked HGCs

4.1 Properties of crosslinked HGCs

According to the preliminary study, it was found that HGCs crosslinked in formaldehyde vapor for 4 h were partially soluble in water, HCl pH 1.2, and PBS pH 6.8 while HGCs crosslinked for at least 6 h were insoluble in these three mediums (Figure 4.1). Thus, HGCs exposed to formaldehyde vapor for 6, 12, and 24 h were investigated. HGCs exposed to formaldehyde vapor for a different length of times showed slight difference in physical appearance. Increasing crosslinking time of capsules, the turbidity of capsules increased. HGCs crosslinked for 24 h had the highest

turbidity compared to non-crosslinked HGCs and HGCs crosslinked for 6 and 12 h (Figure 4.2).

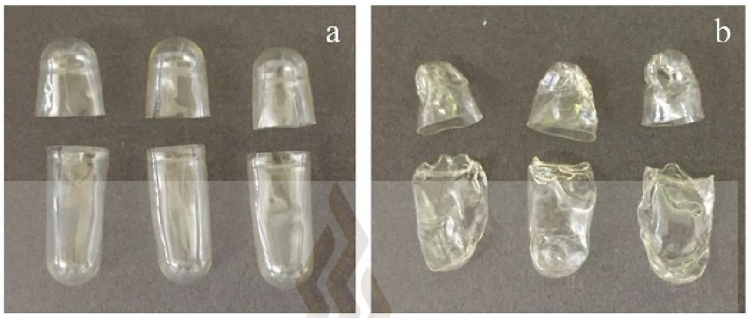


Figure 4.1 Physical appearance of (a) insoluble (exposed to formaldehyde vapor for 6 h) and (b) partially soluble crosslinked HGCs (exposed to formaldehyde vapor for 4 h) after being immersed in water for 24 h.

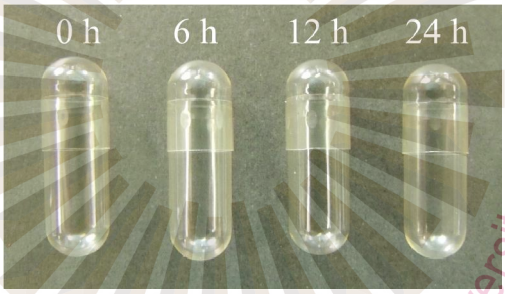


Figure 4.2 Appearance of non-crosslinked HGCs (0 h) and HGCs crosslinked with different lengths of time (6, 12, and 24 h).

The susceptibility of HGCs to aldehyde compounds leading to crosslinking between proteins chain of gelatin is a well-known phenomenon (Salsa et al., 1996). Crosslinking of amino acids in HGCs occurred between lysine and arginine or between two arginine molecules. The crosslinking reaction was initialized by interaction of lysine with formaldehyde forming lysine methylol followed by reacting arginine with formaldehyde causing formation of arginine methylol that reacted with lysine methylol to form lysine/arginine crosslink. In the case of arginine, two arginine methylols also could form an arginine/arginine crosslinks (Salsa et al., 1996) as shown in Figure 2.14 and Figure 2.15. FTIR was a sensitive method to detect crosslinking process in HGCs

(Singh et al., 2002). The spectrum of untreated HGC (non-crosslinked) in this study was similar to the typical spectrum of proteins including amide I, amide II, and amide III. They exhibited the vibrations of amide I (carbonyl stretch) at 1628 cm^{-1}, amide II (N-H bend and C-N stretch) at 1542 cm^{-1}, and amide III (in-phase combination of N-H bend and C-N stretch), a set of three peaks centered at 1242 cm^{-1}. At higher frequencies, there were CH_2 and CH_3 stretching modes, weak peaks in the wavenumber ranged from 2800 to 2950 cm^{-1}. Finally, O-H and N-H stretching vibrates at 3000 to 3600 cm^{-1} (Figure 4.3). The non-crosslinked HGC spectrum in this present study was similar to the previous publication reported by Tengroth et al. (2005), while crosslinking of HGCs could be observed at low to medium frequencies approximately 700-1400 cm^{-1}. There were tentative four peaks representing four functional groups, including ring bend of arginine methylol (800 cm^{-1}), lysine methylol C-O stretch (1020 cm^{-1}), arginine methylol C-O stretch (1095 cm^{-1}), and aromatic amine C-N stretch (1260 cm^{-1}).

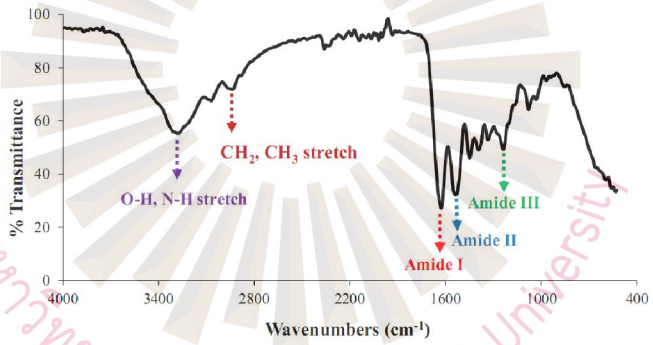


Figure 4.3 FTIR spectrum of untreated (non-crosslinked) HGC.

Figure 4.4(a) shows initial FTIR spectra of non-crosslinked HGCs and crosslinked HGCs. With increasing of crosslinking time, the intensity of lysine methylol C-O stretch at a wavenumber of 1025 cm^{-1} increased while the intensity of arginine methylol C-O stretch at a wavenumber of 1090 cm^{-1} decreased. The increasing peak intensity of crosslinked HGCs at 1025 cm^{-1} might be the combined signal of C-O group of lysine methylol and C-O group of formaldehyde. Furthermore, observed peaks of ring bend of arginine methylol (800 cm^{-1}) and aromatic amine C-N stretch (1270 cm^{-1}) were not affected by crosslinking time. Figure 4.4(b-e) show FTIR spectra of HGCs crosslinked for 0, 6, 12, and 24 h, respectively, at the initial, and after storage for 30,

60, and 90 days. Storage of crosslinked HGCs at ambient condition did not alter the peak intensity of ring bend of arginine methylol, lysine methylol C-O stretch, arginine methylol C-O stretch, and aromatic amine C-N stretch.

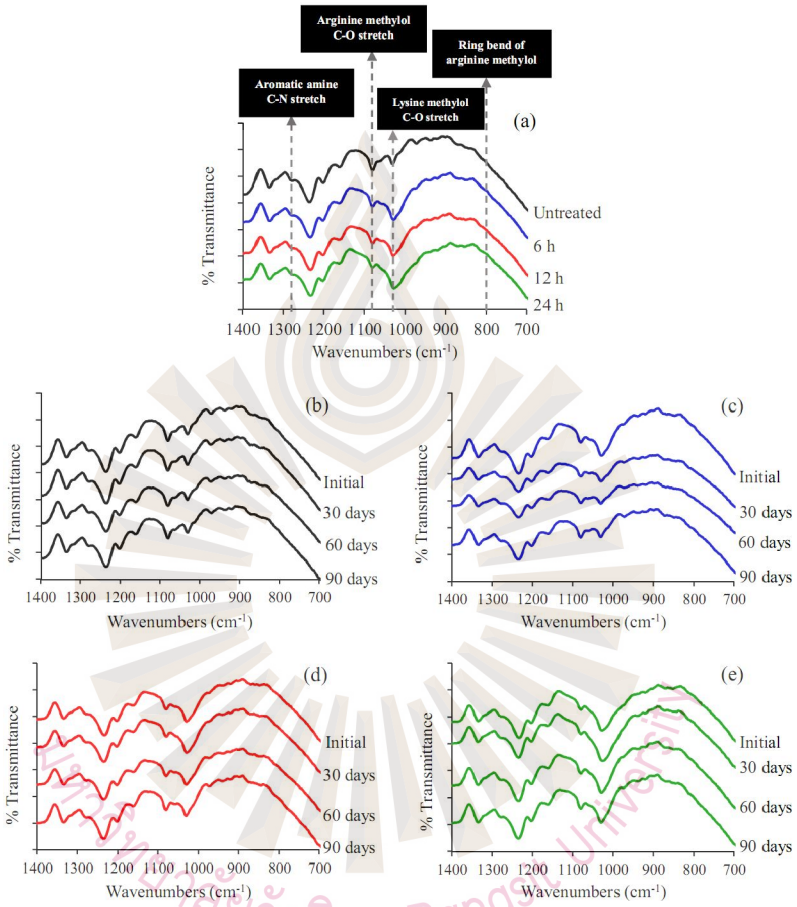


Figure 4.4 FTIR spectra of (a) untreated HGCs (non-crosslinked) and formaldehyde treated HGCs (crosslinked) for 6, 12, and 24 h at initial time; FTIR spectra of (b) non-crosslinked HGCs and formaldehyde treated HGCs shells for (c) 6 h, (d) 12 h, (e) 24 h after storage for 30, 60, and 90 days.

Crosslinked HGCs were immersed in water, HCl pH 1.2, and PBS pH 6.8 mediums for 24 h to evaluate their solubility properties. Non-crosslinked HGCs, in their nature, were soluble in these three mediums (Chiwele, Jones, & Podczeck, 2000), while all crosslinked HGCs were insoluble in all mediums. This implies crosslinked HGCs will remain intact throughout the GI tracts. Percent weight loss of crosslinked HGCs

after immersion in mediums was less than 6% (Figure 4.5). After storage for 90 days, crosslinked HGCs remained insoluble with similar degree of percent weight loss. Negative values of percent weight loss of capsules after immersion in PBS were observed; it might be the residue of buffering agent remained in capsules after drying process.

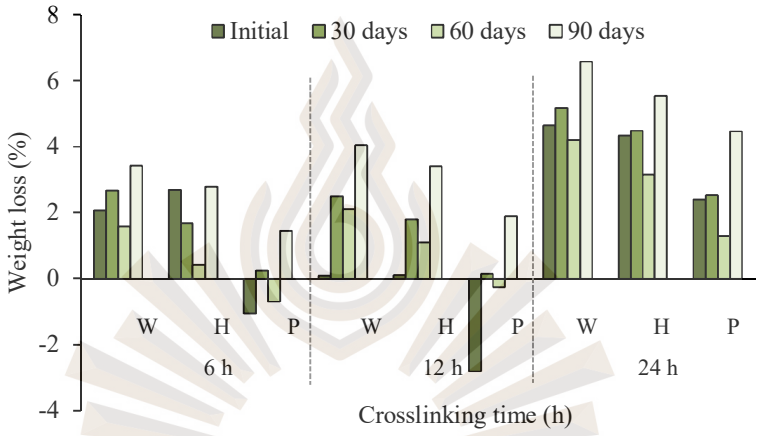


Figure 4.5 Percent weight loss of HGCs crosslinked with different lengths of time after immersion in water (W), HCl pH 1.2 (H), and PBS pH 6.8 (P) when storage for 30, 60, and 90 days.

Dissolution of crosslinked capsules was observed by detection of water soluble protein fraction. Our work used BCA protein assay kit. Four amino acids were reported to be responsible for color formation with BCA i.e. cysteine, cystine, tryptophan, and tyrosine (Wiechelman, Braun, & Fitzpatrick, 1988). Tyrosine was a one amino acid containing in gelatin (Eastoe, 1955; Farris et al., 2010) while cysteine and cystine were slightly found (Singh et al., 2002). However, gelatin contained no tryptophan (Eastoe, 1955; Farris et al., 2010; Singh et al., 2002).

Water soluble protein fraction should be low in crosslinked HGCs compared to non-crosslinked HGCs. Ofner et al. (2001) reported that capsule exposed to formaldehyde level of 120 and 200 ppm causing lower capsule dissolution compared to untreated capsules and capsules treated with 20 and 30 ppm formaldehyde. Results of water soluble protein fraction determination after storing capsule shells for 90 days

are shown in Figure 4.6. Crosslinked HGCs initially dissolved less than 3% and did not dissolve when storage time increased. Our results show that increasing of crosslinking time is not correlated with water soluble protein fraction. Water soluble protein fraction of capsule shells corresponded with the results of weight loss of capsule as described above.

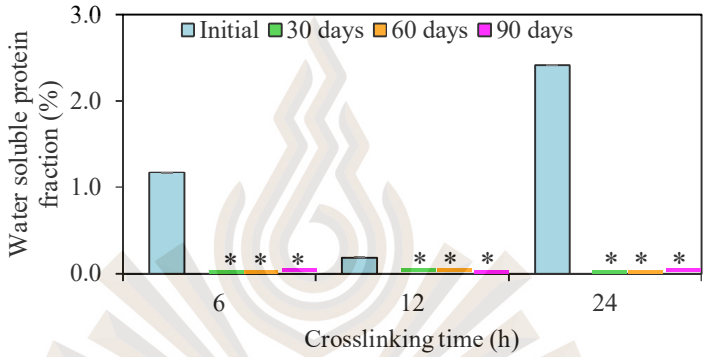


Figure 4.6 Water soluble protein fraction of HGCs crosslinked with different lengths of time when storage for 30, 60, and 90 days (* not detectable at 30, 60, and 90 days).

The moisture remaining in capsules plays the role as a plasticizer of HGC shells. Normally, equilibrium moisture content of plain HGCs was approximately 13-16%w/w, which was the same amount of moisture for non-crosslinked HGCs used in this study. Nevertheless, the moisture content of capsules would depend on the storage condition e.g., temperature, and humidity (Augsburger, 2009). However, loss on drying determination using moisture balance would include both moisture content and residual formaldehyde in capsules. Loss on drying of crosslinked HGCs initially was approximately 7-9 %w/w. After storage at room condition for 90 days, capsules gradually reabsorbed moisture from the atmosphere. Thus, the moisture content of crosslinked HGCs increased; however, moisture was still lower than equilibrium moisture content of non-crosslinked HGCs. Loss on drying of non-crosslinked HGCs and HGCs crosslinked with different storage times at initial and after storage for 30, 60, and 90 days, are shown in Figure 4.7. However, loss on drying data would be a summation of moisture and formaldehyde residue in capsules.

Figure 4.8 shows that increment of crosslinking time increased formaldehyde residue. However, upon increasing storage time to 90 days, formaldehyde residue gradually decreased due to its volatile property. HGCs subjected to crosslinking time for 6-24 h had formaldehyde residue of less than 0.5%w/w. The results suggested that formaldehyde content was very low and it could be negligible; thus, loss on drying results are mostly represented by moisture content rather than formaldehyde content. The United States Environmental Protection Agency reported that limit number of clinical trials reported an association between exposure to formaldehyde and lung/nasopharyngeal cancer. Animal studies indicated that formaldehyde caused nasal squamous cell cancer. In addition, formaldehyde was categorized as a probable human carcinogen (Group B1) (US Environmental Protection Agency, 2000). However, daily intake of formaldehyde for adults was approximately 1.5-14 mg/day by consumption of contaminated food, breathing contaminated indoor air, the smoke of tobacco, or ambient urban air (US Environmental Protection Agency, 2001). Furthermore, US Environmental Protection Agency recommended the oral limit of formaldehyde of 0.2 mg/kg/day or 10 mg/day for a 50 kg person (ICH Expert Working Group, 2015). From our study, the weight of one empty capsule was approximately 75 mg. Thus, crosslinked HGCs may have formaldehyde residue less than 0.375 mg which 26.7 times is lower than the limit of daily intake for formaldehyde. The result shows that crosslinked HGCs had low formaldehyde content indicating the safety of crosslinked HGCs that could be used for the pharmaceutical purpose.

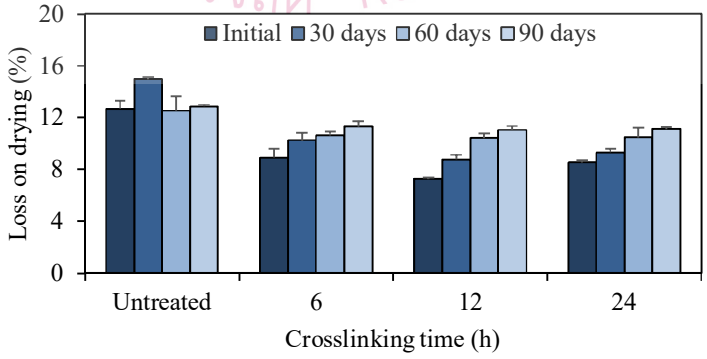


Figure 4.7 Loss on drying of non-crosslinked HGCs and HGCs crosslinked with different lengths of time when storage for 30, 60, and 90 days.

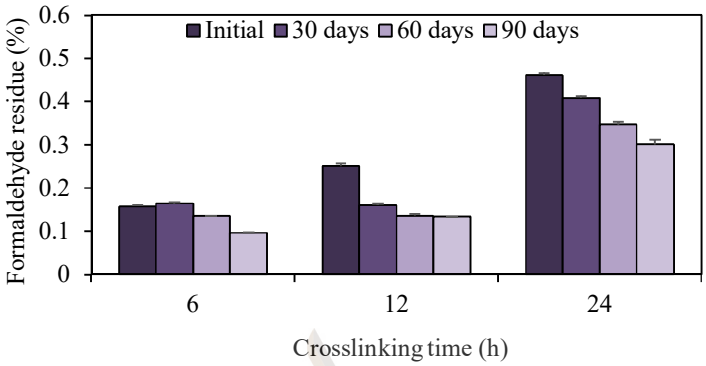


Figure 4.8 Formaldehyde residue of crosslinked HGCs when storage for 30, 60, and 90 days.

Apparent density of capsule was performed only at the initial time point. Non-crosslinked HGCs had apparent density of 1.493±0.009 g/cm^3, which higher than crosslinked HGCs. The apparent density was decreased when crosslinking time increased. Figure 4.9 shows apparent density of HGC crosslinked for 6, 12, and 24 h was 1.441±0.009, 1.473±0.006, and 1.409±0.004 g/cm^3, respectively. The capsule crosslinked for 24 h had the lowest apparent density. The correlation between crosslinking time (0, 6, 12, and 24 h) and apparent density had Pearson correlation value of -0.812 with p-value of 0.001. Among three crosslinking groups (6, 12, and 24 h), the correlation between crosslinking time and apparent density had Pearson correlation value of -0.649 with p-value of 0.009. This correlation result also indicated increasing crosslinking time decreased apparent density.

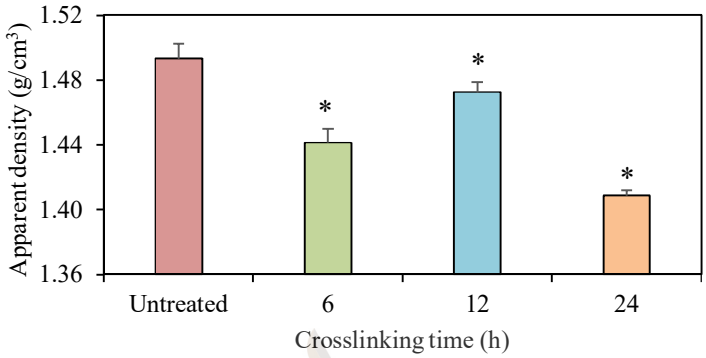


Figure 4.9 Apparent density of non-crosslinked and crosslinked HGCs with different crosslinking times. * = significant difference compared to non-crosslinked HGCs, p-value < 0.05. Difference was investigated using One-Way ANOVA followed by Tukey HSD.

Texture analysis was performed only at initial time point. Crosslinking of HGCs provided significant higher tensile force compared to the non-crosslinked HGCs (Figure 4.10). This result indicated crosslinked HGCs become significant tougher than non-crosslinked HGCs which related to condensed polymer chain of crosslinked HGCs. The correlation between crosslinking time (0, 6, 12, and 24 h) and tensile force had Pearson correlation value of -0.091 with p-value of 0.582. Among three crosslinking groups (6, 12, and 24 h), the correlation between crosslinking time and tensile force had Pearson correlation value of -0.828 with p-value less than 0.0001. It was found that crosslinking time (6, 12, and 24 h) correlated with tensile force.

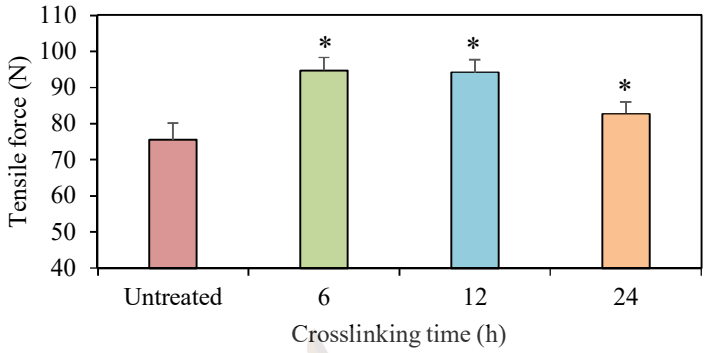


Figure 4.10 Tensile force of non-crosslinked and crosslinked HGCs with different crosslinking times. * = significant difference (p-value < 0.05) compared to non-crosslinked HGCs. Difference was investigated using One-Way ANOVA followed by Tukey HSD.

Crosslinking of HGCs provided significant higher elastic stiffness compared to the non-crosslinked HGCs except HGCs crosslinked for 24 h (Figure 4.11). This result indicated HGCs crosslinked for 6 and 12 h become significant softer than non-crosslinked HGCs. The correlation between crosslinking time (0, 6, 12, and 24 h) and elastic stiffness had Pearson correlation value of -0.100 with p-value of 0.544. Among three crosslinking groups (6, 12, and 24 h), the correlation between crosslinking time and elastic stiffness had Pearson correlation value of -0.651 with p-value less than 0.0001. It was found that crosslinking time (6, 12, and 24 h) correlated with elastic stiffness. The result of tensile force and elastic stiffness specified that crosslinked HGCs become softer when increasing of crosslinking time.

Crosslinking of HGCs provided significant higher elongation at break compare to the non-crosslinked HGCs (Figure 4.12). This result indicated crosslinked HGCs become significant flexible than non-crosslinked HGCs. The correlation between crosslinking time (0, 6, 12, and 24 h) and elongation at break had Pearson correlation value of 0.463 with p-value of 0.003. Among three crosslinking groups (6, 12, and 24 h), the correlation between crosslinking time and elongation at break had Pearson correlation value of 0.025 with p-value less than 0.896. It was found that no correlation between crosslinking time (6, 12, and 24 h) and elongation at break. From the

mechanical property including tensile force, elastic stiffness, and elongation at break, it was found that crosslinking of HGCs let them significant flexible and tougher.

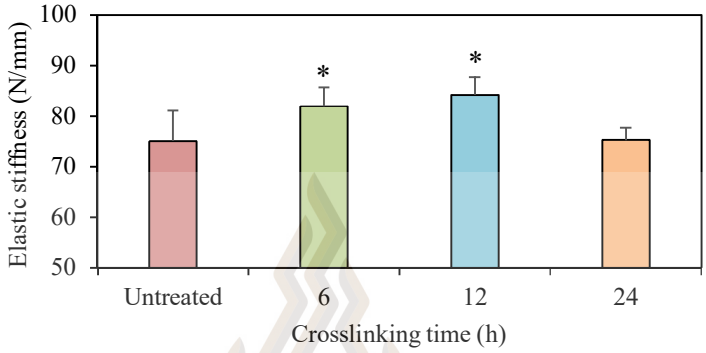


Figure 4.11 Elastic stiffness of non-crosslinked and crosslinked HGCs with different crosslinking times. * = significant difference (p-value < 0.05) compared to non-crosslinked HGCs. Difference was investigated using One-Way ANOVA followed by Tukey HSD.

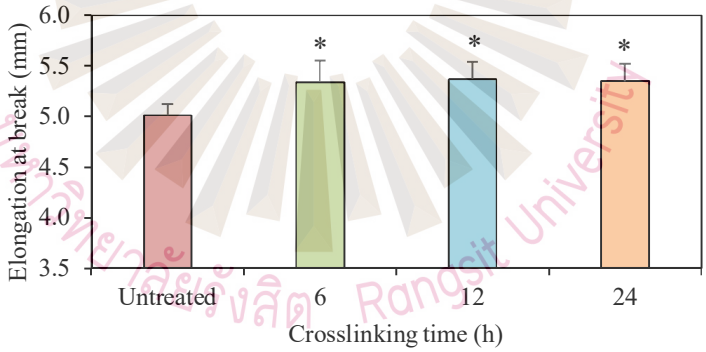


Figure 4.12 Elongation at break of non-crosslinked and crosslinked HGCs with different crosslinking times. * = significant difference (p-value < 0.05) compared to non-crosslinked HGCs. Difference was investigated using One-Way ANOVA followed by Tukey HSD.

EOP was prepared for delivery of freely water soluble and sparingly water soluble model drugs. Figure 4.13(a) and Figure 4.14(a) display drug release of DIL HCl and AMB HCl EOP, respectively. Drug release from EOP prepared using non-

crosslinked HGCs was higher than those of EOP prepared using crosslinked HGCs for both model drugs. The results could be described by the solubility property of capsules. When non-crosslinked HGCs EOP imbibed water, the capsule shell dissolved, drug component easily contacted water without blockade by capsule shell, thus, higher drug release was obtained. From our preliminary study, it was found that EOP prepared using non-crosslinked HGCs as a structural assembly showed high variation of drug release and irreproducible release profiles. This evidence was also reported by Wichianprasit and Kulvanich (2009). The results support the suitability of crosslinked HGCs as a structural assembly for osmotic system. DIL HCl release from EOPs prepared using crosslinked HGCs with different crosslinking time of 6 and 12 h was similar, but EOP prepared using crosslinked HGCs of crosslinking time for 24 h gave slower drug release with a longer lag time.

Figure 4.13(b-d) present effect of storage time of crosslinked HGC shells on DIL HCl release from EOP when crosslinking for 6, 12, and 24 h, respectively. Storage of crosslinked capsule shells for 90 days did not affect DIL HCl release from EOP prepared using HGCs crosslinked for 12 h, while alteration of DIL HCl release was observed in EOP prepared using HGCs crosslinked for 6 and 24 h. Less fluctuation of drug release of EOP prepared using HGCs crosslinked for 12 h might be due to lower water soluble protein fraction of capsule shells (Figure 4.6) resulting in more consistent water imbibition into the EOP system. The result indicated that HGCs crosslinked for 12 h were suitable for use as a structural assembly of EOP due to its consistent drug release profile. AMB HCl release from EOP prepared using crosslinked HGCs was extremely low for all crosslinking times (Figure 4.14(b-d)). Aside from low water solubility of the drug, high density of AMB HCl (1.69 g/cm^3) might cause rapid sedimentation of drug particles within osmotic pump during drug release from the system preventing drug delivery through the delivery orifice. The results indicate that EOP capsule was appropriate for delivery of water soluble drug.

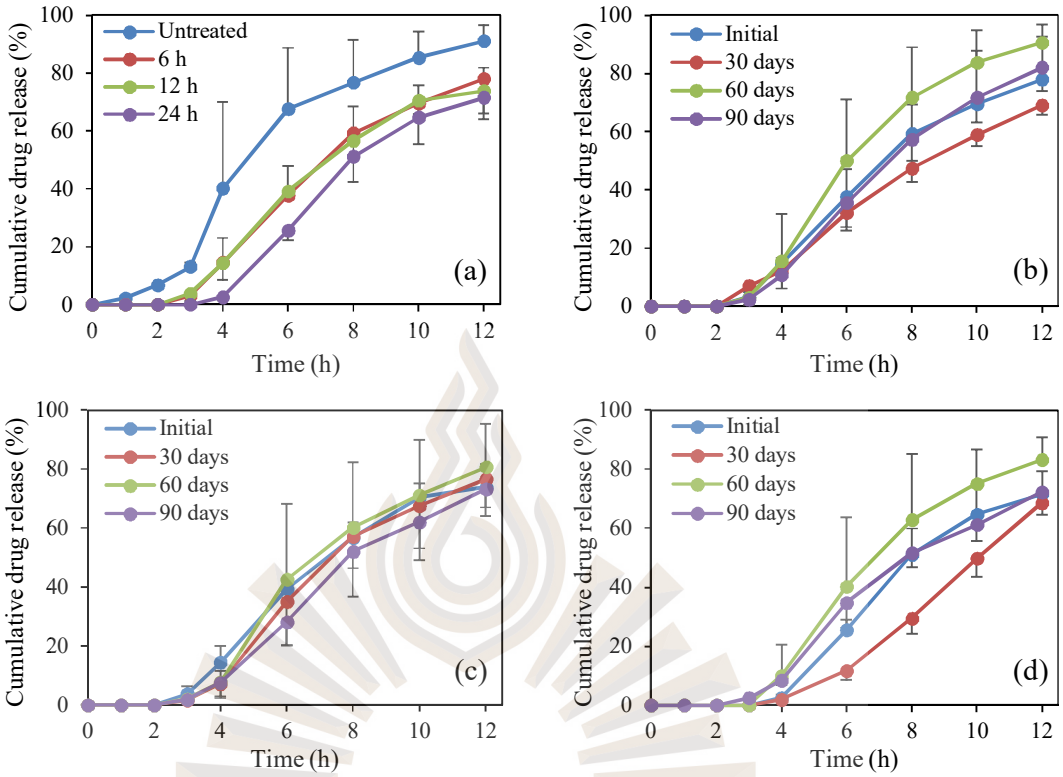


Figure 4.13 Drug release profiles in water of (a) DIL HCl EOP prepared using non-crosslinked HGCs shells and HGCs shells crosslinked for 6, 12, and 24 h at initial time; (b), (c), and (d) display drug release from DIL HCl EOP prepared using HGCs crosslinked for 6, 12, and 24 h, respectively, after keeping crosslinked capsule shells for 30, 60, and 90 days.

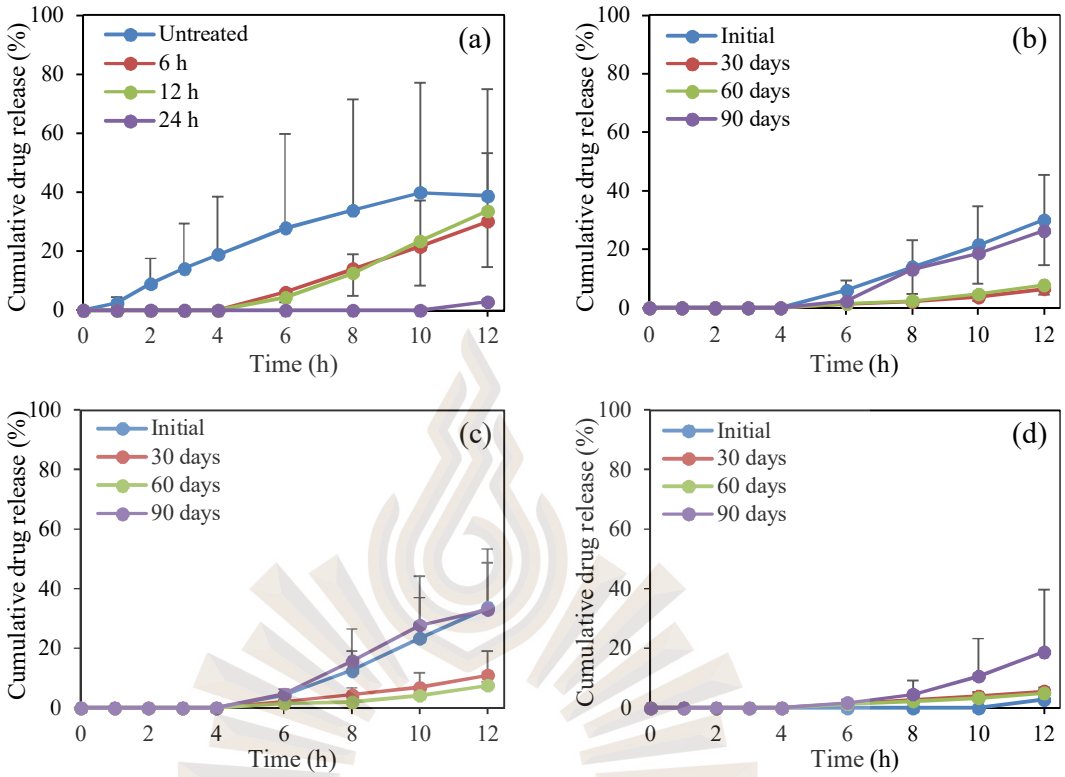


Figure 4.14 Drug release profiles in water of (a) AMB HCl EOP prepared using non-crosslinked HGCs and HGCs shells crosslinked for 6, 12, and 24 h at initial time; (b), (c), and (d) display drug release from AMB HCl EOP prepared using HGCs crosslinked for 6, 12, and 24 h, respectively, after keeping crosslinked capsule shells for 30, 60, and 90 days.

According to the previous reports, drug release from osmotic pumps mostly exhibited zero-order release model (Conley et al., 2006). However, Higuchi's model of drug release of EOP system was also reported (Derakhshandeh & Berenji, 2014). Almost all of EOP capsules developed in this work exhibited Higuchi's release model (Table 4.1). However, DIL HCl release from EOP prepared using HGCs crosslinked for 24 h after storage for 30 days exhibited zero-order release model. As presented in Table 4.1, DIL HCl release from EOP prepared using crosslinked HGCs showed release rate of approximately 37.4-53.5 $\%/h^{-0.5}$ with lag time of 2.5-4.3 h. HGCs crosslinked for 12 h gave DIL HCl release rate of approximately 43.1-48.5 $\%/h^{-0.5}$ with a lag time of 2.6-3.1 h. From overall results, HGCs crosslinked for 12 h was selected for use as a

structural assembly of osmotic pumps in subsequent work due to its insoluble property, low formaldehyde residue, and stability of drug release profiles of those EOP prepared from storage capsule shells.

Table 4.1 Interpretation of drug release data (based on zero-order, first-order, and Higuchi's model) of DIL HCl EOP capsules and determination of release rate, lag time.

Capsule shell storage time, day(s)	Crosslinking time (h)	R^2 of drug release			Release rate ($\%/h^{-1/2}$)*	Lag time (h)*
		Zero-order	First-order	Higuchi		
0	Untreated capsules	0.9183	0.7505	0.9508	41.48	1.21
	6	0.9635	0.7669	0.9876	44.96	2.67
	12	0.9512	0.7749	0.9802	43.09	2.58
	24	0.9531	0.7514	0.9778	49.08	3.62
30	6	0.9867	0.8830	0.9953	37.38	2.54
	12	0.9622	0.7812	0.9825	46.54	2.99
	24	0.9867	0.8980	0.9636	46.33	4.34
60	6	0.9390	0.7372	0.9729	53.51	2.65
	12	0.9475	0.7723	0.9743	48.50	2.88
	24	0.9383	0.7828	0.9707	50.38	2.90
90	6	0.9798	0.7912	0.9937	48.39	2.93
	12	0.9785	0.8135	0.9882	43.62	3.05
	24	0.9542	0.7881	0.9808	42.09	2.86

* calculated based on Higuchi's release model using Microsoft Excel without add-in program.

4.2 Properties of model drugs

DIL HCl, PRO HCl, AMB HCl, and PAR were chosen as model drugs to prepare PPOP capsules. Water solubility determinations (at 37 °C) of DIL HCl, PRO HCl, AMB HCl and PAR were found to be 516.14±22.19, 217.64±9.78, 29.47±4.06, and 20.82±0.69 mg/mL, respectively (Table 4.2). It was indicated that DIL HCl and PRO HCl were freely water soluble while AMB HCl and PAR were sparingly water soluble. In addition, solubility of model drugs in various mediums is also shown in

Table 4.2. DIL HCl was freely soluble in all mediums. PRO HCl was freely soluble in all mediums, but to be sparingly soluble in 3% NaCl. AMB HCl was sparingly soluble in all mediums, except slightly soluble in 3% NaCl. Finally, PAR was sparingly soluble in all mediums. In addition, with increase in osmolality of the medium, the solubility of all model drugs decreased (Table 4.2).

Table 4.2 Solubility of model drugs in various mediums at 37 °C.

Model drugs	Mediums	Solubility (mg/mL)	Class
DIL HCl	Water	516.14±22.19	Freely soluble
	HCl pH 1.2*	491.44±10.13	Freely soluble
	PBS pH 6.8*	500.48±18.37	Freely soluble
	0.45%NaCl	532.14±37.95	Freely soluble
	0.9%NaCl	493.67±33.84	Freely soluble
	3%NaCl	373.76±33.45	Freely soluble
	SGF	499.11±5.82	Freely soluble
	SIF	519.95±8.20	Freely soluble
PRO HCl	Water	217.64±9.78	Freely soluble
	HCl pH 1.2*	142.96±1.08	Freely soluble
	PBS pH 6.8*	172.84±3.03	Freely soluble
	0.45%NaCl	172.21±6.13	Freely soluble
	0.9%NaCl	127.76±8.22	Freely soluble
	3%NaCl	11.74±5.09	Sparingly soluble
AMB HCl	Water	29.47±4.06	Sparingly soluble
	HCl pH 1.2*	12.78±0.19	Sparingly soluble
	PBS pH 6.8*	16.35±0.54	Sparingly soluble
	0.45%NaCl	18.19±0.55	Sparingly soluble
	0.9%NaCl	12.04±0.53	Sparingly soluble
	3%NaCl	4.69±0.26	Slightly soluble
PAR	Water	20.82±0.69	Sparingly soluble
	HCl pH 1.2*	20.86±0.54	Sparingly soluble
	PBS pH 6.8*	19.95±0.65	Sparingly soluble
	0.45%NaCl	19.77±0.54	Sparingly soluble
	0.9%NaCl	19.61±0.41	Sparingly soluble
	3%NaCl	16.93±0.68	Sparingly soluble
	SGF	20.29±0.37	Sparingly soluble
	SIF	19.77±0.33	Sparingly soluble

* = isoosmolality adjusted

Apparent density of each model drug was determined using gas displacement technique. DIL HCl, PRO HCl, AMB HCl, and PAR had apparent density of 1.301±0.000, 1.281±0.001, 1.688±0.002, and 1.206±0.000 g/cm^3 (Figure 4.15). AMB HCl and PAR had the highest and the lowest apparent density, respectively. Apparent density of pulverized AMB HCl was similar to intact AMB HCl to be 1.683±0.001 g/cm^3.

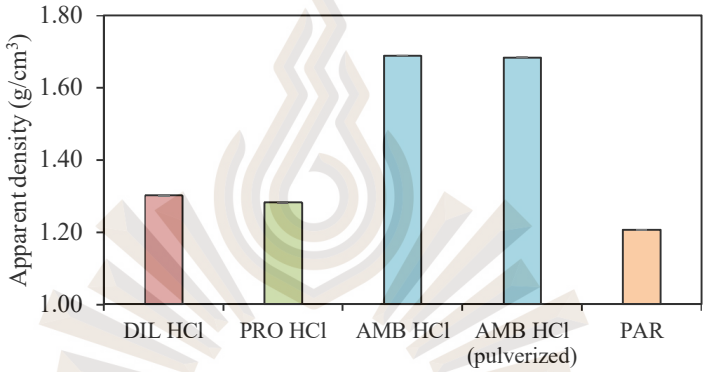


Figure 4.15 Apparent density of model drugs.

Particle size of each model drug is presented in Table 4.3. PAR and AMB HCl had the largest and the lowest particle size, respectively. PAR also had wider particle size distribution, which might be due to the agglomeration of drug particle due to its electrostatic charging (Jallo & Dave, 2015). Particle size of AMB HCl became smaller after pulverization, but they had similar apparent density. SEM photomicrograph of four model drugs is shown in Figure 4.16. DIL HCl particle had rod shape. AMB HCl particle had rhombus shape with some irregular shape. While, both PRO HCl and PAR particle had irregular shape.

Table 4.3 Particle size of model drugs.

Model drugs	Mean diameter (by volume), µm	D10	D50	D90	Span*
DIL HCl	64.48±0.26	9.34±0.09	51.85±0.29	139.28±0.42	2.51
PRO HCl	49.31±0.35	5.60±0.02	26.20±0.41	128.10±0.45	4.68
AMB HCl	39.43±0.25	7.06±0.07	32.58±0.23	81.24±0.53	2.28
AMB HCl (pulverized)	27.16±0.16	2.61±0.02	19.93±0.25	62.84±0.09	3.10
PAR	87.87±1.27	4.60±0.03	26.41±0.31	268.78±2.78	10.00

* Span = D90-D10/D50

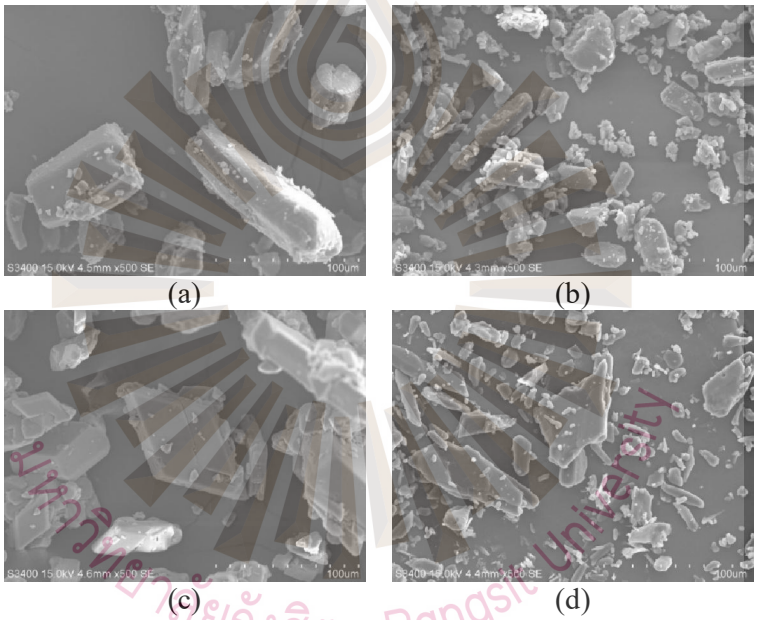


Figure 4.16 SEM photomicrograph of (a) DIL HCl, (b) PRO HCl, (c) AMB HCl, and (d) PAR with magnification ×500.

4.3 Appearance of EOP and PPOP capsules

This section reports the appearance of EOP and PPOP capsules from visual observation and SEM photomicrography. Morphology from SEM photomicrography of delivery orifice before and after CA coating is shown. Cross-section and surface of coating layer are also displayed.

Appearance of EOP and PPOP is shown in Figure 4.17 and Figure 4.18, respectively. Shiny surface found when capsule was subcoated with HPMC while matted surface found when capsule was coated with CA. This work used needle to make delivery orifice of the device. SEM photomicrographs show the smooth orifice on the capsule shell surface before coating process and after coating process (when peeled off the coating film) (Figure 4.19). This result indicated that making delivery orifice using needle did not destroy the crosslinked HGC shells during the osmotic pump preparation.

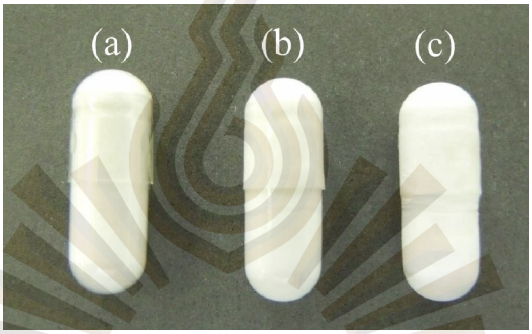


Figure 4.17 Appearance of powder mixture filled crosslinked HGC (a), crosslinked HGC subcoated with HPMC (b), and crosslinked HGC coated with CA (c) of EOP capsules.

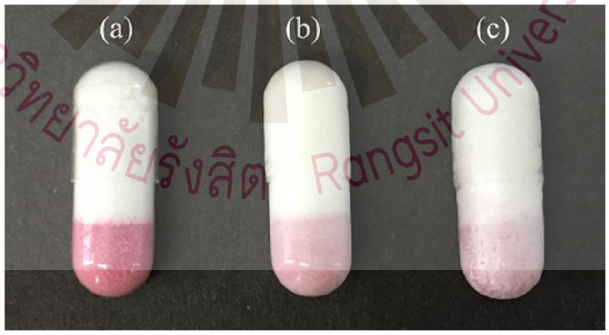
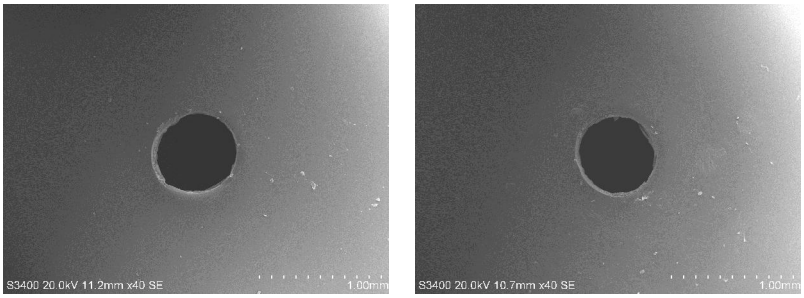


Figure 4.18 Appearance of powder mixture filled crosslinked HGC (a), crosslinked HGC subcoated with HPMC (b), and crosslinked HGC coated with CA (c) of PPOP capsules.



(a) (b)

Figure 4.19 Morphology of delivery orifice (×40) on capsule shell surface (crosslinked for 12 h); (a) before CA coating and (b) after CA coating (when peeled off the coating film).

Morphology of delivery orifice, surface and cross-section of EOP and PPOP are shown in Figure 4.20. The delivery orifices of both EOP and PPOP were round with rough surface due to CA layer had low flexibility than capsule shell, thus it was rougher than the delivery orifice shown in Figure 4.19. Surface of both EOP and PPOP capsule was rough due to drying process by dryer. Capsules were coated with CA for two layers for EOP and eight layers for PPOP capsule. PPOP had higher pressure inside the system, thus it was coated with thicker layers to prevent the rupture of the device. From the preliminary study, we found that coating PPOP with CA for five layers was insufficient to prevent device rupture. Device rupture (dismantled between body and cap) usually found when coating layer was not thick enough as shown in Figure 4.21. However, the preliminary study found that ten layers coating could prevent the rupture of device during the course of drug release testing but retard drug release.

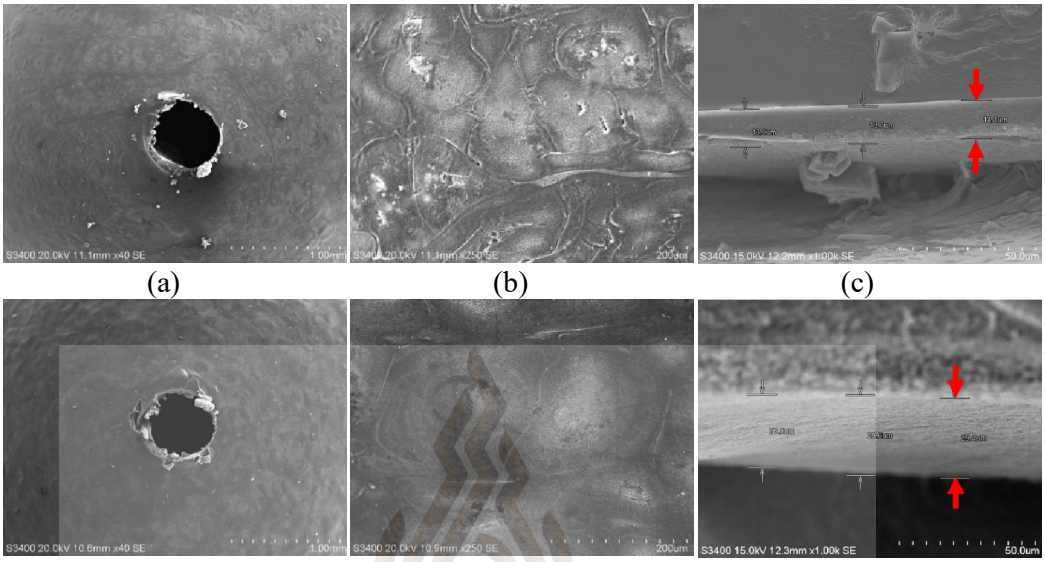


Figure 4.20 Morphology of EOP (top) and PPOP (bottom) showing (a) delivery orifice (×40), (b) on coating surface (×250), and (c) cross-section view of coating layer (×1000). The coating layer was pointed by the red arrows.

Figure 4.21 Separation of capsule body and cap of PPOP capsules during release study.

After 12 h drug release study, EOP was fulfilled with water as well as PPOP was fulfilled with wetting expanded components of push layer. No rupture was found for both EOP and PPOP capsule (Figure 4.22). When withdrawn EOP and PPOP capsule from release medium, the capsules were transparency. But, after keeping them at ambient environment for several minutes, it became dry and opaque.

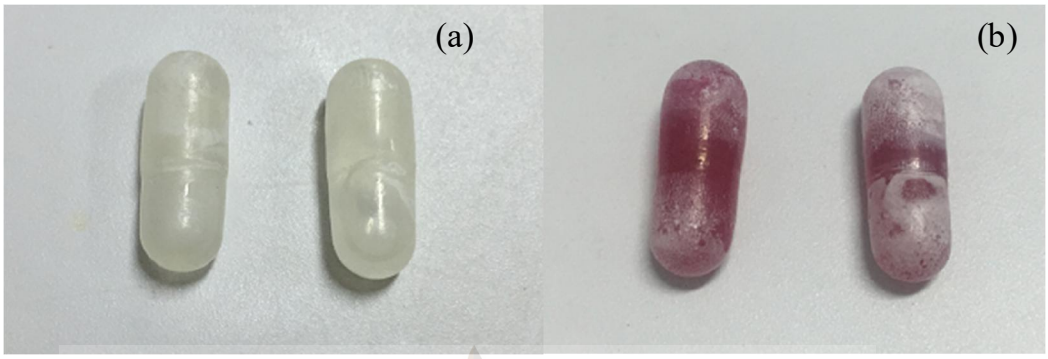


Figure 4.22 Appearance of (a) EOP and (b) PPOP capsules after release study for 12 h without any defect.

4.4 Drug release from EOP and PPOP capsules prepared using non-crosslinked and crosslinked HGCs

DIL HCl and PAR were selected based on drug solubility to investigate the reproducibility of HGCs osmotic pump preparation in relation to their drug release property. Reproducibility of PPOP capsule preparation were evaluated using both two drugs; while for EOP capsules, only DIL HCl was used. Individual three lots of each drug was prepared and drug release was evaluated. Fluctuation of DIL HCl release from EOP capsule prepared using non-crosslinked HGCs was stated in our work, while crosslinked HGCs provided reproducible of drug release profiles (Figure 4.23). However, our study had opposite result to the previous work of Wichianprasit and Kulvanich (2009) that the release rate of DIL HCl was apparently similar for both crosslinked and non-crosslinked HGCs. Drug release from osmotic pump capsules prepared using non-crosslinked HGCs was faster and varied, while crosslinked HGCs gave slower and reproducible drug release as shown in Figure 4.23, Figure 4.24, and Figure 4.25.

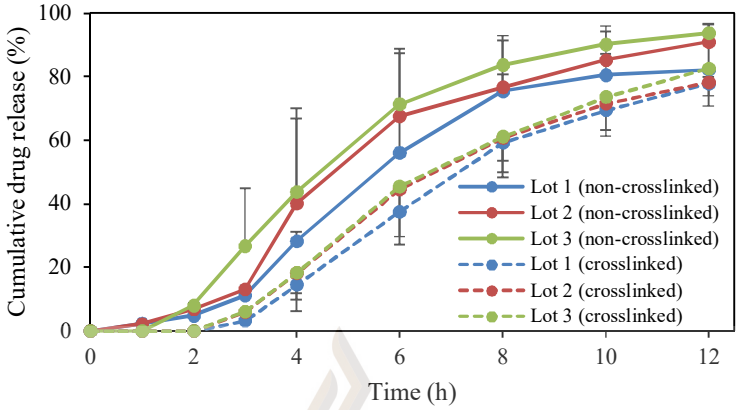


Figure 4.23 Cumulative drug release in water of DIL HCl (100 mg) from EOP capsules prepared using non-crosslinked HGCs compared to crosslinked HGCs from different lots.

Drug release of DIL HCl and PAR from PPOP capsule are shown in Figure 4.24 and Figure 4.25, respectively. Fluctuation of DIL HCl release from PPOP capsules prepared using non-crosslinked HGCs was found, the same as of EOP capsules. However, PAR release from PPOP capsules prepared using non-crosslinked and crosslinked HGCs was similar and reproducible within the group, but using of non-crosslinked HGCs still provided higher drug release compared to crosslinked group. The result confirmed that crosslinking of HGCs was an important process of HGC-based osmotic pump preparation that could reduce the fluctuation of drug release. Variation of drug release from EOP capsule prepared using non-crosslinked HGCs might be affected by the dissolved gelatin-formed viscous mass inside the osmotic device. The viscous mass of dissolved gelatin could be visually observed at the delivery orifice during drug release study.

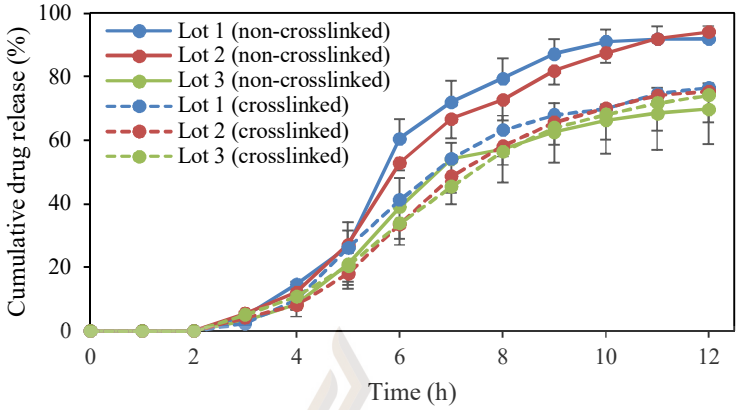


Figure 4.24 Cumulative drug release in water of DIL HCl (10 mg) from PPOP capsules prepared using non-crosslinked HGCs compared to crosslinked HGCs from different lots.

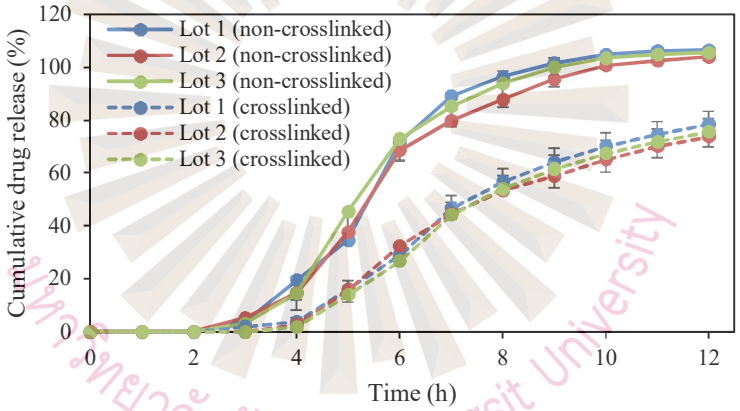


Figure 4.25 Cumulative drug release in water of PAR (10 mg) from PPOP capsules prepared using non-crosslinked HGCs compared to crosslinked HGCs from different lots.

Leakage of drug powder filled in osmotic pump capsule could prevent by closing the delivery orifice with water soluble polymer; HPMC was used in this preliminary study. The result shows that closing of the delivery orifice by HPMC did not alter drug release from the device as shown in Figure 4.26.

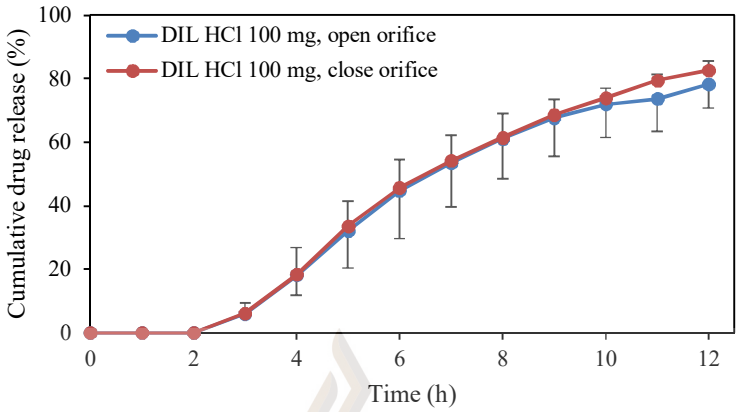


Figure 4.26 Cumulative drug release in water of DIL HCl from EOP capsules compared between with and without HPMC band closing at delivery orifice.

4.5 Effect of drug substance and loading dose on drug release from EOP capsules

This section reports the effect of drug type and loading dose on drug release from EOP capsules. Four model drugs and four loading doses were investigated. Drug doses were varied from 10-100 mg or 2.50-25% of powder mixture filled into EOP capsule. They exhibited different drug release patterns depending on drug type and loading dose. Figure 4.27 shows effect of drug types on drug release When 10 mg and 30 mg of model drug were delivered, PAR was released with the highest release rate followed by DIL HCl, PRO HCl, and AMB HCl, respectively. PAR had the lowest Mw compared to the other drugs, so it diffused faster from EOP capsule. Furthermore, it might generate an osmotic pressure that could motivate drug release. Remarkably, at loading dose of 30 mg, PAR released from EOP capsule exhibited the high drug release with zero-order release kinetic. The result indicated that when an appropriate loading dose of drug is selected, the developed EOP capsule may give drug release with a zero-order release kinetic. However, when 50 mg and 100 mg of model drug were loaded, drug release was correlated to water solubility of the drug. The highest water solubility model drug; DIL HCl exhibited the highest release rate and followed by PRO HCl and PAR, respectively.

Figure 4.28 shows effect of drug loading doses on drug release. DIL HCl release was similar for different drug loading doses. PRO HCl and PAR release were decreased when loading dose increased. This effect was also found in the delivery of nifedipine from monolithic osmotic tablets. Release rate of nifedipine was decreased when drug loading increased (Liu, Khang, Rhee, & Lee, 2000). The results revealed the drug release was dependent of drug type but not correlated to water solubility property. However, EOP capsule could not deliver AMB HCl at all loading doses due to its rather high density (Figure 4.15) with low water solubility. Consequently, sedimentation of drug powder inside the capsule was observed during drug release study. Release kinetic models of drug release are shown in Table 4.4.

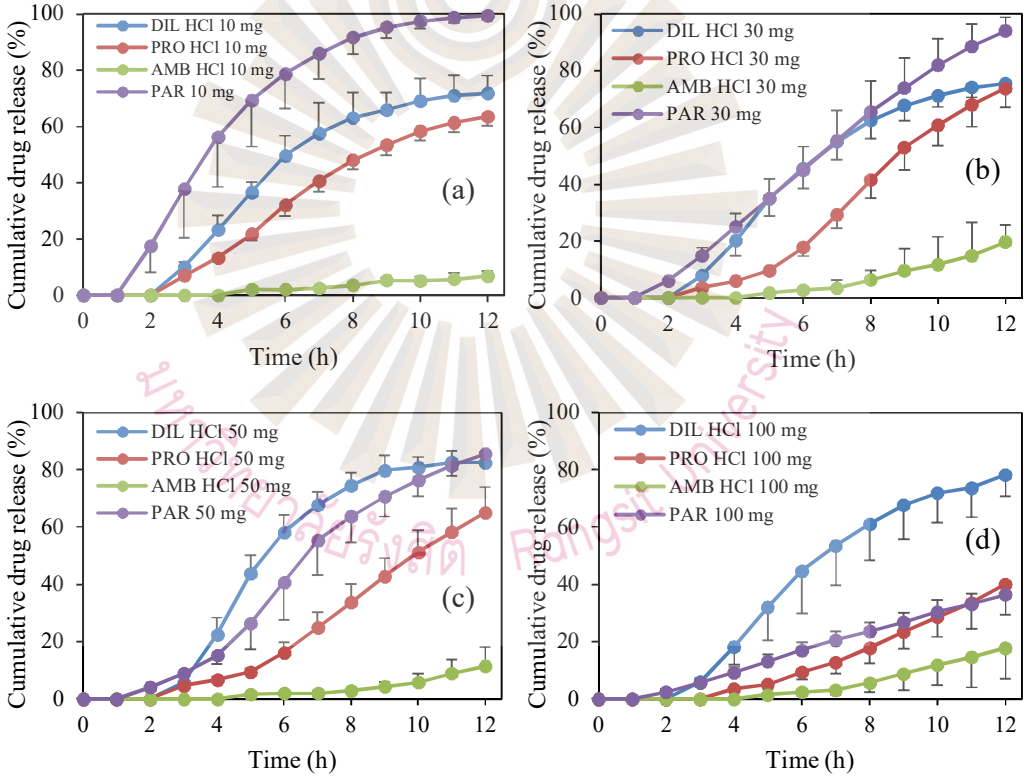


Figure 4.27 Cumulative drug release in water of model drugs with different water solubilities from EOP capsules with loading doses of (a) 10 mg, (b) 30 mg, (c) 50 mg, and (d) 100 mg.

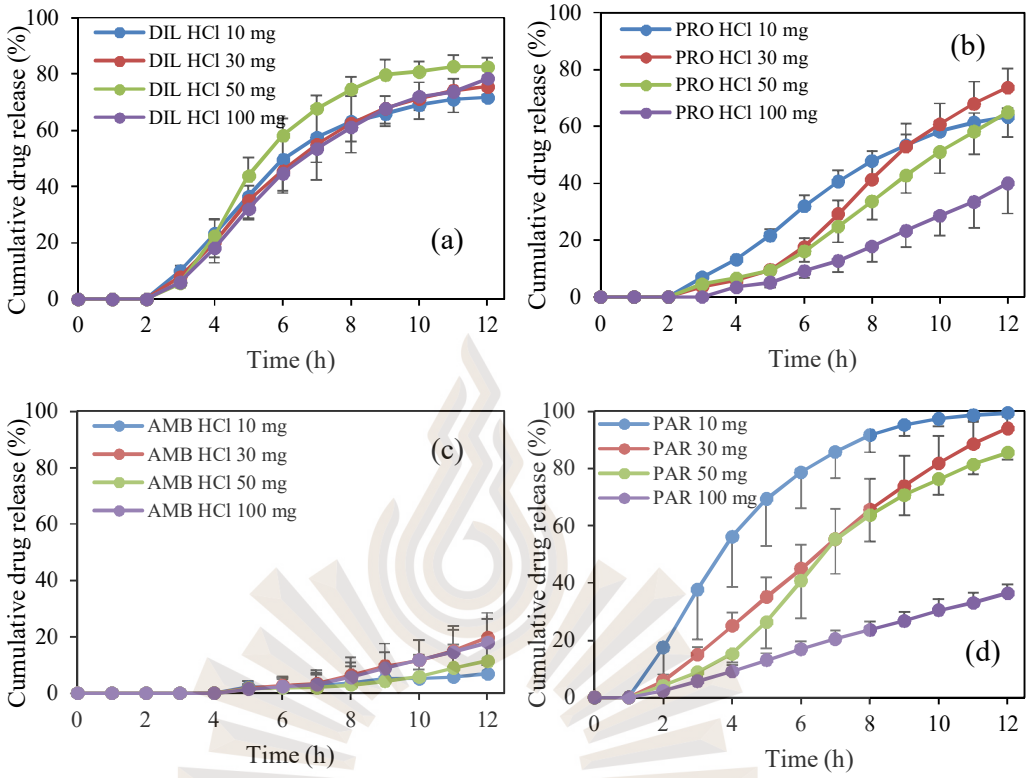


Figure 4.28 Cumulative drug release in water of (a) DIL HCl, (b) PRO HCl, (c) AMB HCl, and (d) PAR from EOP capsules with different loading doses.

Table 4.4 Release kinetic models of model drugs with different loading doses from EOP capsule.

Model drugs	Loading dose	Release rate (%/h$^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi
DIL HCl	10 mg	26.00±2.81	2.94±0.29	0.9350	0.9422	0.9807
	30 mg	27.30±0.91	3.31±0.43	0.9522	0.9353	0.9910
	50 mg	31.27±0.65	3.20±0.32	0.9147	0.9039	0.9770
	100 mg	27.67±3.48	3.49±0.45	0.9547	0.9219	0.9890
PRO HCl	10 mg	22.61±1.44	3.73±0.07	0.9714	0.9433	0.9848
	30 mg	29.02±3.30	5.70±0.09	0.9218	0.8357	0.9790
	50 mg	23.86±4.77	5.41±0.50	0.9255	0.8561	0.9617
	100 mg	14.24±5.10	5.51±1.11	0.9096	0.8719	0.9531

* calculated based on Higuchi's release model.

Table 4.4 Release kinetic models of model drugs with different loading doses from EOP capsule (Cont.).

Model drugs	Loading dose	Release rate (%/$h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi
PAR	10 mg	34.22±1.32	1.60±0.52	0.8783	0.9402	0.9656
	30 mg	30.96±2.19	3.34±0.49	0.9871	0.9159	0.9774
	50 mg	30.06±2.32	3.77±0.21	0.9584	0.8951	0.9754
	100 mg	11.58±1.21	3.37±0.54	0.9908	0.9815	0.9768

* calculated based on Higuchi's release model.

The relationship between loading dose of DIL HCl, PRO HCl, and PAR and Higuchi's release rate is shown in Figure 4.29. They were found that loading dose of DIL HCl did not affect release rate. When water imbibed into the inside of EOP, drug substance rapidly dissolved because of the highly water soluble property of DIL HCl. So, release rate was not altered by the variation of drug dose. In case of PRO HCl and PAR, they exhibited lower water solubility compared to DIL HCl, so their drug release rates were affected by loading dose especially at high dose (100 mg). However, when low to medium dose (10-50 mg) was used, release rates of DIL HCl, PRO HCl, and PAR were comparable. Figure 4.30 shows the relationship between loading dose of DIL HCl, PRO HCl, and PAR and lag time of drug release from EOP capsules. Lag time of DIL HCl release seems to be stable and independent of loading dose, while lag time of PRO HCl and PAR release was varied in the range of 2 h.

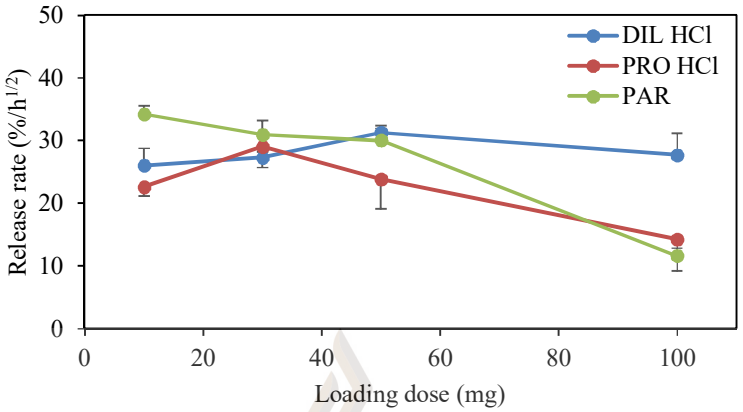


Figure 4.29 The relationship between loading dose of DIL HCl, PRO HCl, and PAR and Higuchi's release rate of EOP capsules.

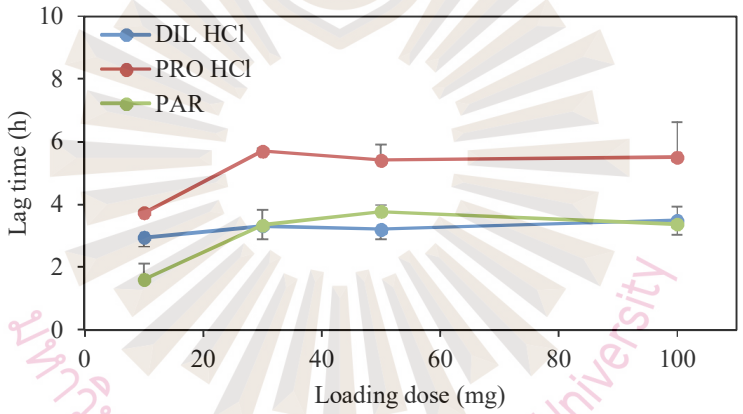


Figure 4.30 The relationship between loading dose of DIL HCl, PRO HCl, and PAR and lag time of drug release from EOP capsules

Drug release from osmotic devices usually approached zero-order release kinetic. However, most of drug release profiles in our work fitted to Higuchi's model. The Higuchi's kinetic model could be found in the other report. Drug release of ropinirole HCl from osmotic pump tablets fitted with Higuchi's model, due to constant release at early time point and then drug diffusion in later release time point during osmotic pressure decreased (Li et al., 2016). However, some formulations approached zero-order release kinetic, especially PRO HCl EOP capsules. According to release pattern, after lag time period, drug release rate was high due to large difference of

osmotic pressure inside and outside the osmotic device. After that, drug release rate decreased due to lowering of osmotic pressure difference (Özdemir & Sahin, 1997; Sinchaipanid, Pongwai, Limsuwan, & Mitrevej, 2003), which clearly found in DIL HCl release.

4.6 Effect of drug substance and loading dose on drug release from PPOP capsules

The PPOP system could delivery both water soluble and water insoluble drugs. EOP was suitable for water soluble drugs due to its simple preparation process, while low water soluble drug had difficulty to deliver by EOP. So PPOP was a suitable device instead of EOP in the delivery of low water soluble drugs. In this work, PPOP was used for delivery of both high and low water soluble drugs to evaluate the release characteristics of many drugs via developed PPOP capsule. Drug loading was an important factor affecting the drug release from the PPOP system especially poorly water soluble drug. Malaterre, Ogorka, Loggia, and Gurny (2009b) evaluated tablet core factors influencing isradipine release kinetics and loadability of PPOP system of which loading dose was a factor studied in that work. They found that over 90% isradipine was released within 14 h when the drug was loaded up to 20%, but this was not achieved for 30% drug loading.

Our study showed that drug doses varied from 10-50 mg or 3.70-18.52% of powder mixture filled into PPOP capsule had a similar drug release pattern. It was observed that drug release was independent of drug substances (Figure 4.31) and loading doses (Figure 4.32) for all three model drugs except AMB HCl. The result indicated that PPOP capsule allows drug release independent of drug characteristics (Malaterre et al., 2009b). A few previous publications showed that drug release from PPOP system was independent of drug loading, which is similar to our findings. Waterman et al. (2011) developed universal PPOP CA capsule for delivery of several drugs. They found that drug delivery from universal PPOP capsule did not depend on the dose. Furthermore, Missaghi et al. (2014) investigated the critical core formulation

of PPOP tablet by varying dose and water solubility properties of four model drugs including glipizide, theophylline, PAR, and verapamil HCl, a practically insoluble, slightly water soluble, sparingly water soluble, and water soluble drug, respectively. Delivery of low dose of glipizide, theophylline, and PAR provided similar drug release, but drug releases were different when a medium dose was used. According to the variation of theophylline dose, drug release was comparable when low and medium doses of theophylline were used, while drug release for high dose of theophylline was dramatically low. This result of theophylline was similar to verapamil HCl.

AMB HCl and PAR had low water solubility. The apparent density of AMB HCl was higher than other model drugs so the PEO Mw 200K could not suspend the drug particles during the operation. Finally, drug suspension could not be pumped out through the delivery orifice of PPOP capsule. In addition, pulverized AMB HCl was also used in PPOP capsule formulation (Figure 4.32(c)). The particle size of AMB HCl was decreased from 39.43±0.25 to 27.16±0.16 μm after pulverization, however, the apparent density of pulverized AMB HCl was the same as of the intact AMB HCl. Both intact AMB HCl and pulverized AMB HCl could not be delivered using this developed PPOP capsule due to its high density, thus AMB HCl PPOP capsule was not further investigated in the other topics. It was indicated that the apparent density had a major effect rather than particle size on drug delivery through orifice. As presented in Table 4.2, Table 4.3, and Figure 4.15, PAR had a lower water solubility, lower apparent density, and larger particle size, but it exhibited a good release profile compared to AMB HCl. Release kinetic models are shown in Table 4.5. Release rate of high water soluble drugs; DIL HCl and PRO HCl was independent of loading dose. In case of PAR, a low water soluble drug, release rate was apparently decreased while loading dose increased (Figure 4.33). Lag time of drug release seems to be stable and independent of loading dose (Figure 4.34).

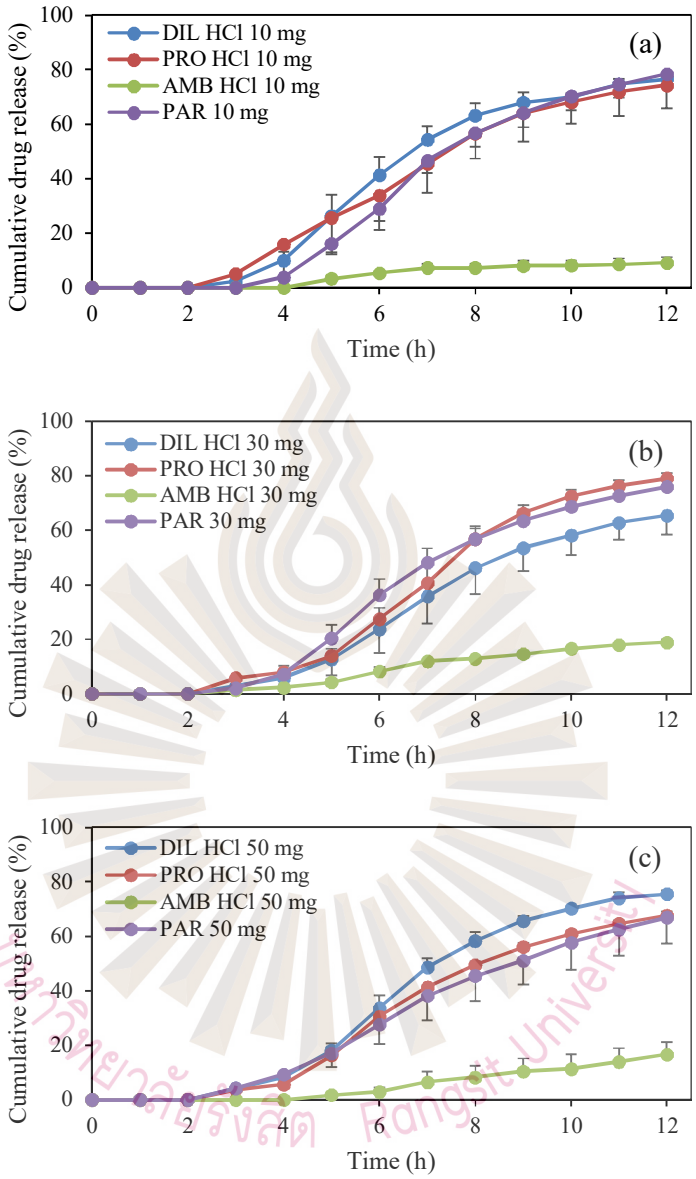


Figure 4.31 Cumulative drug release in water of several drugs with different water solubilities from PPOP capsules with loading dose of (a) 10 mg, (b) 30 mg, and (c) 50 mg.

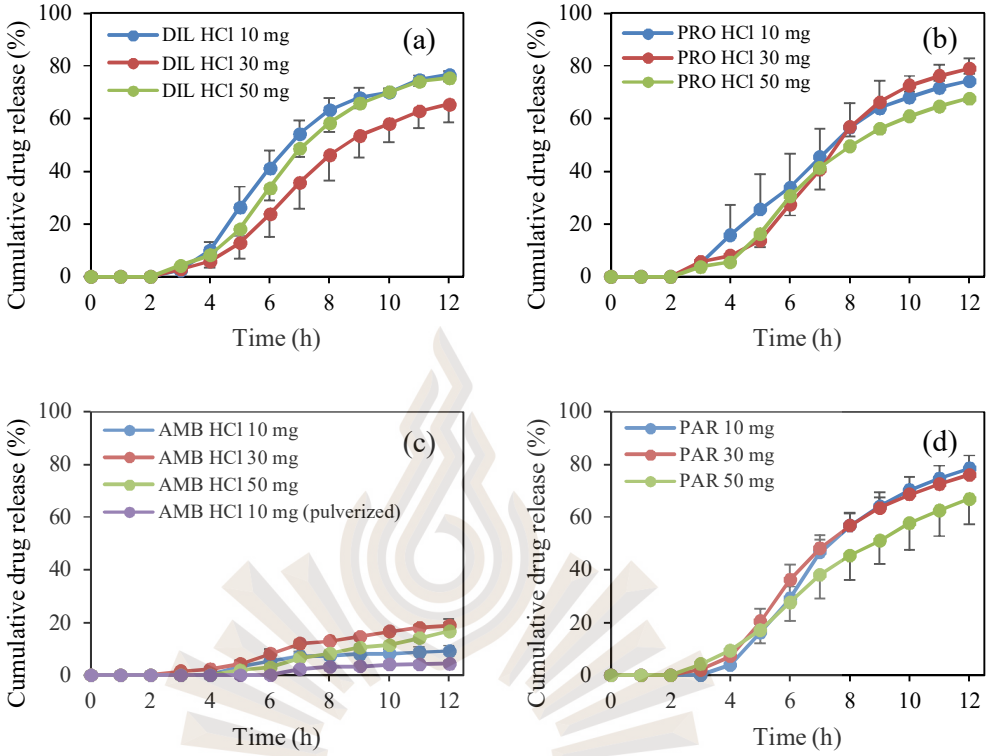


Figure 4.32 Cumulative drug release in water of (a) DIL HCl, (b) PRO HCl, (c) AMB HCl, and (d) PAR from PPOP capsules with different loading doses.

Table 4.5 Release kinetic models of model drugs with different loading doses from PPOP capsules.

Model drugs	Loading dose	Release rate (%/h$^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi
DIL HCl	10 mg	29.20±0.22	4.06±0.46	0.9355	0.8920	0.9891
	30 mg	25.55±2.85	4.95±0.58	0.9376	0.8807	0.9877
	50 mg	29.62±0.73	4.56±0.20	0.9426	0.8813	0.9850
PRO HCl	10 mg	27.33±1.75	4.01±0.95	0.9528	0.9057	0.9811
	30 mg	30.59±1.00	4.83±0.10	0.9298	0.8457	0.9764
	50 mg	25.80±0.48	4.56±0.05	0.9455	0.8951	0.9900
PAR	10 mg	30.06±2.02	4.73±0.11	0.9346	0.8576	0.9931
	30 mg	28.09±0.91	4.20±0.48	0.9447	0.8867	0.9944
	50 mg	23.31±3.55	4.16±0.46	0.9643	0.9142	0.9836

* calculated based on Higuchi's release model.

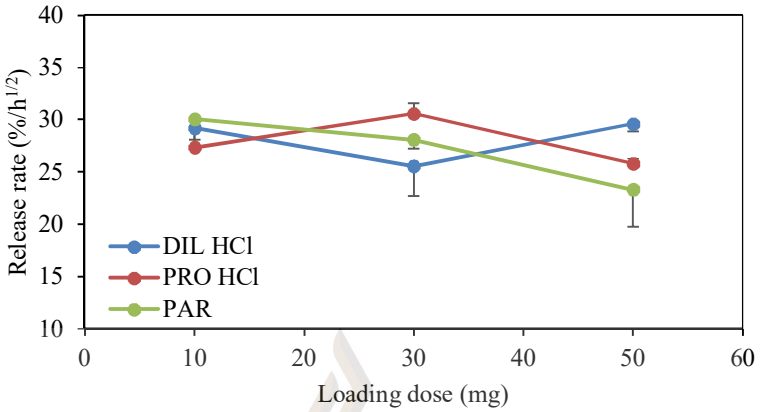


Figure 4.33 The relationship between loading dose of DIL HCl, PRO HCl, and PAR and Higuchi's release rate of PPOP capsules.

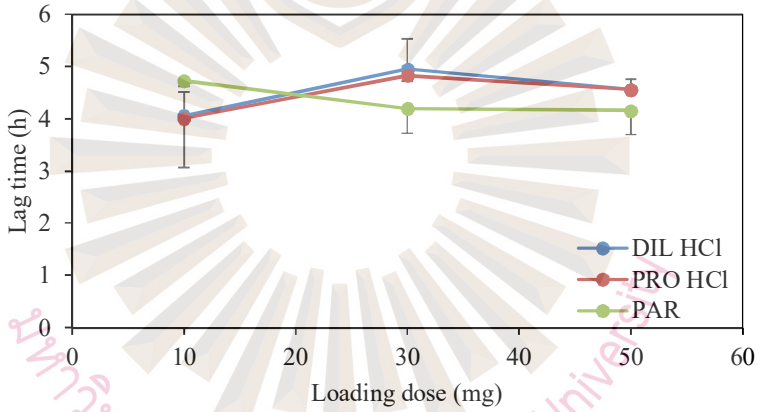


Figure 4.34 The relationship between loading dose of DIL HCl, PRO HCl, and PAR and lag time of drug release from PPOP capsules.

4.7 Effect of PEO Mw 200K amount on drug release from PPOP capsules

PEO was a kind of water-soluble polymer that could be used to generate a high osmotic pressure. Uniformity of swelling rate of PEO ensured that the release rate of the drug from the delivery device was relatively constant. Owing to its inherent properties, PEO is one of the most popular materials for the osmotic pump (Ma et al.,

2014; Verma et al., 2002). The increment of PEO amount in the pull layer of PPOP increased the viscosity of drug suspension or drug solution within the system, which increased its stability by inhibiting the aggregation of precipitation of low water soluble drugs (Nie et al., 2007). Nie et al. (2007) prepared the osmotic pump tablets of allopurinol. The PEO Mw 100K in pull layer was varied from 200 to 300 mg to which the total weight of pull layer was 452 mg. The author mentioned that usage of PEO for 200 and 250 mg had no influence on drug release, while 300 mg had a lower drug release. However, similarity factor when compared every two groups was higher than 50, indicating that all three groups had a similar drug release pattern. There were other studies that gave the same result. The increase of amount of PEO Mw 100K in pull layer could reduce drug release rate (Wu et al., 2014; Zhang et al., 2009).

This present work is contrary to the previous works. The amount of PEO Mw 200K in pull layer of PPOP capsule was varied between 90 and 170 mg. Drug release from PPOP capsule containing different amount of PEO Mw 200K was similar for all model drugs. Usage of 90 mg PEO Mw 200K reduced the drug release dramatically (Figure 4.35). The result was similar to the study of Li et al. (2015), when they varied the amount of PEO Mw 200K in the pull layer of a tri-layer ascending osmotic pump tablet. They described that a small amount of PEO could not entirely suspend the drug particles. Furthermore, we proposed that the optimal osmotic pressure difference between pull and push layer was an important factor affecting drug release. The larger difference of osmotic pressure due to low content of PEO Mw 200K in pull layer could provide lower drug release from the osmotic device.

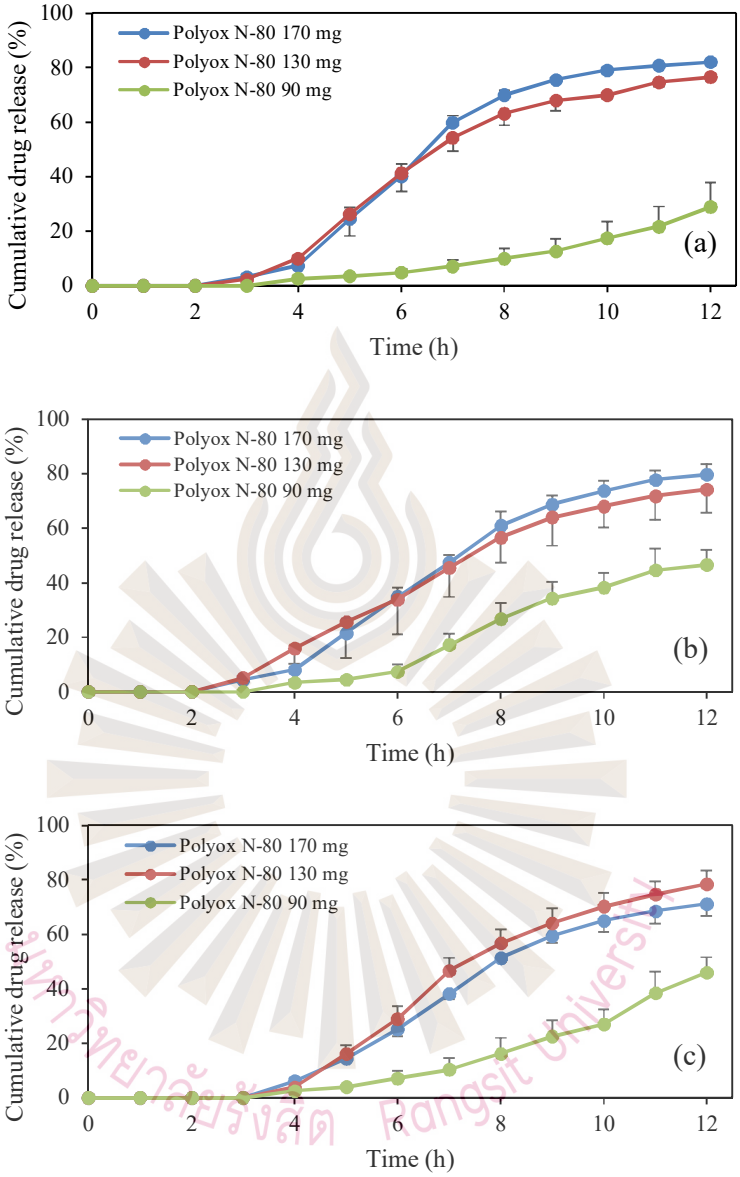


Figure 4.35 Cumulative drug release in water of (a) DIL HCl, (b) PRO HCl, and (c) PAR (10 mg) from PPOP capsules using a different amount of PEO Mw 200K.

According to our result, the highly water soluble drug was unnecessary to suspend within the device because it rapidly dissolved when in contact with water. However, amount of PEO Mw 200K had a similar effect for low water soluble drugs. The result indicated that the amount of PEO Mw 200K contained in pull layer provide the same influencing effect on drug release of each drug type in our developed PPOP

capsule. Release kinetic models are shown in Table 4.6. Amount of PEO Mw 200K played a positive effect on release rate of high water soluble drugs; DIL HCl and PRO HCl. Increasing amount of PEO Mw 200K increased drug release rate. Amount of PEO Mw 200K seems to increase release rate of PAR as well. Increasing of PEO Mw 200K from 90 mg to 130 mg, release rate increased. However, when PEO Mw 200K was increased from 130 mg to 170 mg, the release rate was slightly decreased (Figure 4.36). Figure 4.37 shows the relationship between amount of PEO Mw 200K and lag time of drug release from PPOP capsules. Using PEO Mw 200K 90 mg gave longer lag time compared to 130 mg and 170 mg. The use of low amount of PEO (90 mg) exhibited the lowest release rate, the drug spent more time to release from PPOP capsule, so longer lag time was found in this case.

Table 4.6 Release kinetic models of model drugs with different amount of PEO Mw 200K from PPOP capsules.

Model drugs	Amount of PEO Mw 200K	Release rate ($\%/h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi
DIL HCl	170 mg	32.11±1.13	4.15±0.38	0.9247	0.8615	0.9834
	130 mg	29.20±0.22	4.06±0.46	0.9355	0.8920	0.9891
	90 mg	10.14±3.01	6.47±0.52	0.8318	0.8050	0.8788
PRO HCl	170 mg	30.16±2.66	4.33±0.40	0.9473	0.8763	0.9872
	130 mg	27.33±1.75	4.06±0.95	0.9528	0.9057	0.9811
	90 mg	18.93±2.84	5.87±0.08	0.8865	0.8376	0.9790
PAR	170 mg	27.46±1.50	4.79±0.02	0.9401	0.8728	0.9876
	130 mg	30.06±2.02	4.73±0.11	0.9346	0.8576	0.9931
	90 mg	18.07±4.14	6.83±0.99	0.8276	0.7795	0.9305

* calculated based on Higuchi's release model.

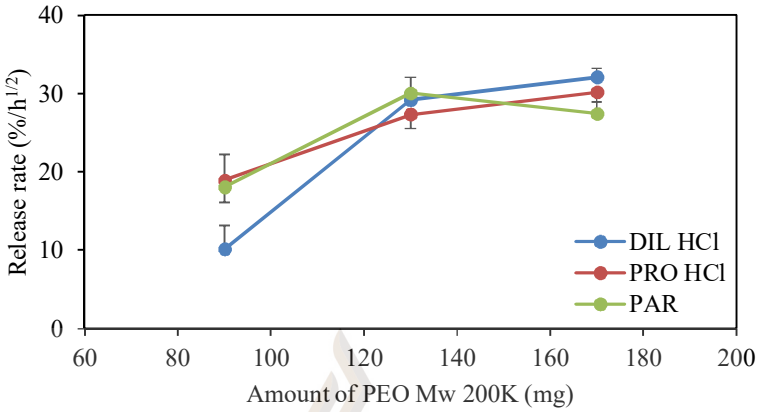


Figure 4.36 The relationship between amount of PEO Mw 200K and Higuchi's release rate of PPOP capsules.

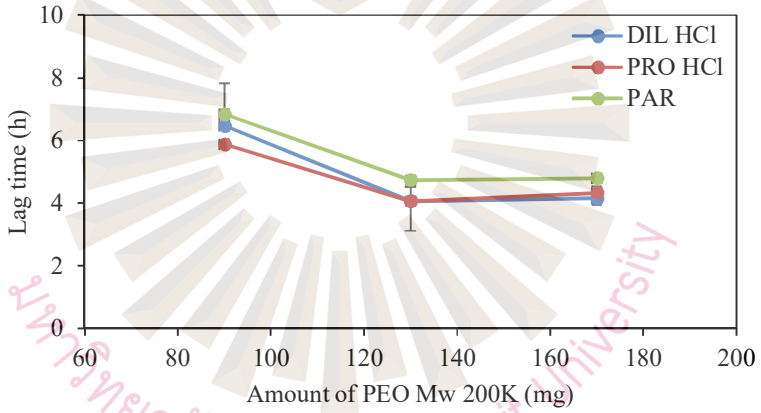


Figure 4.37 The relationship between amount of PEO Mw 200K and lag time of drug release from PPOP capsules.

4.8 Effect of sodium chloride in push layer on drug release from PPOP capsules

Drug layer of PPOP could deliver through the delivery orifice by the shear of the hydrostatic pressure from the push layer (Waterman et al., 2011). This topic presents the effect of sodium chloride in push layer on drug release. Although expansion of PEO

Mw 5,000K in push layer could pump a drug outside the device, but the drug release is not achieved when lacks of sodium chloride. As shown in Figure 4.38, less drug release was seen in PPOP capsule without sodium chloride in push layer. The result indicated that osmotic inducing agent combined with swelling polymer containing in push layer to increase the hydrostatic pressure inside the osmotic device by accelerated wetting and expansion of swelling polymer, thus it could enhance drug release as reported by Wu et al. (2014).

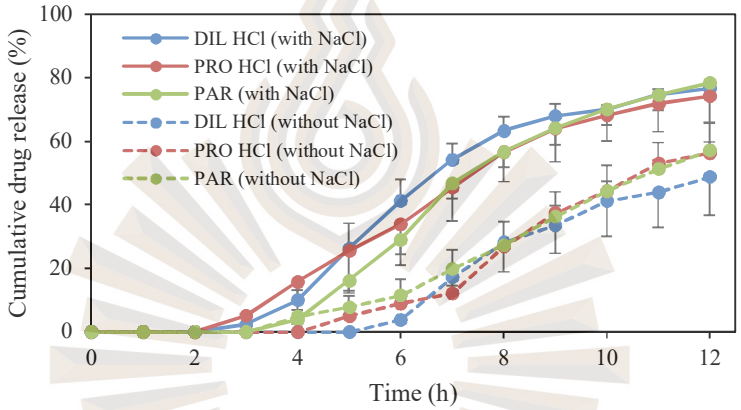


Figure 4.38 Cumulative drug release in water of each model drug (10 mg) from PPOP capsule with or without sodium chloride in push layer.

4.9 Effect of capsule size on drug release from PPOP capsules

Capsule size was one factor investigated in this study. Both capsule no. 1 and 2 were drilled to have the same delivery orifice of 0.6 mm, which indicated that surface area of delivery orifice per surface area of the device of capsule no.2 was relatively higher than capsule no.1. The powder mixture was loaded in crosslinked HGC no.1 and 2 as proportion, thus drug content in capsule no. 2 (8.33 mg/capsule) was slightly lower than capsule no.1 (10 mg/capsule). Figure 4.39 displays the similarity in drug release from both capsule no.1 and 2 for all model drugs. Thus, it is unlikely that the size of capsule had an effect on drug release.

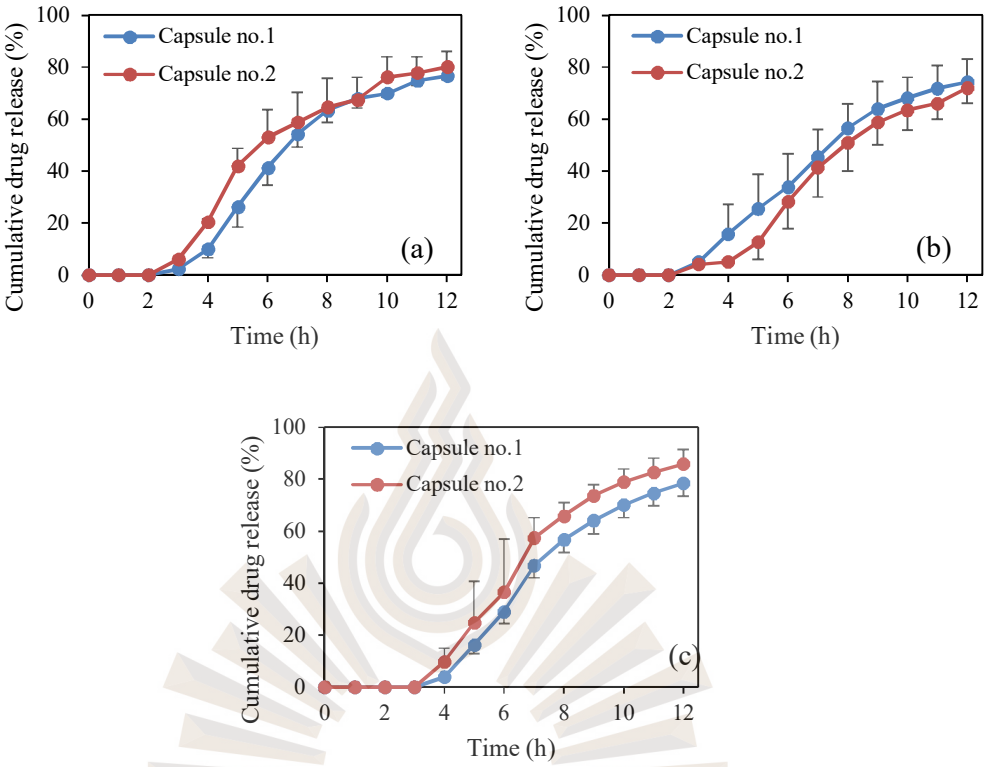


Figure 4.39 Effect of capsule size on cumulative drug release in water from PPOP capsule of (a) DIL HCl, (b) PRO HCl, and (c) PAR.

4.10 Effect of CA coating on drug release from EOP and PPOP capsules

CA was a polymer used for controlling water penetration into osmotic devices. DIL HCl release from EOP capsules with CA coating was similar to those of without CA (Figure 4.40a) as well as PPOP capsules (Figure 4.40d). These results indicated that DIL HCl release from EOP and PPOP capsules was independent from CA coating. This occurrence might associate with the highly water solubility property of DIL HCl. In case of PRO HCl and PAR, CA retarded drug release from both EOP and PPOP capsules. However, CA coating was still necessary in this work because it could prevent the separation of capsule body and cap during release study.

There were numerous works showed that increasing of CA content provided release retardation of many drugs from EOP systems e.g. carbamazepine (Rabti et al., 2014), diclofenac sodium (Emara et al., 2012), felodipine/metoprolol tartrate (Zhao et al., 2016), glipizide (Mehramizi, Alijani, Pourfarzib, Dorkoosh, & Rafiee -Tehrani, 2007; Verma & Garg, 2004), indomethacin (Shokri et al., 2008), isosorbide mononitrate (Verma, Kaushal, & Garg, 2003), ketorolac tromethamine (Ali & Sayed, 2013), metformin hydrochloride/glipizide (Defang et al., 2005; Pan, Jing, Yang, Pan, & Chen, 2017), nifedipine (Liu et al., 2000; Nokhodchi, Momin, Shokri, Shahsavari, & Rashidi, 2008), PRO HCl (Mohammadi-Samani, Adrangui, Siahi-Shadbad, & Nokhodchi, 2000), risperidone (Gong, Liu, Mei D., Yang, & Mei X., 2015), ropinirole hydrochloride (Li et al., 2016), tramadol hydrochloride (Kumar, Singh, & Mishra, 2009), ziprasidone (Yanfei, Guoguang, Lili, & Pingkai, 2015), etc. and PPOP systems e.g. acetaminophen (Waterman et al., 2011), felodipine (Wu et al., 2014), gliclazide (Li et al., 2008), glipizide (Missaghi et al., 2014), paliperidone (Xu et al., 2013), valsartan (Shaikh et al., 2013), etc.

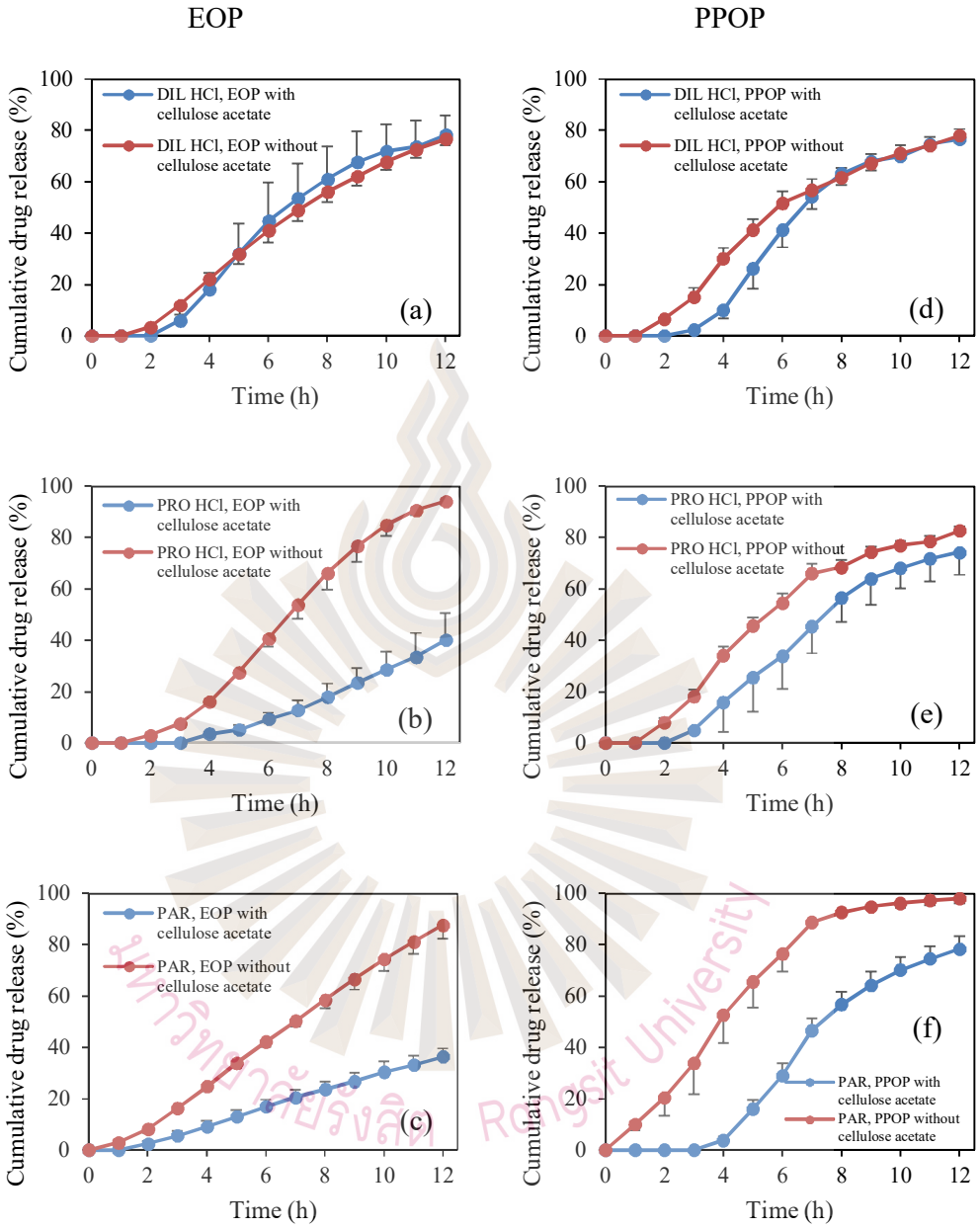


Figure 4.40 Cumulative drug release in water of DIL HCl (a, d), PRO HCl (b, e), and PAR (c, f) from EOP and PPOP capsule with and without CA coating. Drug loading of EOP and PPOP was 100 mg and 10 mg, respectively.

4.11 Effect of osmolality of release medium on drug release from EOP and PPOP capsules

Osmolality plays an important role on drug release from the osmotic pump system. According to Poiseuille's law of lamina flow, the pressure difference between the device and release medium could affect the drug release rate (Lu et al., 2003; Malaterre et al., 2009a; Xu et al., 2013; Zhang et al., 2009). An increase in NaCl concentration (or osmolality) of release medium provided a slower drug release rate. Hill, Geiβler, Weigandt, and Mäder (2012) investigated the osmolality difference between formulation and medium. The result showed that the higher difference of osmolality provided higher drug release rate.

Figure 4.41 shows drug release in mediums with different osmolalities. It was found that 0.45% NaCl, 0.9% NaCl, and 3% NaCl had osmolality value of 143.67±0.58, 286.00±2.00, and 948.00±3.61 mOsm/kg, respectively. The variation of osmolality of release medium was used in order to mimic the effect of osmolality variation in the GI tract that might be affecting drug release from the osmotic pump capsule. For PPOP capsule, the results showed that the drug release patterns were similar for all model drugs. The medium without NaCl provided the highest drug release rate while increment of NaCl concentration (or osmolality value) caused a lower drug release rate for all determination time points. A lower rate and a lower amount of drug release was found in 3% NaCl medium. It might be due to the lower pressure difference between the device and release medium following Poiseuille's law as described above. It could be concluded that drug release from PPOP capsule in a medium with different osmolality was independent of drug type but dependent on osmolality of release medium. In case of EOP capsules, osmolality affected drug release less than those of PPOP capsules. This occurrence could be described by less osmolality difference between EOP capsule and medium because EOP capsule contained higher amount of NaCl compared to PPOP capsule. However, increase of osmolality of release medium tend to decrease drug release from EOP capsule as well (Verma & Garg, 2004). Release kinetic models are shown in Table 4.7 and Table 4.8.

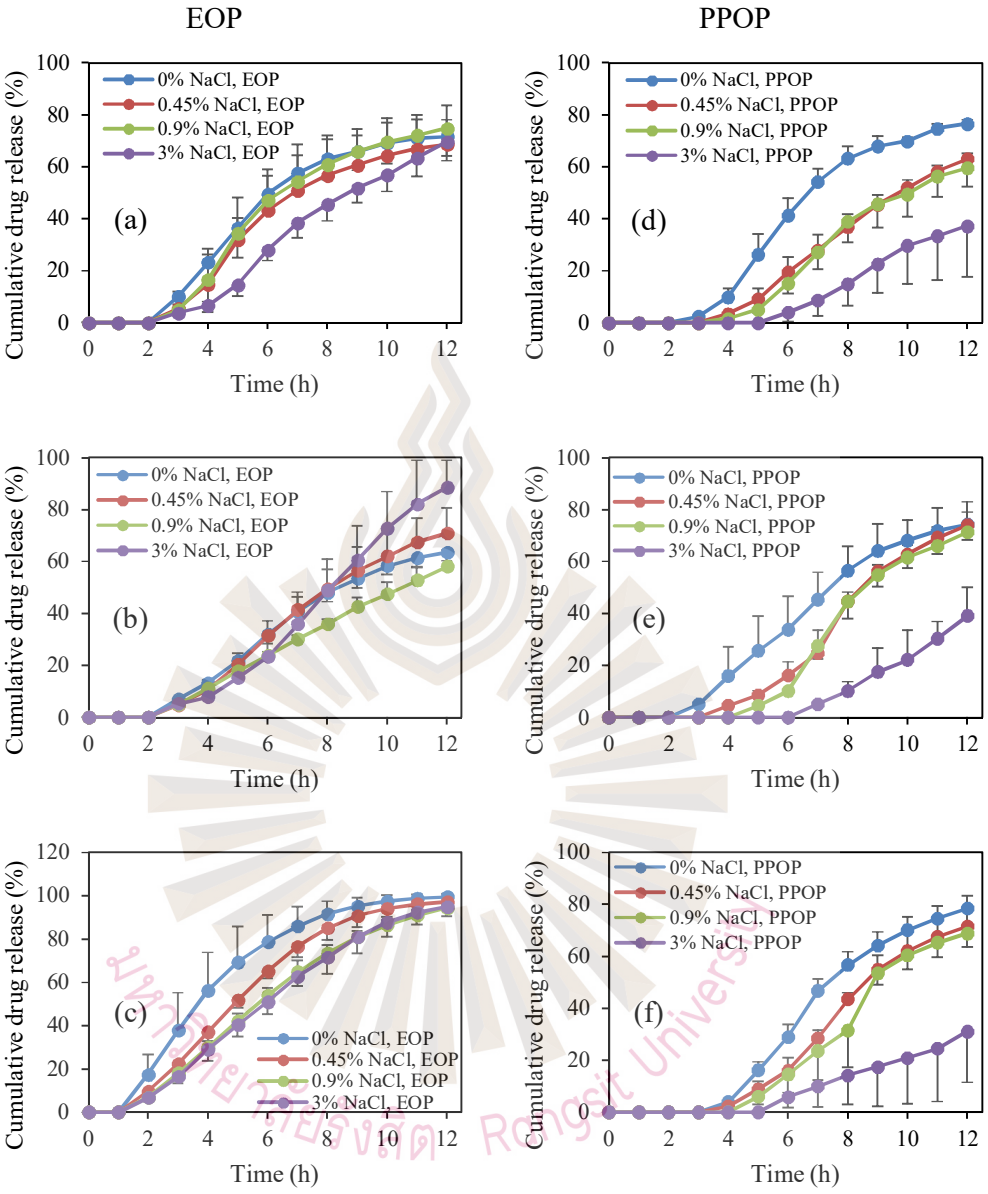


Figure 4.41 Effect of osmolality of release medium on cumulative drug release of (a, d) DIL HCl, (b, e) PRO HCl, and (c, f) PAR (10 mg) from EOP and PPOP capsule.

Table 4.7 Release kinetic models of model drugs from EOP capsule in mediums with different osmolalities.

Model drugs	Mediums	Release rate (%/$h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi
DIL HCl	0% NaCl	26.00±2.81	2.94±0.29	0.9350	0.9422	0.9807
	0.45% NaCl	24.98±2.09	3.35±0.52	0.9387	0.9288	0.9884
	0.9% NaCl	27.23±4.22	3.40±0.80	0.9339	0.9167	0.9871
	3% NaCl	24.64±3.15	4.49±0.52	0.9543	0.8973	09914
PRO HCl	0% NaCl	22.61±1.44	3.73±0.07	0.9713	0.9434	0.9848
	0.45% NaCl	24.98±2.86	4.08±0.42	0.9664	0.9143	0.9887
	0.9% NaCl	18.76±1.75	3.79±0.06	0.9814	0.9469	0.9801
	3% NaCl	33.20±3.96	5.44±0.57	0.9214	0.8102	0.9682
PAR	0% NaCl	34.18±1.25	1.59±0.52	0.8783	0.9402	0.9642
	0.45% NaCl	33.66±1.82	2.42±0.42	0.9503	0.9284	0.9855
	0.9% NaCl	31.34±1.03	2.77±0.07	0.9791	0.9333	0.9911
	3% NaCl	31.97±1.06	3.05±0.45	0.9832	0.9212	0.9862

* calculated based on Higuchi's release model.

Table 4.8 Release kinetic models of model drugs from PPOP capsule in mediums with different osmolalities.

Model drugs	Mediums	Release rate (%/$h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
				Zero-order	First-order	Higuchi model
DIL HCl	0% NaCl	29.20±0.22	4.01±0.46	0.9355	0.8920	0.9891
	0.45% NaCl	24.04±0.96	5.34±0.57	0.9296	0.8663	0.9898
	0.9% NaCl	23.99±9.02	5.77±0.86	0.8943	0.8278	0.9872
	3% NaCl	16.55±9.78	6.76±0.92	0.8406	0.7975	0.9852
PRO HCl	0% NaCl	27.33±1.75	4.06±0.95	0.9528	0.9057	0.9811
	0.45% NaCl	31.06±1.01	6.02±0.58	0.8993	0.8101	0.9790
	0.9% NaCl	29.57±1.91	5.89±0.03	0.8864	0.7992	0.9920
	3% NaCl	17.82±5.18	7.80±1.00	0.7319	0.6940	0.8874
PAR	0% NaCl	30.06±2.02	4.73±0.11	0.9346	0.8576	0.9931
	0.45% NaCl	28.48±4.59	5.49±0.59	0.9102	0.8264	0.9895
	0.9% NaCl	29.13±4.48	6.01±0.81	0.8728	0.7905	0.9767
	3% NaCl	11.25±7.79	6.15±1.50	0.8164	0.7881	0.8812

* calculated based on Higuchi's release model.

Figure 4.42 displays the relationship between osmolality of release medium and Higuchi's release rate of EOP and PPOP capsules. Osmolality of release medium had less effect on release rate of EOP capsule, while a high osmolality medium could decrease release rate of PPOP capsule. At a high osmolality medium, a large decrease of release rate was found in the delivery of PRO HCl and PAR, while DIL HCl was slightly decreased. This result indicated that osmolality of release medium had less effect on release rate of a highly water soluble drug but showed a large effect on low water soluble drug. The lag time of drug release from EOP capsule and PPOP capsule seem to increase when osmolality of release medium increased (Figure 4.43). The occurrence could be described by the osmotic pressure difference across the membrane. Medium at a high osmolality provided a low osmotic pressure difference between outside and inside of the membrane as well as between two sides of delivery orifice, difficulty of drug pumped out into the release medium was expected, so release rate was decreased and lag time was increased.

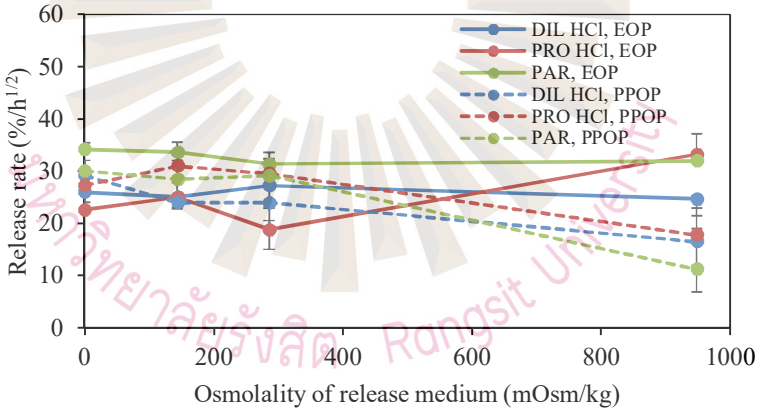


Figure 4.42 The relationship between osmolality of release medium and Higuchi's release rate of EOP and PPOP capsules.

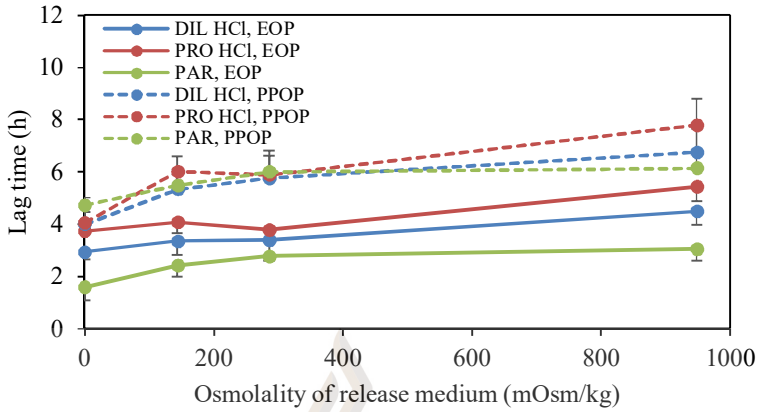


Figure 4.43 The relationship between osmolality of release medium and lag time of drug release from EOP and PPOP capsules.

4.12 Effect of pH of release medium with or without enzyme on drug release from EOP and PPOP capsules

The other factor investigated in this work was the pH of release mediums. Some previous works reported the effect of pH of release medium on drug release. Naproxen release from monolithic osmotic tablet was independent from pH of the medium between pH 1.2-6.8 (Lu et al., 2003). Nifedipine release from monolithic osmotic tablet in water, SGF, and SIF was media-independent manner (Liu et al., 2000). Verma et al. (2003) showed the result of isosorbide mononitrate EOP, drug release from buffer pH 4.5, SGF, and SIF was similar. The similar results were also found in the release of several drugs from EOP such as atenolol (Liu & Che, 2006; Liu & Wang, 2008), diethylcarbamazine citrate (Khan, Tripathi, & Mishra, 2011), flurbiprofen (Patel & Mehta, 2014), glipizide (Verma & Garg, 2004), ketorolac tromethamine (Ali & Sayed, 2013), metformin/glipizide (Ouyang et al., 2005), PRO HCl (Wang et al., 2008), risperidone (Gong et al., 2015), ropinirole (Li et al., 2016), and ziprasidone solid dispersion (Yanfei et al., 2015).

DIL HCl release from EOP capsule in PBS pH 6.8 was lower than HCl pH 1.2 (Figure 4.44a). This result was similar to DIL HCl release from EOP capsule in medium with enzyme; DIL HCl release in SIF was lower than SGF (Figure 4.45a). PRO HCl EOP had similar release, less release in PBS pH 6.8 compared to HCl pH 1.2 (Figure 4.44b). In case of PAR EOP, drug release in HCl pH 1.2 and PBS pH 6.8 were comparable as well as SGF and SIF (Figure 4.44c and Figure 4.45c).

In case of PPOP, no effect of the medium pH 1.0, 6.8, and 7.4 on allopurinol release from PPOP tablets was reported (Nie et al., 2007). Liu & Xu (2008) prepared PPOP tablet containing nifedipine and evaluated drug release in different mediums. The result showed that a significant difference was not found in drug release in different mediums. In addition, acetaminophen release from tablets-filled PPOP capsules in 0.01 N HCl and SIF were also similar (Waterman et al., 2011). Those previous works were similar to the report of Liu et al. (2014), it was found that micronized nimodipine loaded PPOP tablets in three different mediums were also similar. All of above results indicated that drug release from an osmotic pump system was independent of pH of release medium. According to our work, DIL HCl and PAR released from PPOP capsule were similar for all four mediums. DIL HCl release in SGF was fairly different from another mediums and PAR release in PBS pH 6.8 was slightly lower than HCl pH 1.2. There are previous reports about the reversibility of crosslinking of HGCs by an enzyme in the GI tract (Brown et al., 1998; Marchais et al., 2003). However, we mentioned that enzymes in the release medium did not alter drug release from a high crosslinking degree of crosslinked HGCs as demonstrated in Figure 4.44 and Figure 4.45. Our result was comparable to the previous report of Jain and Naik (1984) that prepared crosslinked HGCs to make GI tract-resistant capsules. The crosslinked HGCs exhibited *in vitro* resistance in both SGF and SIF as well as in a human volunteer study. Release kinetic models are shown in Table 4.9.

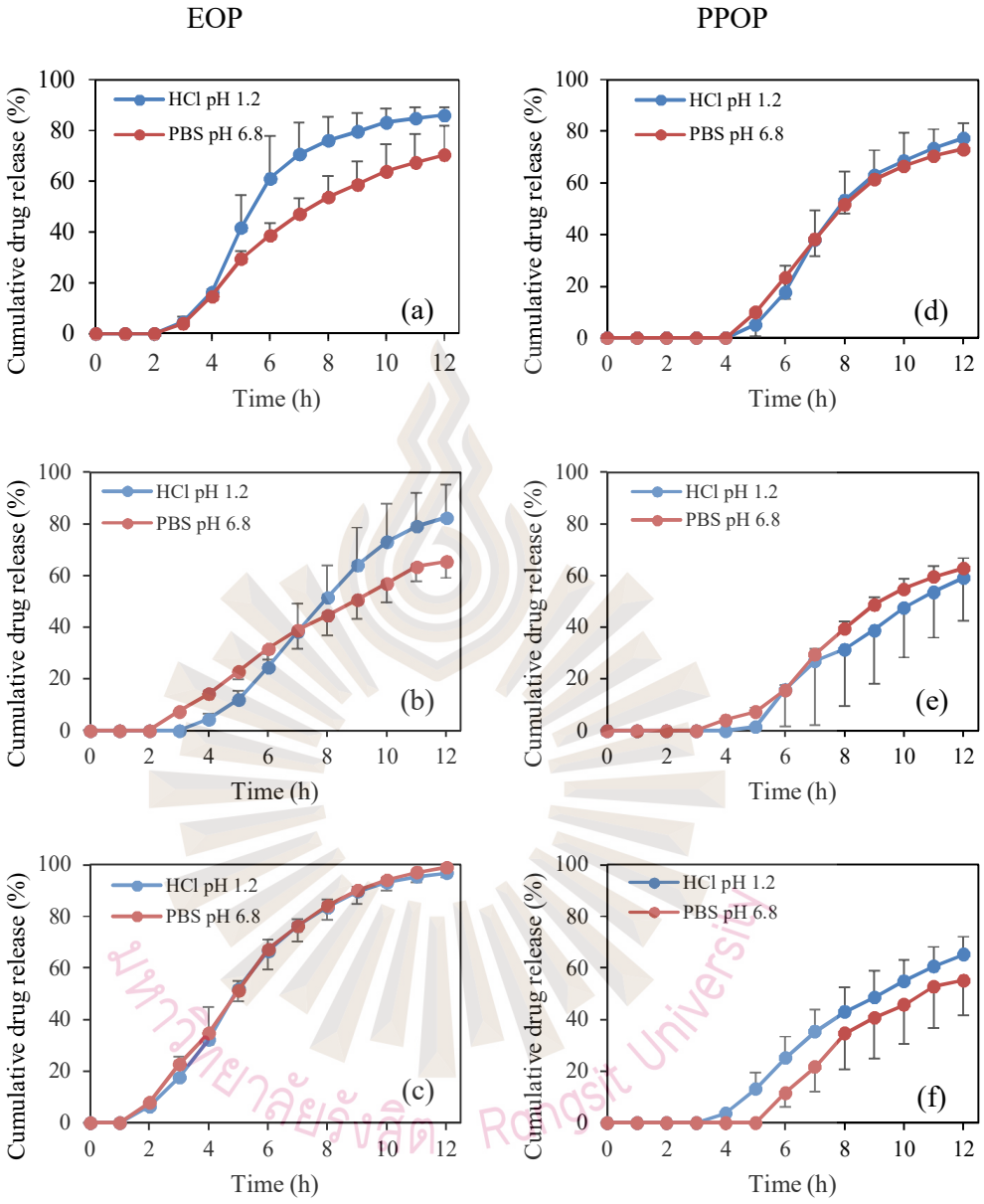


Figure 4.44 Effect of pH of isoosmolality release medium on cumulative drug release of (a, d) DIL HCl, (b, e) PRO HCl, and (c, f) PAR (10 mg) from EOP and PPOP capsule.

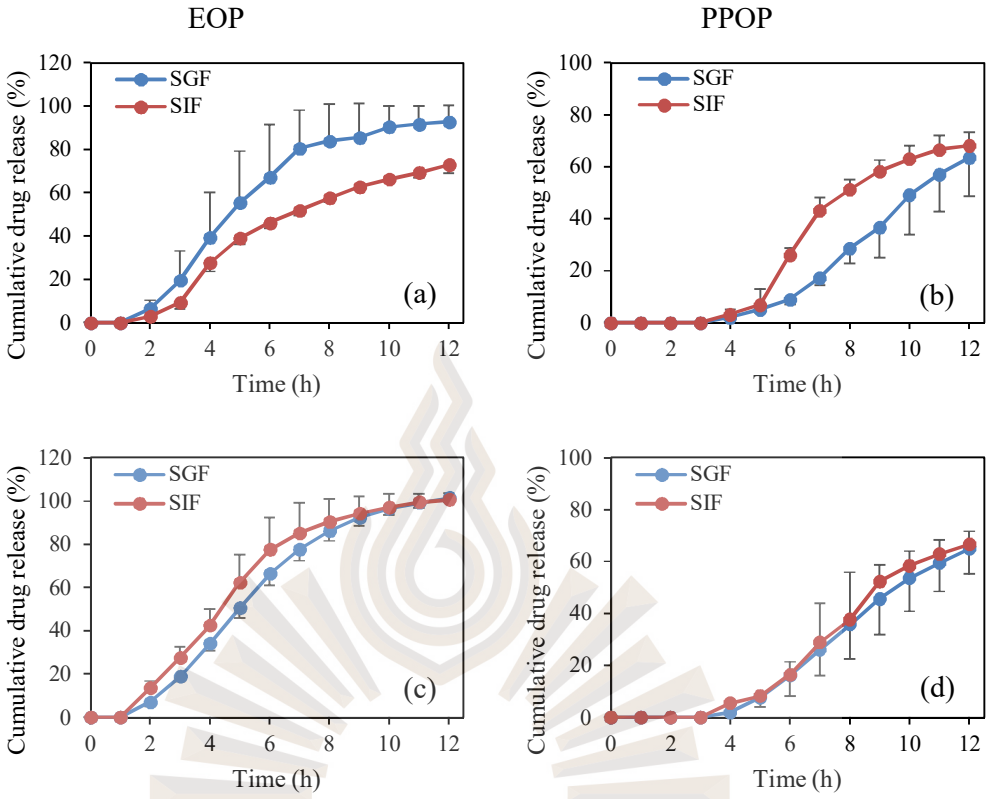


Figure 4.45 Effect of SGF and SIF on cumulative drug release of (a, b) DIL HCl and (c), (d) PAR (10 mg) from EOP and PPOP capsule.

Table 4.9 Release kinetic models of model drugs from EOP and PPOP capsule in mediums with different pH.

Model drugs/mediums	Release rate (%/h$^{1/2}$)*	Lag time (h)*	R^2 of drug release		
			Zero-order	First-order	Higuchi
DIL HCl, EOP					
HCl pH 1.2**	32.67±1.99	3.42±0.38	0.9065	0.8792	0.9673
PBS pH 6.8**	24.97±4.16	3.57±0.22	0.9584	0.9315	0.9955
SGF	34.03±2.28	2.73±0.95	0.8834	0.8987	0.9494
SIF	24.80±0.26	2.79±0.17	0.9498	0.9559	0.9927
PRO HCl, EOP					
HCl pH 1.2**	31.99±6.97	5.14±0.51	0.9288	0.8279	0.9874
PBS pH 6.8**	22.33±2.75	3.71±0.06	0.9790	0.9455	0.9849

* calculated based on Higuchi's release model. ** isoosmolality adjusted.

Table 4.9 Release kinetic models of model drugs from EOP and PPOP capsule in mediums with different pH (Cont.).

Model drugs/mediums	Release rate (%/$h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
			Zero-order	First-order	Higuchi
PAR, EOP					
HCl pH 1.2**	34.37±1.63	2.73±0.05	0.9432	0.9156	0.9849
PBS pH 6.8**	34.90±1.42	2.77±0.39	0.9481	0.9216	0.9848
SGF	35.40±1.26	2.74±0.09	0.9541	0.9094	0.9883
SIF	34.86±1.89	2.10±0.46	0.9164	0.9232	0.9728
DIL HCl, PPOP					
HCl pH 1.2**	31.90±2.72	5.53±0.57	0.8899	0.7965	0.9943
PBS pH 6.8**	29.15±2.56	5.02±1.01	0.9221	0.8483	0.9887
SGF	26.71±2.99	6.66±1.29	0.8799	0.8019	0.9785
SIF	27.35±2.42	4.87±0.17	0.9140	0.8524	0.9913
PRO HCl, PPOP					
HCl pH 1.2**	30.54±7.60	7.82±0.93	0.7384	0.6594	0.9951
PBS pH 6.8**	24.46±7.19	6.10±1.43	0.8623	0.7989	0.9873
PAR, PPOP					
HCl pH 1.2**	23.75±3.17	4.70±0.28	0.9438	0.8884	0.9928
PBS pH 6.8**	22.96±5.86	6.02±0.54	0.8815	0.8184	0.9887
SGF	25.30±3.23	5.71±0.91	0.9075	0.8347	0.9845
SIF	27.01±3.36	5.56±1.11	0.8956	0.8256	0.9664

* calculated based on Higuchi's release model. ** isoosmolality adjusted.

4.13 Effect of storage time of crosslinked HGC shells on drug release from EOP and PPOP capsules

Crosslinked HGCs were stored for 12 months, after that they were used to freshly prepare of EOP and PPOP capsules at every three months to evaluate the effect of storage time on drug release. Drug release of both DIL HCl and PAR from EOP and PPOP capsules had similar release pattern (Figure 4.46). However, variation of drug release of some formulations could be found. The highest variation was observed in drug release of DIL HCl from PPOP capsules. Delivery of DIL HCl using PPOP capsule might not appropriate for freely water soluble drug. DIL HCl dissolves well in water

by its nature, so drug release could be affected by its solubilization property besides the PPOP system. Release kinetic models are shown in Table 4.10.

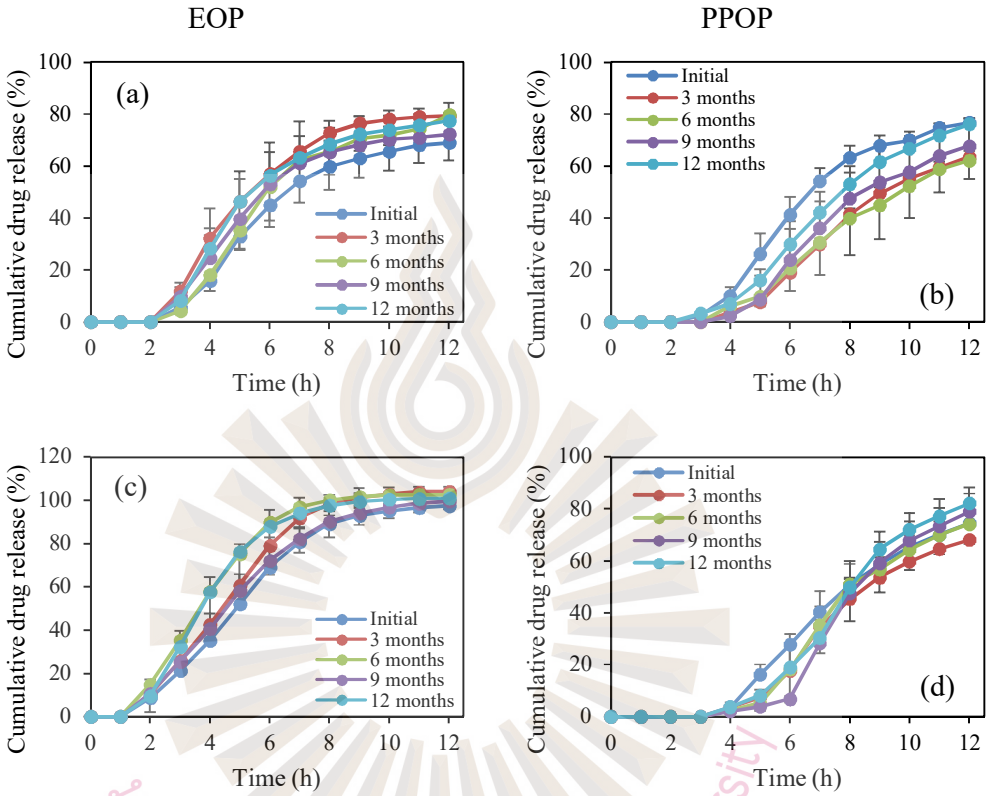


Figure 4.46 Effect of storage time of crosslinked HGCs on cumulative drug release in water of (a, b) DIL HCl and (c, d) PAR from EOP and PPOP capsule.

Table 4.10 Release kinetic models of model drugs from EOP and PPOP capsule prepared from stored crosslinked HGC shells.

Model drugs/ Storage time	Release rate (%/$h^{1/2}$)*	Lag time (h)*	R^2 of drug release		
			Zero-order	First-order	Higuchi
DIL HCl, EOP					
Initial	25.70±3.46	3.57±0.39	0.9351	0.9302	0.9830
3 months	29.43±0.30	2.84±0.65	0.9088	0.9304	0.9687
6 months	28.86±1.32	3.42±0.42	0.9223	0.9067	0.9787
9 months	26.67±2.28	2.86±0.45	0.9091	0.9303	0.9762
12 months	28.25±2.95	2.85±0.04	0.9113	0.9303	0.9802
DIL HCl, PPOP					
Initial	29.20±0.22	4.06±0.46	0.9361	0.8934	0.9897
3 months	24.52±1.85	5.15±0.45	0.9277	0.8648	0.9915
6 months	23.51±3.42	5.14±0.61	0.9270	0.8699	0.9805
9 months	26.48±4.54	5.14±0.70	0.9126	0.8481	0.9926
12 months	28.64±1.14	4.70±0.20	0.9491	0.8793	0.9918
PAR, EOP					
Initial	35.04±1.57	2.62±0.11	0.9347	0.9142	0.9775
3 months	37.01±2.25	2.19±0.46	0.9141	0.9024	0.9729
6 months	36.89±0.90	1.73±0.14	0.8578	0.9162	0.9534
9 months	34.30±1.98	2.10±0.30	0.9321	0.9245	0.9799
12 months	36.65±1.10	1.87±0.61	0.8407	0.9005	0.9399
PAR, PPOP					
Initial	27.76±2.24	4.71±0.14	0.9425	0.8709	0.9945
3 months	27.44±1.64	5.35±0.52	0.9072	0.8373	0.9938
6 months	30.02±3.70	5.46±0.52	0.9009	0.8165	0.9948
9 months	33.82±3.73	6.19±0.39	0.8684	0.7629	0.9924
12 months	34.46±5.25	5.79±0.94	0.8997	0.7955	0.9893

* calculated based on Higuchi's release model.

Figure 4.47 displays the relationship between storage time of crosslinked HGC shells and Higuchi's release rate of EOP and PPOP capsules. Variation of drug release rates from EOP and PPOP capsules prepared from storage crosslinked HGC shells were in the range of 10 %/$h^{1/2}$. The lag time of drug release from EOP capsule was apparently consistent (Figure 4.48). It seems storage time of crosslinked HGC shells did not affected release rate and lag time of drug release from EOP and PPOP capsule.

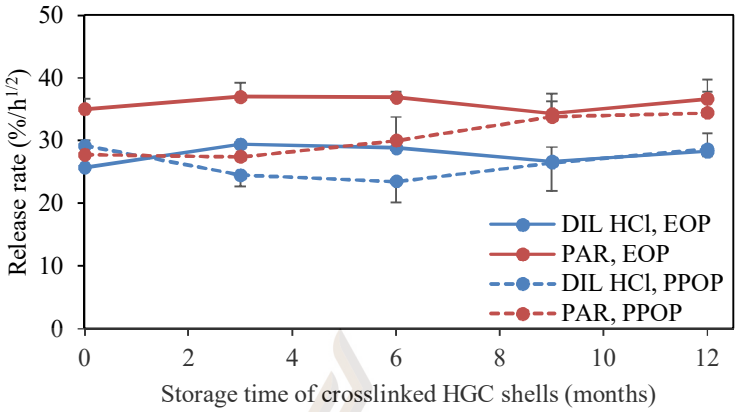


Figure 4.47 The relationship between storage time of crosslinked HGC shells and Higuchi's release rate of EOP and PPOP capsules.

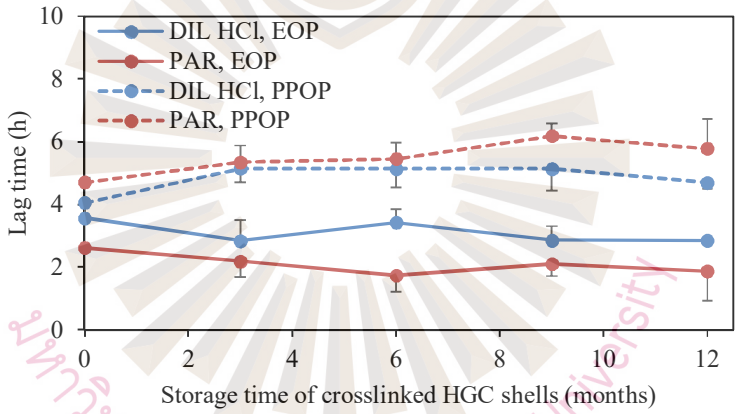


Figure 4.48 The relationship between storage time of crosslinked HGC shells and lag time of EOP and PPOP capsules.

CHAPTER 5

CONCLUSIONS

The objectives of this study were to prepare and evaluate properties of crosslinked HGCs using formaldehyde as crosslink inducing agent, to use as a structural assembly of EOP and PPOP capsules for the delivery of model drugs with different water solubilities. HGCs crosslinked in formaldehyde vapor exhibited insoluble characteristic. Crosslinking formation of HGCs by detection of lysine methylol and arginine methylol was achieved from the FTIR spectra. Low formaldehyde residue (less than 0.5%w/w) was observed in all crosslinked HGCs, which indicated the safety of crosslinked HGCs that could be used for the pharmaceuticals. HGCs crosslinked for 12 h exhibited the most consistent drug release profile after storage for 90 days. So, HGCs crosslinked for 12 h was selected to prepare EOP and PPOP capsules for the delivery of model drugs with different water solubilities i.e. DIL HCl, PRO HCl, AMB HCl, and PAR.

Drug release from EOP capsules was dependent of drug substance and loading dose except DIL HCl. While, drug release from PPOP capsules was independent of drug substance, loading dose, and capsule size. But it was dependent of amount of PEO Mw 200K. The osmolality of release medium had a greater effect on the drug release from PPOP capsules than EOP capsules. Release mediums with or without enzymes gave similar drug release. Drug release from EOP and PPOP capsules prepared from crosslinked HGCs stored for 12 months seemed to provide reproducible release profiles. Almost all of the formulations approached Higuchi's release kinetic. However, both EOP and PPOP capsules failed to delivery of AMB HCl due to its low water solubility and high apparent density. In conclusion, the developed EOP and PPOP capsules were an alternative device that could be used in drug delivery systems and were applicable for several drugs with different water solubilities.

The future work to be investigated is that particle size and density should be controlled to ensure that the drug release was dependent of drug water solubility rather than particle size and density. The *in vivo* study should be performed to evaluate the pharmacokinetic parameters of the osmotic pump capsules. Moreover, tablet-filled crosslinked HGC should be used to prepare osmotic pump capsules to increase the loading dose of the drug substance.

REFERENCES

Ali, A. A., & Sayed, O. M. (2013). Development and characterization of ketorolac tromethamine osmotic pump tablets. *Journal of Drug Delivery Science and Technology, 23*(3), 275-281. doi:10.1016/S1773-2247(13)50041-4

Allen, L. V., Popovich, N. G., & Ansel, H. C. (Ed.). (2010). *Ansel's pharmaceutical dosage forms and drug delivery systems* (9th ed.). China: Lippincott Williams & Wilkins.

Augsburger, L. L. (2009). Hard- and soft-shell capsules. In A. T. Florence, & J. Siepmann (Eds.), *Modern pharmaceutics-basic principles and systems* (p. 511). Florida: CRC Press.

Baker, R. W., & Brooke, J. W. (1987). *Pharmaceutical delivery system*. US patent no.4687660.

Bindschaedler, C., Gurny, R., & Doelker, E. (1986). Osmotically controlled drug delivery systems produced from organic solutions and aqueous dispersions of cellulose acetate. *Journal of Controlled Release, 4*(3), 203-212. doi:10.1016/0168-3659(86)90004-0

Brown, J., Madit, N., Cole, E. T., Wilding, I. R., & Cade, D. (1998). The effect of cross-linking on the *in vivo* disintegration of hard gelatin capsules. *Pharmaceutical Research, 15*(7), 1026-1030. doi:10.1023/A:1011973909815

Chiwele, I., Jones, B. E., & Podczeck, F. (2000). The shell dissolution of various empty hard capsules. *Chemical and Pharmaceutical Bulletin, 48*(7), 951-956.

Cole, G. (1998). Evaluating development and production costs: Tablets versus capsules. *Pharmaceutical Technology Europe, 5,* 17-26.

Collett, J., & Moreton, C. (2002). Modified release peroral dosage forms. In M. E. Aulton (Ed.), *Pharmaceutics: The science of dosage form design*. Spain: Churchill Livingstone.

Colorcon. (2012). *Polyox™ water soluble resins*. Retrieved from https://www.colorcon. com/products-formulation/all-products/download/782/2124/34?method=view

REFERENCES (CONT.)

Conley, R., Gupta, S. K., & Sathyan, G. (2006). Clinical spectrum of the osmotic-controlled release oral delivery system (OROS), an advanced oral delivery form. *Current Medical Research and Opinion, 22*(10), 1879-1892. doi:10.1185/030079906x132613

Cortese, R., & Theeuwes, F. (1982). *Osmotic device with hydrogel driving member*. US patent no.4327725.

Council of Europe. (2005). *Free formaldehyde*. Retrieved from http://lib.njutcm.edu.cn/yaodian/ep/EP5.0/02_methods_of_analysis/2.4.__limit_tests/2.4.18.%20Free%20formaldehyde.pdf

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*(3), 217-223.

Davar, N., Barcley, B., & Gupta, S. (2008). Osmotic systems. In L. L. Augsburger, & S. W. Hoag (Eds.), *Pharmaceutical dosage forms: Tablets* (pp. 493-507). New York: Informa Healthcare.

Defang, O., Shufang, N., Wei, L., Hong, G., Hui, L., & Weisan, P. (2005). In vitro and in vivo evaluation of two extended release preparations of combination metformin and glipizide. *Drug Development and Industrial Pharmacy, 31*(7), 677-685. doi:10.1080/03639040500216410

Derakhshandeh, K., & Berenji, M. G. (2014). Development and optimization of buspirone oral osmotic pump tablet. *Research in Pharmaceutical Sciences, 9*(4), 233-241.

Digenis, G. A., Gold, T. B., & Shah, V. P. (1994). Cross-linking of gelatin capsules and its relevance to their *in vitro-in vivo* performance. *Journal of Pharmaceutical Sciences, 83*(7), 915-921. doi:10.1002/jps.2600830702

REFERENCES (CONT.)

Ding, H. (2016). Modified-release drug products and drug devices. In L. Shargel, & A. B. C. Yu (Eds.), *Applied biopharmaceutics & pharmacokinetics*. New York: McGraw-Hill Education. Retrieved from http://accesspharmacy.mhmedical.com/content.aspx?bookid=1592&sectionid=100674031

Duconseille, A., Astruc, T., Quintana, N., Meersman, F., & Sante-Lhoutellier, V. (2015). Gelatin structure and composition linked to hard capsule dissolution: A review. *Food Hydrocolloids, 43,* 360-376. doi:10.1016/j.foodhyd.2014.06.006

Durect Corporation. (2015). *Alzet® osmotic pumps*. Retrieved from http://www.alzet.com/ products/ALZET_Pumps/howdoesitwork.html

Eastoe, J. E. (1955). The amino acid composition of mammalian collagen and gelatin. *Biochemical Journal, 61*(4), 589-600. doi:10.1042/bj0610589

Emara, L. H., Taha, N. F., Badr, R. M., & Mursi, N. M. (2012). Development of an osmotic pump system for controlled delivery of diclofenac sodium. *Drug Discoveries & Therapeutics., 6*(5), 269-277. doi:10.5582/ddt.2012.v6.5.269

Fara, J. W., & Ray, N. (1988). Osmotic pumps. In P. Tyle (Ed.), *Drug delivery devices; Fundamentals and applications* (pp. 137-175). New York: Marcel Dekker.

Farris, S., Song, J., & Huang, Q. (2010). Alternative reaction mechanism for the cross-linking of gelatin with glutaraldehyde. *Journal of Agricultural and Food Chemistry, 58*(2), 998-1003. doi:10.1021/jf9031603

Garg, A., Gupta, M., & Bhargava, H. N. (2007). Effect of formulation parameters on the release characteristics of propranolol from asymmetric membrane coated tablets. *European Journal of Pharmaceutics and Biopharmaceutics, 67*(3), 725-731. doi:10.1016/j.ejpb.2007.04.009

REFERENCES (CONT.)

Gerk, P. M., Yu, A. B. C., & Shargel, L. (2016). Physiologic factors related to drug absorption. In L. Shargel, & A. B. C. Yu (Eds.), *Applied biopharmaceutics & pharmacokinetics*. New York: McGraw-Hill Education. Retrieved from http://accesspharmacy.mhmedical.com/content.aspx?bookid=1592&sectionid=100672699.

Gong, W., Liu, Y., Mei, D. Y., Yang, M., & Mei, X. G. (2015). Preparation, release and pharmacokinetics of a risperidone elementary osmotic pump system. *Drug Development and Industrial Pharmacy, 41*(3), 464-469. doi:10.3109/03639045.2013.877923

Higuchi, T., & Leeper, H. M. (1973). *Improved osmotic dispenser employing magnesium sulphate and magnesium chloride*. US patent no.3760804.

Higuchi, T., & Leeper, H. M. (1976). *Osmotic dispenser with means for dispensing active agent responsive to osmotic gradient*. US patent no.3995631.

Hill, A., Geißler, S., Weigandt, M., & Mäder, K. (2012). Controlled delivery of nanosuspensions from osmotic pumps: Zero order and non-zero order kinetics. *Journal of Controlled Release, 158*(3), 403-412. doi:10.1016/j.jconrel.2011.12.005

ICH Expert Working Group. (2005). *Validation of analytical procedures: Text and methodology Q2(R1)*. Retrieved from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf

ICH Expert Working Group. (2015). *Addendum to ICH M7: Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk: Application of the principles of the ICH M7 guideline to calculation of compound-specific acceptable intakes M7(R1)*. Retrieved from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M7/M7_Addendum_Step_2.pdf

Jagdale, S. C., Padekar, S. S., Bhadoriya, A. S., Ghorpade, S. A., & Kuchekar, B. S. (2010). Intragastric floating drug delivery of ambroxol hydrochloride using pectin and carbonate salts. *Der Pharmacia Lettre, 2*(4), 217-226.

REFERENCES (CONT.)

Jain, N. K., & Naik, S. U. (1984). Design of a slow-release capsule using laser drilling. *Journal of Pharmaceutical Sciences, 73*(12), 1806-1811. doi:10.1002/jps.2600731237

Jain, P. S. (2010). Stability-indicating HPTLC determination of ambroxol hydrochloride in bulk drug and pharmaceutical dosage form. *Journal of Chromatographic Science, 48*(1), 45-48. doi:10.1093/chromsci/48.1.45

Jallo, L. J., & Dave, R. N. (2015). Explaining electrostatic charging and flow of surface-modified acetaminophen powders as a function of relative humidity through surface energetics. *Journal of Pharmaceutical Sciences, 104*(7), 2225-2232. doi:10.1002/jps.24479

Jensen, J. L., Appel, L. E., Clair, J. H., & Zentner, G. M. (1995). Variables that affect the mechanism of drug release from osmotic pumps coated with acrylate/methacrylate copolymer latexes. *Journal of Pharmaceutical Sciences, 84*(5), 530-533. doi:10.1002/jps.2600840503

Kalantzi, L., Reppas, C., Dressman, J. B., Amidon, G. L., Junginger, H. E., Midha, K. K., . . . Barends, D. M. (2006). Biowaiver monographs for immediate release solid oral dosage forms: Acetaminophen (paracetamol). *Journal of Pharmaceutical Sciences, 95*(1), 4-14. doi:10.1002/jps.20477

Khan, Z. A., Tripathi, R., & Mishra, B. (2011). Floating elementary osmotic pump tablet (FEOPT) for controlled delivery of diethylcarbamazine citrate: a water-soluble drug. *AAPS PharmSciTech, 12*(4), 1312-1323. doi:10.1208/s12249-011-9699-6

Kumar, P., Singh, S., & Mishra, B. (2009). Development and evaluation of elementary osmotic pump of highly water soluble drug: tramadol hydrochloride. *Current Drug Delivery, 6*(1), 130-139.

Laidler, P., Maslin, S. C., & Gilhome, R. W. (1985). What's new in Osmosin and intestinal perforation? *Pathology - Research and Practice, 180*(1), 74-76. doi:10.1016/s0344-0338(85)80078-9

REFERENCES (CONT.)

Li, G., Wang, Y., Chen, H., Leng, D., Ma, P., Dong, Y., . . . He, Z. (2015). Can semipermeable membranes coating materials influence *in vivo* performance for paliperidone tri-layer ascending release osmotic pump tablet: *In vitro* evaluation and *in vivo* pharmacokinetics study. *Asian Journal of Pharmaceutical Sciences, 10*(2), 128-137. doi:10.1016/j.ajps.2014.12.002

Li, W., Du, G., Yang, X., Zhang, Z., Nie, S., Peng, B., & Pan, W. (2008). In vitro and in vivo evaluation of a novel push–pull osmotic pump with orifices on both side surfaces. *Drug Development and Industrial Pharmacy, 34*(12), 1350-1355. doi:10.1080/10673220802122928

Li, Z., Yu, F., Di, Z., Zhao, X., Zhao, S., Liu, Y., . . . Mei, X. (2016). Development and uniform evaluation of ropinirole osmotic pump tablets with REQUIP XL both in vitro and in beagle dogs. *Drug Development and Industrial Pharmacy, 42*(1), 12-18. doi:10.3109/03639045.2015.1020219

Liu, D., Yu, S., Zhu, Z., Lyu, C., Bai, C., Ge, H., . . . Pan, W. (2014). Controlled delivery of carvedilol nanosuspension from osmotic pump capsule: *In vitro* and *in vivo* evaluation. *International Journal of Pharmaceutics, 475*(1–2), 496-503. doi:10.1016/j.ijpharm.2014.09.008

Liu, L., & Che, B. (2006). Preparation of monolithic osmotic pump system by coating the indented core tablet. *European Journal of Pharmaceutics and Biopharmaceutics, 64*(2), 180-184. doi:10.1016/j.ejpb.2006.05.004

Liu, L., Khang, G., Rhee, J. M., & Lee, H. B. (2000). Monolithic osmotic tablet system for nifedipine delivery. *Journal of Controlled Release, 67*(2), 309-322. doi:10.1016/S0168-3659(00)00222-4

Liu, L., Ku, J., Khang, G., Lee, B., Rhee, J. M., & Lee, H. B. (2000). Nifedipine controlled delivery by sandwiched osmotic tablet system. *Journal of Controlled Release, 68*(2), 145-156. doi:10.1016/S0168-3659(00)00243-1

Liu, L., & Wang, X. (2008). Solubility-modulated monolithic osmotic pump tablet for atenolol delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 68*(2), 298-302. doi:10.1016/j.ejpb.2007.04.020

REFERENCES (CONT.)

Liu, L., & Xu, X. (2008). Preparation of bilayer-core osmotic pump tablet by coating the indented core tablet. *International Journal of Pharmaceutics, 352*(1), 225-230. doi:10.1016/j.ijpharm.2007.10.047

Liu, X., Wang, S., Chai, L., Zhang, D., Sun, Y., Xu, L., & Sun, J. (2014). A two-step strategy to design high bioavailable controlled-release nimodipine tablets: The push–pull osmotic pump in combination with the micronization/solid dispersion techniques. *International Journal of Pharmaceutics, 461*(1–2), 529-539. doi:10.1016/j.ijpharm.2013.12.023

Lu, E.-X., Jiang, Z.-Q., Zhang, Q.-Z., & Jiang, X.-G. (2003). A water-insoluble drug monolithic osmotic tablet system utilizing gum arabic as an osmotic, suspending and expanding agent. *Journal of Controlled Release, 92*(3), 375-382. doi:10.1016/S0168-3659(03)00371-7

Ma, L., Deng, L., & Chen, J. (2014). Applications of poly(ethylene oxide) in controlled release tablet systems: a review. *Drug Development and Industrial Pharmacy, 40*(7), 845-851. doi:10.3109/03639045.2013.831438

Malaterre, V., Ogorka, J., Loggia, N., & Gurny, R. (2009a). Approach to design push–pull osmotic pumps. *International Journal of Pharmaceutics, 376*(1–2), 56-62. doi:10.1016/j.ijpharm.2009.04.015

Malaterre, V., Ogorka, J., Loggia, N., & Gurny, R. (2009b). Evaluation of the tablet core factors influencing the release kinetics and the loadability of push–pull osmotic systems. *Drug Development and Industrial Pharmacy, 35*(4), 433-439. doi:10.1080/03639040802425230

Malaterre, V., Ogorka, J., Loggia, N., & Gurny, R. (2009c). Oral osmotically driven systems: 30 years of development and clinical use. *European Journal of Pharmaceutics and Biopharmaceutics, 73*(3), 311-323. doi:10.1016/j.ejpb.2009.07.002

Marchais, H., Cayzeele, G., Legendre, J.-Y., Skiba, M., & Arnaud, P. (2003). Cross-linking of hard gelatin carbamazepine capsules: effect of dissolution conditions on in vitro drug release. *European Journal of Pharmaceutical Sciences, 19*(2–3), 129-132. doi:10.1016/S0928-0987(03)00070-8

REFERENCES (CONT.)

Marroum, P. J. (2008). Setting dissolution specifications. In L. L. Augsburger, & S. W. Hoag (Eds.), *Pharmaceutical dosage forms: Tablets* (pp. 191-205). New York: Informa Healthcare.

McEvoy, G. K. (Ed.). (2014). *AHFS drug information 2014*. Maryland: American Society of Health-System Pharmacist.

Mehramizi, A., Alijani, B., Pourfarzib, M., Dorkoosh, F. A., & Rafiee -Tehrani, M. (2007). Solid carriers for improved solubility of glipizide in osmotically controlled oral drug delivery system. *Drug Development and Industrial Pharmacy, 33*(8), 812-823. doi:10.1080/03639040601128753

Meyer, M. C., Straughn, A. B., Mhatre, R. M., Hussain, A., Shah, V. P., Bottom, C. B., . . . Williams, R. L. (2000). The effect of gelatin cross-linking on the bioequivalence of hard and soft gelatin acetaminophen capsules. *Pharmaceutical Research, 17*(8), 962-966. doi:10.1023/A:1007579221726

Missaghi, S., Patel, P., Farrell, T., Huatan, H., & Rajabi-Siahboomi, A. (2014). Investigation of critical core formulation and process parameters for osmotic pump oral drug delivery. *AAPS PharmSciTech, 15*(1), 149-160. doi:10.1208/s12249-013-0040-4

Mohammadi-Samani, S., Adrangui, M., Siahi-Shadbad, M. R., & Nokhodchi, A. (2000). An approach to controlled-release dosage form of propranolol hydrochloride. *Drug Development and Industrial Pharmacy, 26*(1), 91-94.

Nie, S.-f., Li, W., Luan, L., Pan, W., & Wang, X. (2007). Studies on bi-layer osmotic pump tablets of water-insoluble allopurinol with large dose: *In vitro* and *in vivo*. *Drug Development and Industrial Pharmacy, 33*(9), 1024-1029. doi:10.1080/03639040601179897

Nokhodchi, A., Momin, M. N., Shokri, J., Shahsavari, M., & Rashidi, P. A. (2008). Factors affecting the release of nifedipine from a swellable elementary osmotic pump. *Drug Delivery, 15*(1), 43-48. doi:10.1080/10717540701829028

REFERENCES (CONT.)

Ofner, C. M., Zhang, Y.-E., Jobeck, V. C., & Bowman, B. J. (2001). Crosslinking studies in gelatin capsules treated with formaldehyde and in capsules exposed to elevated temperature and humidity. *Journal of Pharmaceutical Sciences, 90*(1), 79-88. doi:10.1002/1520-6017(200101)90:1<79::aid-jps9>3.0.co;2-l

Ouyang, D., Nie, S., Li, W., Guo, H., Liu, H., & Pan, W. (2005). Design and evaluation of compound metformin/glipizide elementary osmotic pump tablets. *Journal of Pharmacy and Pharmacology, 57*(7), 817-820. doi:10.1211/0022357056370

Özdemir, N., & Sahin, J. (1997). Design of a controlled release osmotic pump system of ibuprofen. *International Journal of Pharmaceutics, 158*(1), 91-97. doi:10.1016/S0378-5173(97)00250-0

Pan, H., Jing, H., Yang, X., Pan, W., & Chen, T. (2017). Synchronized and controlled release of metformin hydrochloride/glipizide from elementary osmotic delivery. *Drug Development and Industrial Pharmacy, 43*(5), 780-788. doi:10.1080/03639045.2016.1200071

Passetti, G. (2012). *Aqueous solution of ambroxol*. WO patent no.2012085185.

Patel, K. N., & Mehta, T. A. (2014). Formulation design and characterization of an elementary osmotic pump tablet of flurbiprofen. *PDA Journal of Pharmaceutical Science and Technology, 68*(4), 333-346. doi:10.5731/pdajpst.2014.00984

Prabakaran, D., Singh, P., Kanaujia, P., & Vyas, S. P. (2003). Effect of hydrophilic polymers on the release of diltiazem hydrochloride from elementary osmotic pumps. *International Journal of Pharmaceutics, 259*(1–2), 173-179. doi:10.1016/S0378-5173(03)00230-8

Prabhu, N. B., Marathe, A. S., Jain, S., Singh, P. P., Sawant, K., Rao, L., & Amin, P. D. (2008). Comparison of dissolution profiles for sustained release resinates of BCS class I drugs using USP Apparatus 2 and 4: A technical note. *AAPS PharmSciTech, 9*(3), 769-773. doi:10.1208/s12249-008-9110-4

REFERENCES (CONT.)

Rabti, H., Mohammed Salmani, J. M., Elamin, E. S., Lammari, N., Zhang, J., & Ping, Q. (2014). Carbamazepine solubility enhancement in tandem with swellable polymer osmotic pump tablet: A promising approach for extended delivery of poorly water-soluble drugs. *Asian Journal of Pharmaceutical Sciences, 9*(3), 146-154. doi:10.1016/j.ajps.2014.04.001

Ramakrishna, N., & Mishra, B. (2002). Plasticizer effect and comparative evaluation of cellulose acetate and ethylcellulose-HPMC combination coatings as semipermeable membranes for oral osmotic pumps of naproxen sodium. *Drug Development and Industrial Pharmacy, 28*(4), 403-412. doi:10.1081/ddc-120003001

Rose, S., & Nelson, J. F. (1955). A continuous long-term injector. *Australian Journal of Experimental Biology and Medical Science, 33*(4), 415-419. doi:10.1038/icb.1955.44

Rowe, R. C., Sheskey, P. J., & Quinn, M. E. (2009). *Handbook of pharmaceutical excipients* (6th ed.). London: Pharmaceutical Press.

Sadeghi, F., Navidpour, L., Bayat, S., & Afshar, M. (2013). Validation and uncertainty estimation of an ecofriendly and stability-indicating HPLC method for determination of diltiazem in pharmaceutical preparations. *Journal of Analytical Methods in Chemistry, 2013,* 353814. doi:10.1155/2013/353814

Salsa, T., Pina, M. E., & Teixeira-Dias, J. J. C. (1996). Crosslinking of gelatin in the reaction with formaldehyde: An FT-IR spectroscopic study. *Applied Spectroscopy, 50*(10), 1314-1318. doi:10.1366/0003702963904881

Santus, G., & Baker, R. W. (1995). Osmotic drug delivery: a review of the patent literature. *Journal of Controlled Release, 35*(1), 1-21. doi:10.1016/0168-3659(95)00013-X

Shaikh, W., Deshmukh, P. K., Patil, G. B., Chatap, V. K., & Bari, S. B. (2013). Design and statistical optimization of osmotically driven capsule based on push-pull technology. *Pharmaceutical Development and Technology, 18*(2), 515-524. doi:10.3109/10837450.2012.726999

REFERENCES (CONT.)

Shamblin, S. L. (2010). Controlled release using bilayer osmotic tablet technology: Reducing theory to practice. In H. Wen, & K. Park (Eds.), *Oral controlled release formulation design and drug delivery: Theory to practice* (pp. 129-153). New Jersey: John Wiley & Sons, Inc.

Shokri, J., Ahmadi, P., Rashidi, P., Shahsavari, M., Rajabi-Siahboomi, A., & Nokhodchi, A. (2008). Swellable elementary osmotic pump (SEOP): an effective device for delivery of poorly water-soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics, 68*(2), 289-297. doi:10.1016/j.ejpb.2007.06.006

Sinchaipanid, N., Pongwai, S., Limsuwan, P., & Mitrevej, A. (2003). Design of salbutamol EOP tablets from pharmacokinetics parameters. *Pharmaceutical Development and Technology, 8*(2), 135-142. doi:10.1081/pdt-120018479

Singh, S., Rao, K. V. R., Venugopal, K., & Manikandan, R. (2002). Alteration in dissolution characteristics of gelatin-containing formulations: A review of the problem, test methods, and solutions. *Pharmaceutical Technology, 26*(4), 36-58.

Srikonda, S., Kotamraj, P., & Barclay, B. (2006). Osmotic controlled drug delivery systems. In X. Li, & B. R. Jasti (Eds.), *Design of controlled release drug delivery systems* (pp. 203-229). New York: McGraw-Hill.

Stetinova, V., Smetanova, L., Kholova, D., Svoboda, Z., & Kvetina, J. (2009). Transepithelial transport of ambroxol hydrochloride across human intestinal Caco-2 cell monolayers. *General Physiology and Biophysics, 28*(3), 309-315. doi:10.4149/gpb_2009_03_309

Suleiman, M. S., Abdulhameed, M. E., Najib, N. M., & Muti, H. Y. (1990). Degradation kinetics of diltiazem. *Drug Development and Industrial Pharmacy, 16*(4), 685-694. doi:10.3109/03639049009104411

REFERENCES (CONT.)

Sun, Y., Zhu, S., Lu, W., Chen, J., Sun, C., Guo, Y., . . . Hu, R. (2019). A novel enteric positioning osmotic pump capsule-based controlled release system of sinomenine hydrochloride: In vitro and in vivo evaluation. *Journal of Drug Delivery Science and Technology, 49,* 188-194. doi:10.1016/j.jddst.2018.11.005

Tengroth, C., Gasslander, U., Andersson, F. O., & Jacobsson, S. P. (2005). Cross-linking of gelatin capsules with formaldehyde and other aldehydes: An FTIR spectroscopy study. *Pharmaceutical Development and Technology, 10*(3), 405-412. doi:10.1081/pdt-65693

The International Agency for Research on Cancer. (2016). *Paracetamol (Acetaminophen)*. Retrieved from https://monographs.iarc.fr/ENG/Monographs /vol50/mono50-20.pdf

Theeuwes, F. (1975). Elementary osmotic pump. *Journal of Pharmaceutical Sciences, 64*(12), 1987-1991. doi:10.1002/jps.2600641218

Theeuwes, F., & Yum, S. I. (1976). Principles of the design and operation of generic osmotic pumps for the delivery of semisolid or liquid drug formulations. *Annals of Biomedical Engineering, 4*(4), 343-353. doi:10.1007/bf02584524

US Environmental Protection Agency. (2000). *Formaldehyde*. Retrieved from http://www3.epa.gov/airtoxics/hlthef/formalde.html

US Environmental Protection Agency. (2001). *Formaldehyde*. Retrieved from http://www.euro.who.int/__data/assets/pdf_file/0014/123062/AQG2ndEd_5_8Formaldehyde.pdf

Uwai, K., Tani, M., Ohtake, Y., Abe, S., Maruko, A., Chiba, T., . . . Takeshita, M. (2005). Photodegradation products of propranolol: The structures and pharmacological studies. *Life Sciences, 78*(4), 357-365. doi:10.1016/j.lfs.2005.04.033

Venkatraman, S., Davar, N., Chester, A., & Kleiner, L. (2000). An overview of controlled release systems. In D. L. Wise (Ed.), *Handbook of pharmaceutical controlled release technology* (pp. 431-463). New York: Marcel Dekker.

REFERENCES (CONT.)

Verma, R. K., & Garg, S. (2004). Development and evaluation of osmotically controlled oral drug delivery system of glipizide. *European Journal of Pharmaceutics and Biopharmaceutics, 57*(3), 513-525.

Verma, R. K., Kaushal, A. M., & Garg, S. (2003). Development and evaluation of extended release formulations of isosorbide mononitrate based on osmotic technology. *International Journal of Pharmaceutics, 263*(1), 9-24. doi:10.1016/S0378-5173(03)00360-0

Verma, R. K., Krishna, D. M., & Garg, S. (2002). Formulation aspects in the development of osmotically controlled oral drug delivery systems. *Journal of Controlled Release, 79*(1–3), 7-27. doi:10.1016/S0168-3659(01)00550-8

Verma, R. K., Mishra, B., & Garg, S. (2000). Osmotically controlled oral drug delivery. *Drug Development and Industrial Pharmacy, 26*(7), 695-708. doi:10.1081/ddc-100101287

Vogelpoel, H., Welink, J., Amidon, G. L., Junginger, H. E., Midha, K. K., Möller, H., . . . Barends, D. M. (2004). Biowaiver monographs for immediate release solid oral dosage forms based on biopharmaceutics classification system (BCS) literature data: Verapamil hydrochloride, propranolol hydrochloride, and atenolol. *Journal of Pharmaceutical Sciences, 93*(8), 1945-1956. doi:10.1002/jps.20131

Walker, J. M. (2002). The bicinchoninic acid (BCA) assay for protein quantitation. In J. M. Walker (Ed.), *The protein protocols handbook*. New Jersey: Humana Press.

Wang, C., Chen, F., Heng, P. W., Li, J. Z., Li, X., Ye, G. H., . . . Pan, W. S. (2008). A novel time-controlled release system based on drug-resin complexes and elementary osmotic pump. *Chemical and Pharmaceutical Bulletin, 56*(4), 457-463.

Wang, W., Xie, X., Yang, D., & Chen, X. (2009). Swelling property of common hydrophilic polymers and their use in push-pull osmotic-pump tablets. *Zhongguo Zhong Yao Za Zhi, 34*(18), 2319-2321.

REFERENCES (CONT.)

Waterman, K. C., Goeken, G. S., Konagurthu, S., Likar, M. D., MacDonald, B. C., Mahajan, N., & Swaminathan, V. (2011). Osmotic capsules: A universal oral, controlled-release drug delivery dosage form. *Journal of Controlled Release, 152*(2), 264-269. doi:10.1016/j.jconrel.2011.02.001

Wichianprasit, N., & Kulvanich, P. (2009, October). *Osmotically controlled drug delivery system using a crosslinked and non-crosslinked hard gelatin capsule.* Poster session presented at the Asian Federation of Pharmaceutical Sciences, Fukuoka, Japan.

Wiechelman, K. J., Braun, R. D., & Fitzpatrick, J. D. (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Analytical Biochemistry, 175*(1), 231-237. doi:10.1016/0003-2697(88)90383-1

Wong, P. S. L., Gupta, S. K., & Stewart, B. E. (2003). Osmotically controlled tablets. In M. J. Rathbone, J. Hadgraft, & M. S. Roberts (Eds.), *Modified-release drug delivery technology* (pp. 101-114). New York: Informa Healthcare.

Wong, P. S. L., Theeuwes, F., Barclay, B. L., & Dealey, M. H. (1991). *Osmotic dosage system for liquid drug delivery*. WO patent no.1991015196.

Wong, P. S. L., Theeuwes, F., Barclay, B. L., & Dealey, M. H. (1994). *Osmotic dosage system for delivering a formulation comprising liquid carrier and drug*. US patent no.5324280.

Wong, P. S. L., Theeuwes, F., Barclay, B. L., & Dealey, M. H. (1995). *Osmotic dosage system for liquid drug delivery*. US patent no.5413572.

Wu, C., Zhao, Z., Zhao, Y., Hao, Y., Liu, Y., & Liu, C. (2014). Preparation of a push–pull osmotic pump of felodipine solubilized by mesoporous silica nanoparticles with a core–shell structure. *International Journal of Pharmaceutics, 475*(1–2), 298-305. doi:10.1016/j.ijpharm.2014.08.033

REFERENCES (CONT.)

Xu, H., Li, Z., Pan, H., Zhang, Z., Liu, D., Tian, B., . . . Pan, W. (2013). A novel bi-layer ascending release osmotic pump tablet: *In vitro* investigation and *in vivo* investigation in pharmacokinetic study and IVIVC evaluation. *International Journal of Pharmaceutics, 458*(1), 181-187. doi:10.1016/j.ijpharm.2013.09.031

Yanfei, M., Guoguang, C., Lili, R., & Pingkai, O. (2015). Controlled release of ziprasidone solid dispersion systems from osmotic pump tablets with enhanced bioavailability in the fasted state. *Drug Development and Industrial Pharmacy, 41*(8), 1353-1362.

Zentner, G. M., McClelland, G. A., & Sutton, S. C. (1991). Controlled porosity solubility- and resin-modulated osmotic drug delivery systems for release of diltiazem hydrochloride. *Journal of Controlled Release, 16*(1–2), 237-243. doi:10.1016/0168-3659(91)90047-H

Zentner, G. M., Rork, G. S., & Himmelstein, K. J. (1990). *Controlled porosity osmotic pump*. US patent no.4968507.

Zhang, Y., Zhang, Z., & Wu, F. (2003). A novel pulsed-release system based on swelling and osmotic pumping mechanism. *Journal of Controlled Release, 89*(1), 47-55. doi:10.1016/S0168-3659(03)00092-0

Zhang, Z.-h., Li, W., Nie, S.-f., Tang, X., Peng, B., Tian, L., & Pan, W.-s. (2009). Overcome side identification in PPOP by making orifices on both layers. *International Journal of Pharmaceutics, 371*(1–2), 1-7. doi:10.1016/j.ijpharm.2008.12.006

Zhao, S., Yu, F., Liu, N., Di, Z., Yan, K., Liu, Y., . . . Mei, X. (2016). Synchronous delivery of felodipine and metoprolol tartrate using monolithic osmotic pump technology. *Drug Development and Industrial Pharmacy, 42*(11), 1723-1731. doi:10.3109/03639045.2016.1171332

APPENDICES

APPENDIX A

METHOD VALIDATION RESULTS

Results of method validation for determination of water soluble protein fraction

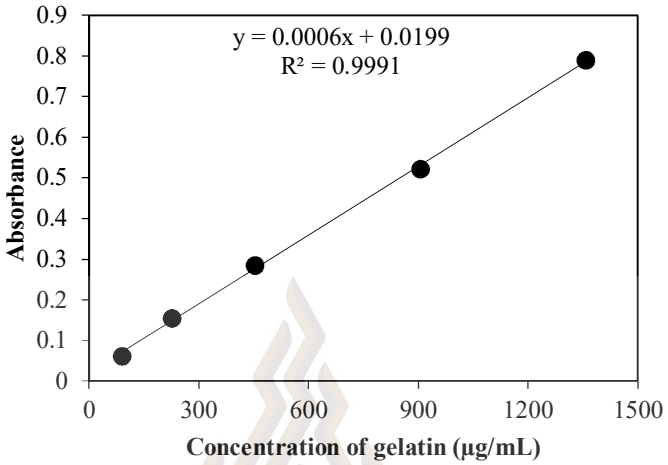


Figure A1 Calibration curve of gelatin aqueous solution (90.5-1357.8 µg/mL) which LOD and LOQ were 24.68 and 74.80 µg/mL, respectively.

Table A1 Precision and accuracy results.

Conc. (µg/mL)	Precision (%RSD)		Spike amount (µg/mL)	Accuracy
	Intraday	Inter-day		Recovery (%)
226	1.35	4.09	226	107.81±1.67
453	0.93	2.12	453	106.04±1.06
905	1.26	1.85	905	100.42±1.31

Results of method validation for determination of formaldehyde content

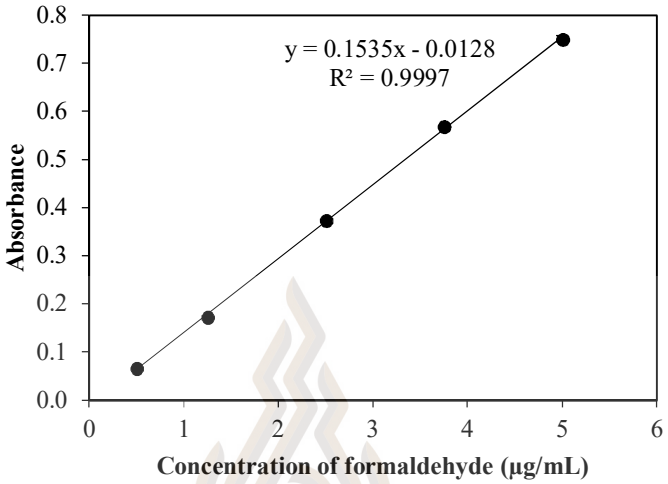


Figure A2 Calibration curve of formaldehyde aqueous solution (0.5-5.0 µg/mL) which LOD and LOQ were 0.06 and 0.19 µg/mL, respectively.

Table A2 Precision and accuracy results.

Conc.	Precision (%RSD)		Spike amount	Accuracy
(µg/mL)	Intraday	Inter-day	(µg/mL)	Recovery (%)
1.25	0.67	2.44	1.25	102.91±0.83
2.50	0.46	2.49	2.50	98.53±2.39
3.75	0.41	1.08	3.75	98.81±1.38

Results of method validation for determination of model drugs content

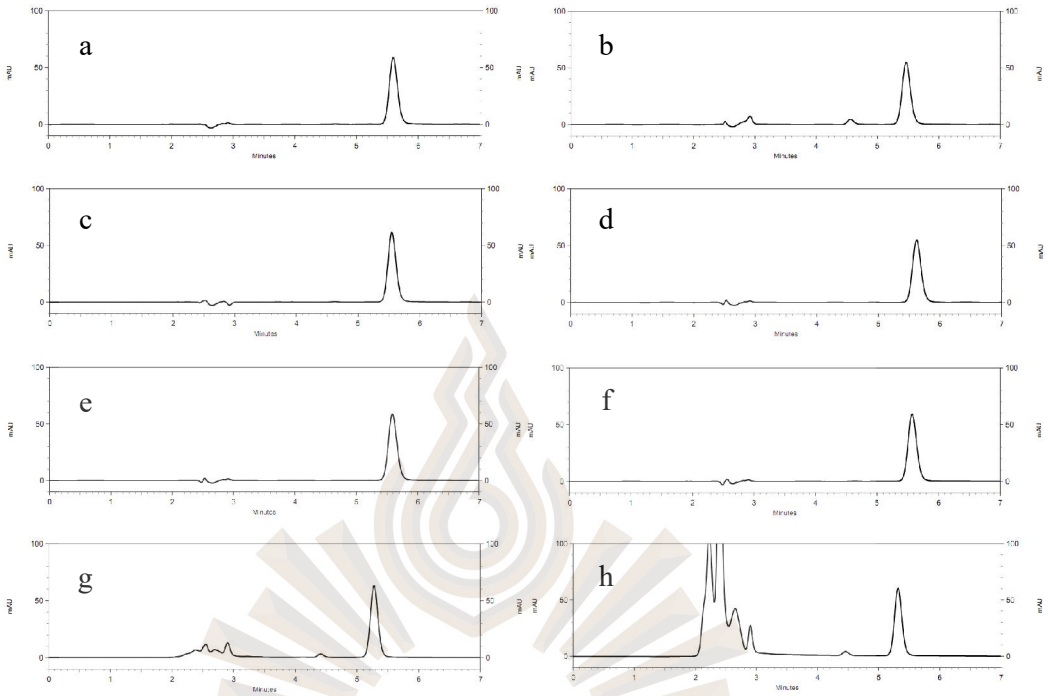


Figure A3 HPLC chromatogram of 20 µg/mL DIL HCl (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, (f) 3%NaCl, (g) SGF, and (h) SIF.

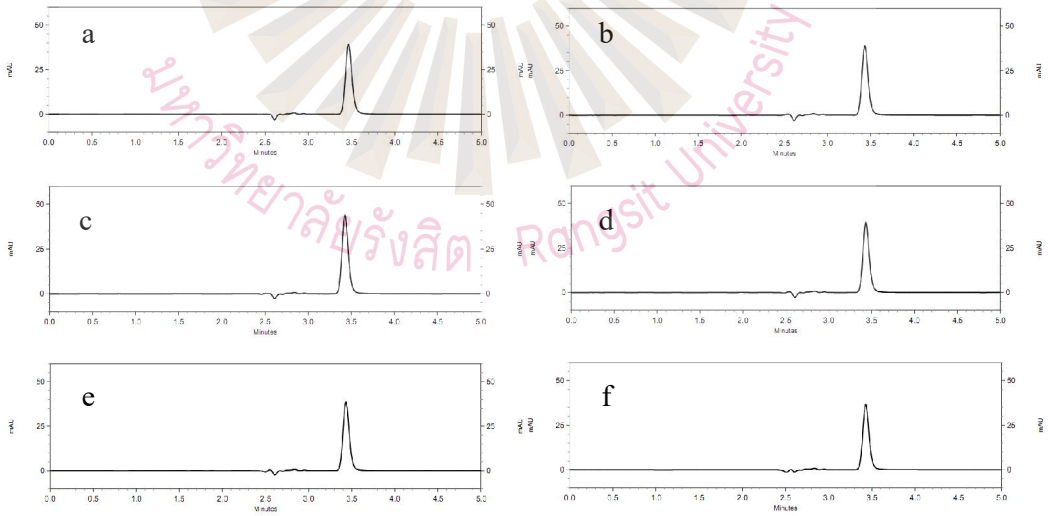


Figure A4 HPLC chromatogram of 20 µg/mL PRO HCl (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, and (f) 3%NaCl.

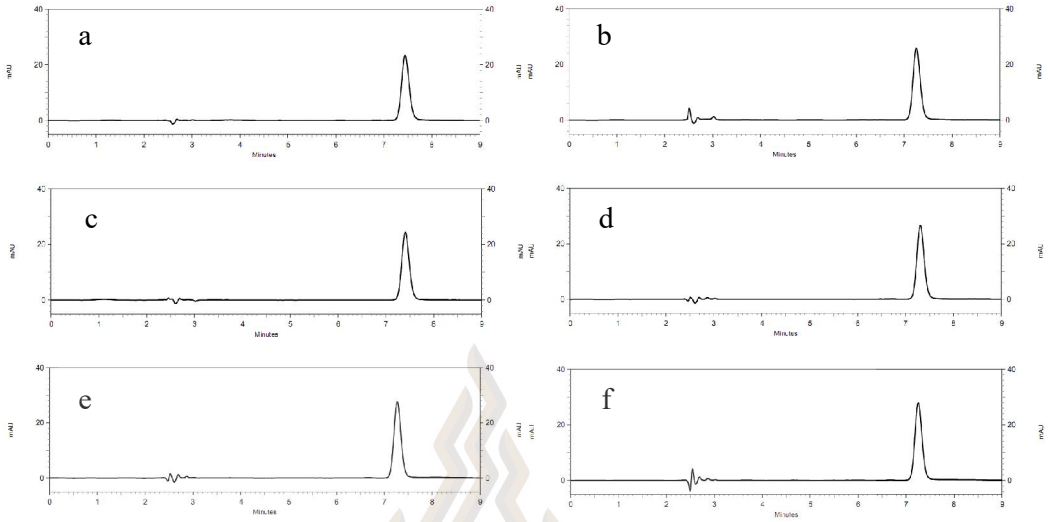


Figure A5 HPLC chromatogram of 20 µg/mL AMB HCl (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, and (f) 3%NaCl.

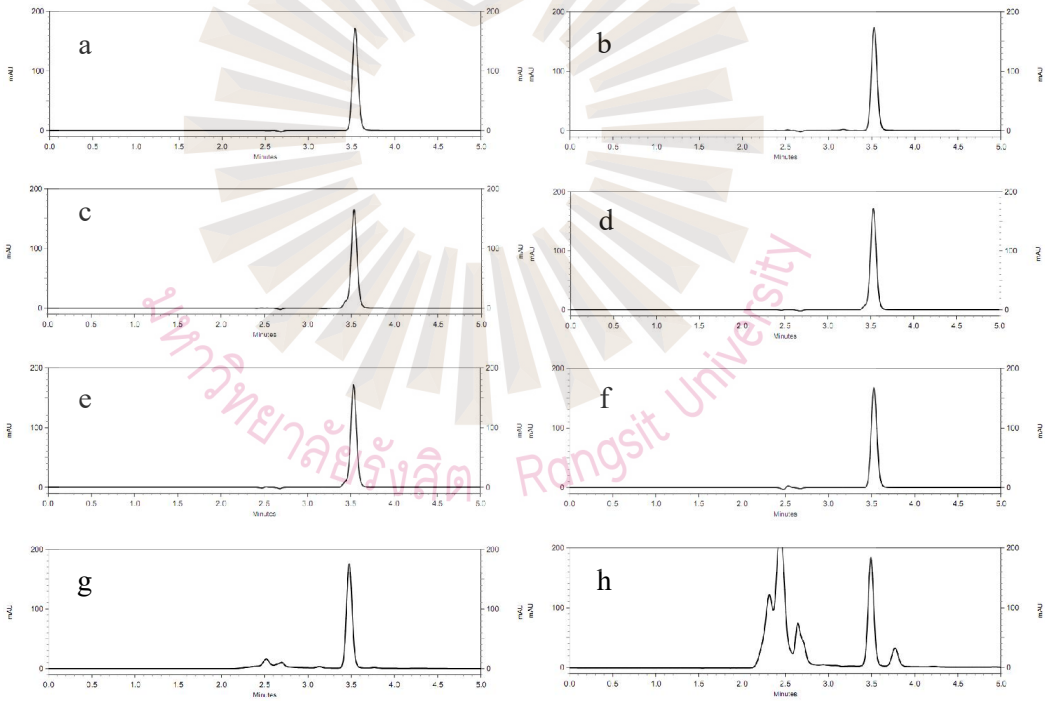


Figure A6 HPLC chromatogram of 20 µg/mL PAR (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, (f) 3%NaCl, (g) SGF, and (h) SIF.

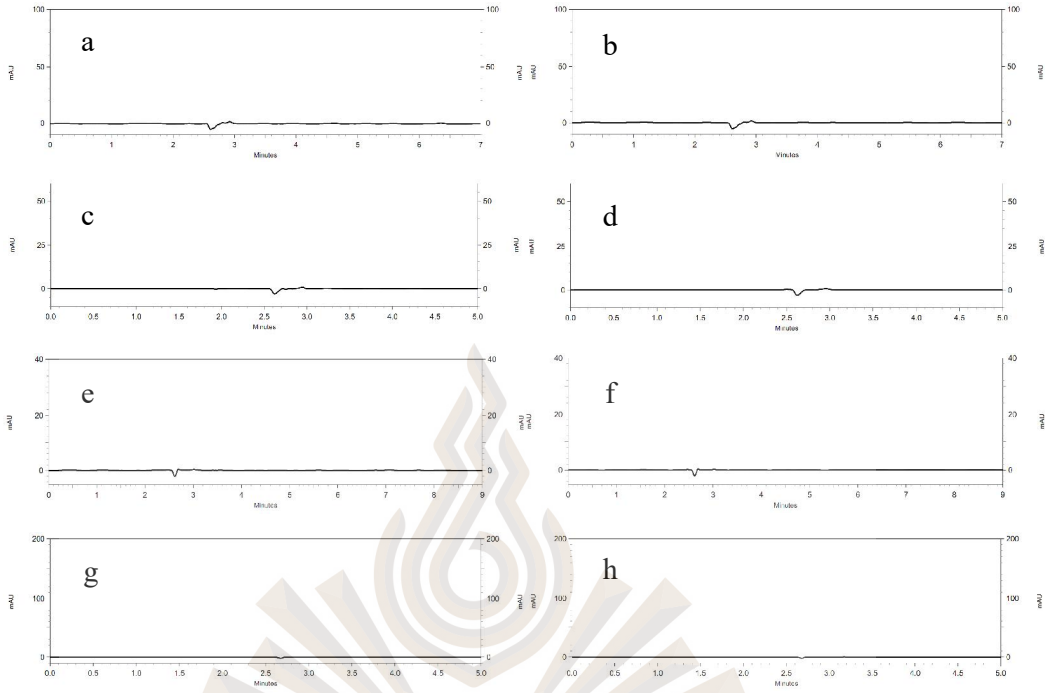


Figure A7 Chromatogram of the blank formulation (in aqueous medium) of EOP (left) and PPOP (right) containing no DIL HCl (a-b), PRO HCl (c-d), AMB HCl (e-f), and PAR (g-h), which no peak found at the same retention time of each model drug as same as other solvents including HCl pH 1.2, PBS pH 6.8, 0.45%NaCl, 0.9%NaCl, and 3%NaCl.

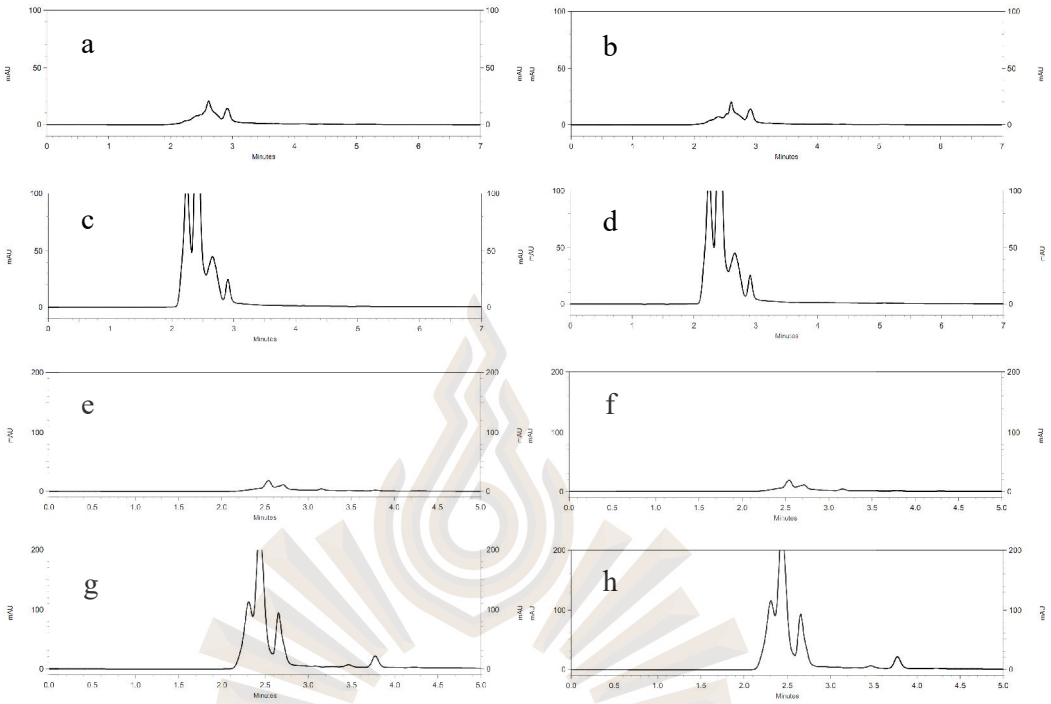


Figure A8 Chromatogram of the blank formulation of EOP (left) and PPOP (right) containing no DIL HCl (a-d) and PAR (e-h) dissolved in SGF (a, b, e, and f) and SIF (c,d, g, and h), which no peak found at the same retention time of each model drug.

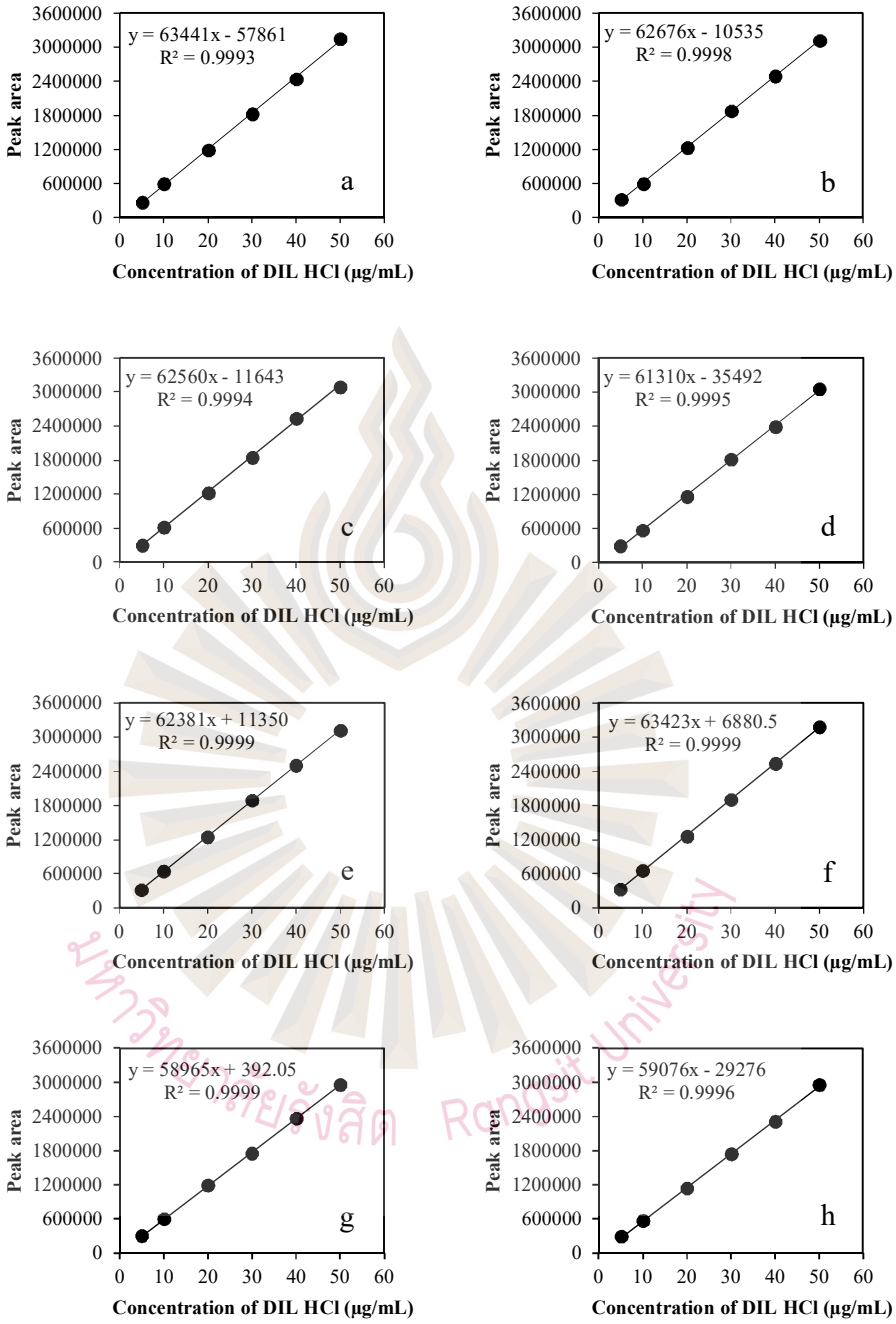


Figure A9 Calibration curves of DIL HCl in (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, (f) 3%NaCl, (g) SGF, and (h) SIF.

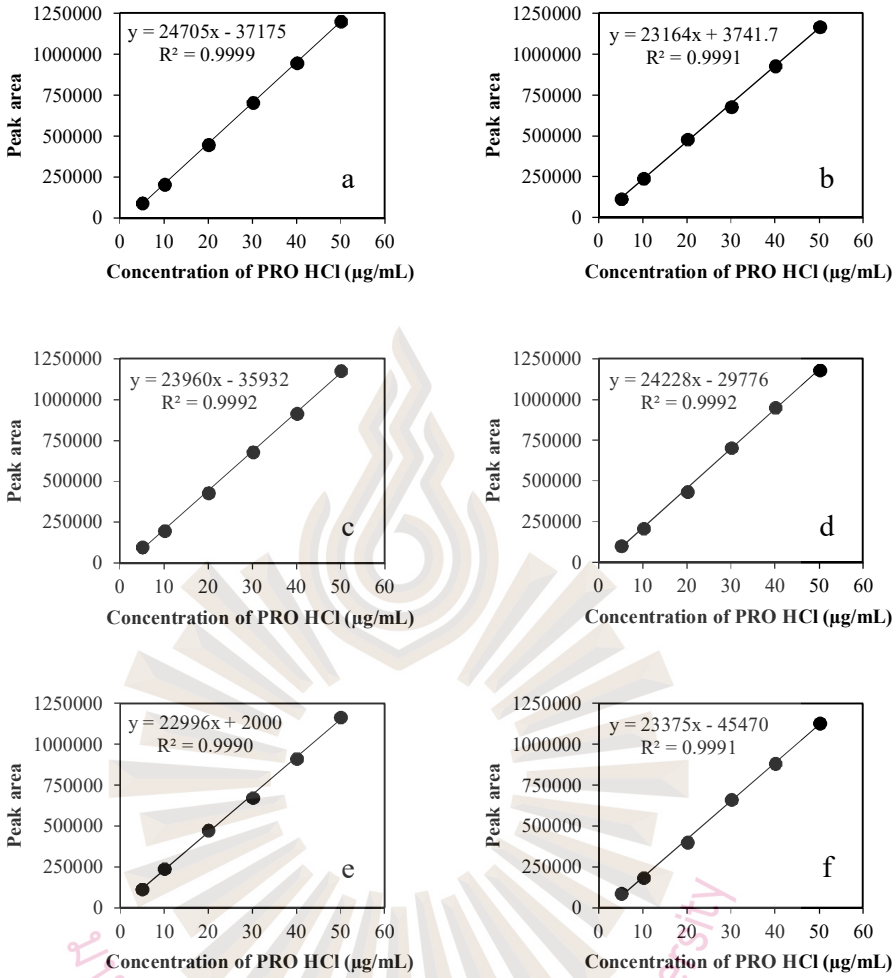


Figure A10 Calibration curves of PRO HCl in (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, and (f) 3%NaCl.

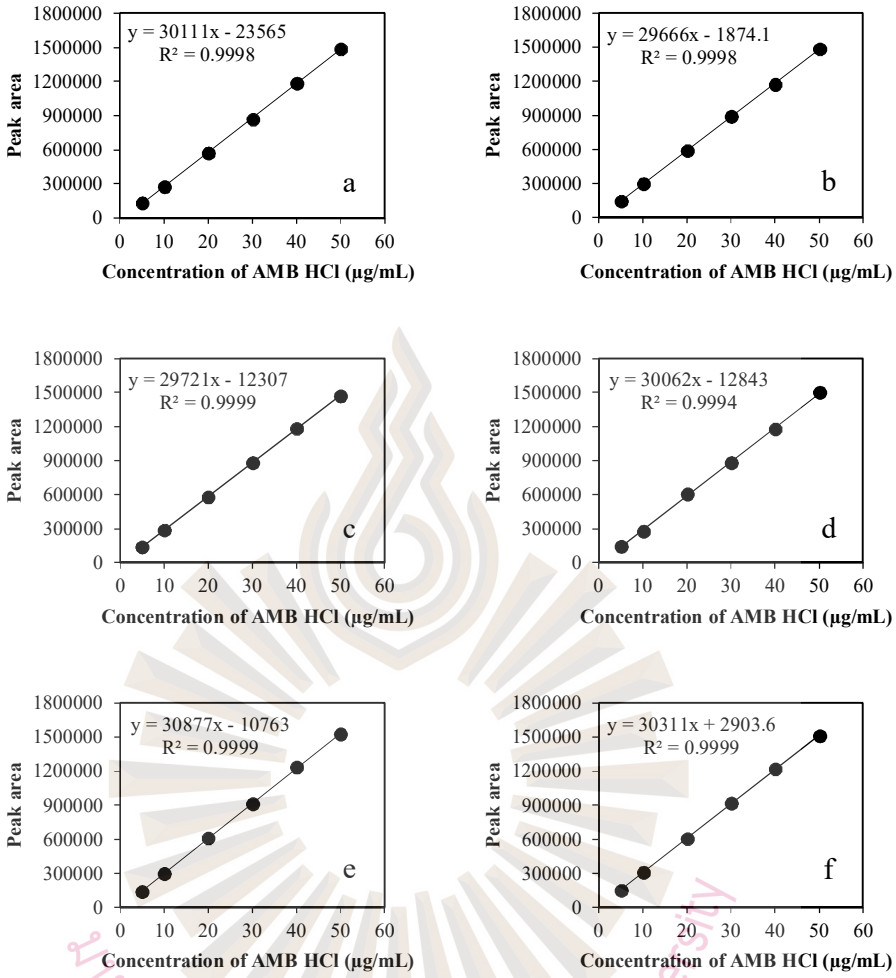


Figure A11 Calibration curves of AMB HCl in (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, and (f) 3%NaCl.

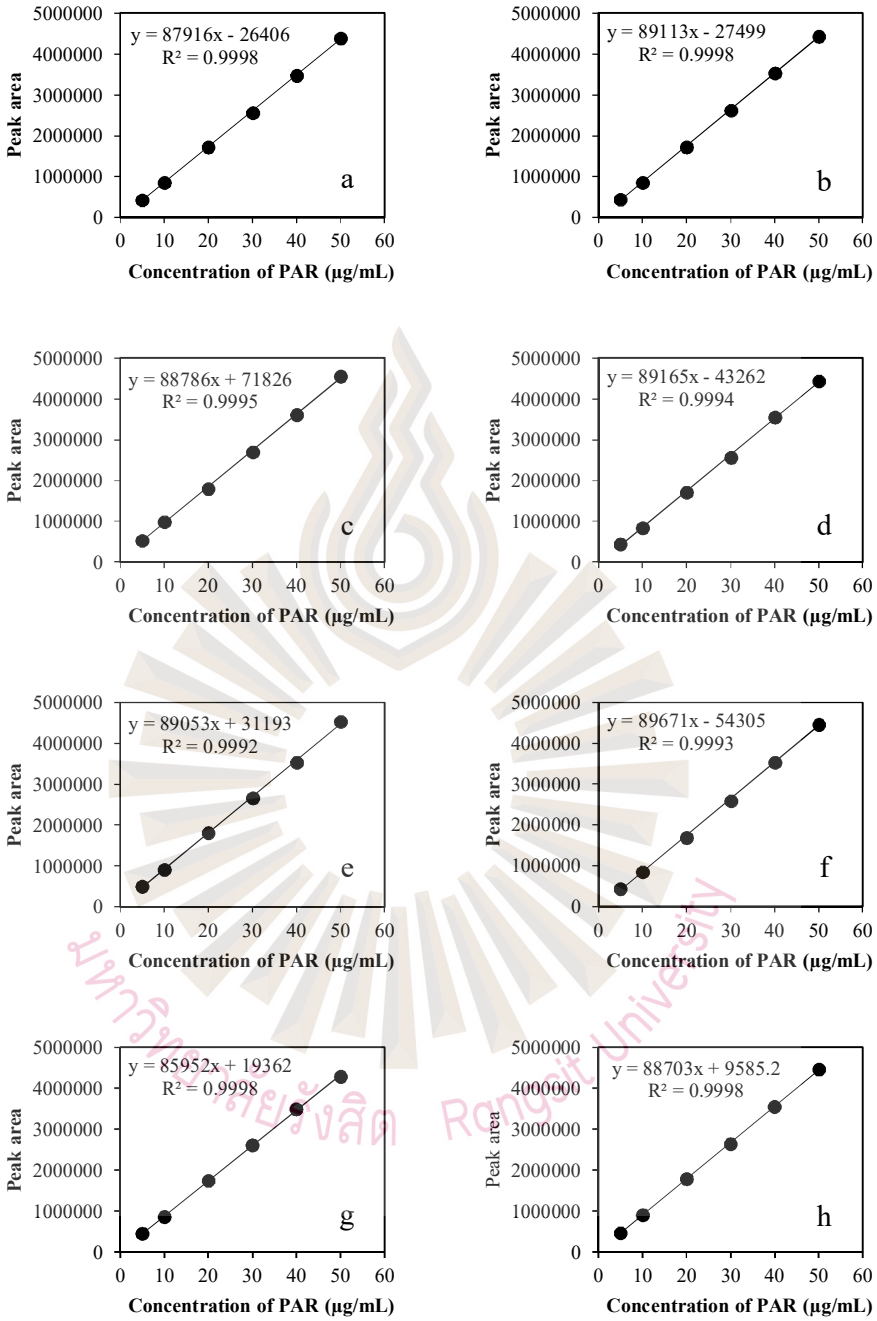


Figure A12 Calibration curves of PAR in (a) water, (b) HCl pH 1.2, (c) PBS pH 6.8, (d) 0.45%NaCl, (e) 0.9%NaCl, (f) 3%NaCl, (g) SGF, and (h) SIF.

Table A3 Linearity parameters and LOD and LOQ of model drugs in different solvents.

Compounds	Solvents	Linear equation	Test range (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)
DIL HCl	Water	y = 63441x-57861	5-50	0.9993	0.67	2.04
	HCl pH 1.2	y = 62676x-10535	5-50	0.9998	0.34	1.04
	PBS pH 6.8	y = 62560x-11643	5-50	0.9994	0.64	1.93
	0.45% NaCl	y = 61310x-35492	5-50	0.9995	0.58	1.76
	0.9% NaCl	y = 62381x+11350	5-50	0.9999	0.29	0.88
	3% NaCl	y = 63423x+6880.5	5-50	0.9999	0.24	0.73
	SGF	y = 58965x+392.05	5-50	0.9999	0.30	0.90
	SIF	y = 59076x-29276	5-50	0.9996	0.54	1.63
PRO HCl	Water	y = 24705x-37175	5-50	0.9999	0.32	0.97
	HCl pH 1.2	y = 23164x+3741.7	5-50	0.9991	0.75	2.27
	PBS pH 6.8	y = 23960x-35932	5-50	0.9992	0.71	2.17
	0.45% NaCl	y = 24228x-29776	5-50	0.9992	0.73	2.21
	0.9% NaCl	y = 22996x+2000	5-50	0.9990	0.82	2.48
	3% NaCl	y = 23375x-45470	5-50	0.9991	0.76	2.31
AMB HCl	Water	y = 30111x-23565	5-50	0.9998	0.35	1.06
	HCl pH 1.2	y = 29666x-1874.1	5-50	0.9998	0.37	1.12
	PBS pH 6.8	y = 29721x-12307	5-50	0.9999	0.24	0.73
	0.45% NaCl	y = 30062x-12843	5-50	0.9994	0.65	1.98
	0.9% NaCl	y = 30877x-10763	5-50	0.9999	0.32	0.96
	3% NaCl	y = 30311x+2903.6	5-50	0.9999	0.28	0.86

Table A3 Linearity parameters and LOD and LOQ of model drugs in different solvents (cont.).

Compounds	Solvents	Linear equation	Test range (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)
PAR	Water	y = 87916x-26406	5-50	0.9998	0.38	1.15
	HCl pH 1.2	y = 89113x-27499	5-50	0.9998	0.35	1.06
	PBS pH 6.8	y = 88786x+71826	5-50	0.9995	0.57	1.74
	0.45% NaCl	y = 89165x-43262	5-50	0.9994	0.64	1.93
	0.9% NaCl	y = 89053x+31193	5-50	0.9992	0.69	2.09
	3% NaCl	y = 89671x-54305	5-50	0.9993	0.65	1.97
	SGF	y = 85952x+19362	5-50	0.9998	1.32	3.99
	SIF	y = 88703x+9585.2	5-50	0.9998	0.34	1.03

Table A4 Precision and accuracy results of DIL HCl.

Solvents	Conc. (µg/mL)	Precision (%RSD)		Spike amount (µg/mL)	Accuracy
		Intraday	Inter-day		Recovery (%)
Water	10	0.52	0.84	10	100.93±1.26
	20	0.99	3.14	20	100.81±1.19
	40	0.37	3.04	40	96.72±0.20
HCl pH 1.2	10	0.95	1.65	10	108.82±0.87
	20	0.31	0.66	20	103.73±1.50
	40	0.42	0.90	40	105.85±0.23
PBS pH 6.8	10	1.85	2.41	10	100.08±0.20
	20	0.44	1.95	20	100.39±0.35
	40	0.21	2.59	40	99.42±0.32
0.45% NaCl	10	1.61	4.20	10	109.58±1.01
	20	0.27	2.61	20	105.27±0.22
	40	0.36	0.48	40	104.10±0.40
0.9% NaCl	10	0.43	1.18	10	102.95±0.83
	20	1.14	0.78	20	103.28±1.27
	40	0.36	0.63	40	104.52±0.47
3% NaCl	10	0.36	2.98	10	95.39±1.23
	20	0.15	0.46	20	102.63±0.81
	40	0.24	0.67	40	102.38±0.71
SGF	10	0.58	2.77	10	107.19±0.19
	20	0.34	2.79	20	102.42±0.29
	40	0.10	2.43	40	100.22±0.05
SIF	10	0.44	2.31	10	91.46±1.63
	20	0.43	2.72	20	94.33±0.61
	40	0.30	2.18	40	96.16±0.62

Table A5 Precision and accuracy results of PRO HCl.

Solvents	Conc. (µg/mL)	Precision (%RSD)		Spike amount (µg/mL)	Accuracy
		Intraday	Inter-day		Recovery (%)
Water	10	0.88	3.40	10	99.70±1.39
	20	0.44	4.50	20	98.13±1.12
	40	0.40	3.69	40	99.67±0.99
HCl pH 1.2	10	0.99	0.79	10	103.38±1.82
	20	0.42	0.53	20	99.34±0.69
	40	0.24	2.38	40	104.27±0.43
PBS pH 6.8	10	0.11	2.41	10	96.61±0.70
	20	0.30	1.29	20	99.54±0.44
	40	0.33	3.96	40	102.70±0.24
0.45% NaCl	10	0.66	1.56	10	104.77±0.08
	20	0.06	0.44	20	97.14±0.13
	40	0.96	2.04	40	99.45±0.16
0.9% NaCl	10	0.23	1.05	10	96.10±0.93
	20	0.17	1.70	20	96.77±0.38
	40	0.31	3.65	40	96.36±1.59
3% NaCl	10	0.30	1.51	10	97.46±0.31
	20	0.30	1.42	20	99.31±0.57
	40	0.22	4.80	40	101.73±0.26

Table A6 Precision and accuracy results of AMB HCl.

Solvents	Conc. (µg/mL)	Precision (%RSD)		Spike amount (µg/mL)	Accuracy
		Intraday	Inter-day		Recovery (%)
Water	10	0.51	2.99	10	102.76±0.65
	20	0.12	1.67	20	96.03±0.23
	40	0.46	1.39	40	95.94±0.14
HCl pH 1.2	10	0.81	0.79	10	99.57±0.32
	20	0.46	1.06	20	105.64±0.14
	40	0.19	1.60	40	104.12±0.01
PBS pH 6.8	10	0.95	3.09	10	101.79±1.29
	20	0.65	0.69	20	93.74±0.27
	40	0.37	0.28	40	97.46±0.39
0.45% NaCl	10	0.91	2.42	10	98.07±1.03
	20	0.51	1.29	20	98.23±0.74
	40	0.77	1.32	40	97.54±0.41
0.9% NaCl	10	0.73	1.05	10	98.29±0.56
	20	0.27	0.62	20	99.70±0.23
	40	0.28	0.65	40	98.91±0.54
3% NaCl	10	0.99	0.85	10	93.22±0.05
	20	1.01	0.99	20	97.78±0.60
	40	0.28	0.61	40	99.05±0.36

Table A7 Precision and accuracy results of PAR.

Solvents	Conc. (µg/mL)	Precision (%RSD)		Spike amount (µg/mL)	Accuracy
		Intraday	Inter-day		Recovery (%)
Water	10	0.20	3.03	10	101.09±0.19
	20	0.06	2.58	20	106.49±0.05
	40	0.08	2.39	40	105.27±0.08
HCl pH 1.2	10	0.10	2.33	10	103.71±0.18
	20	0.04	1.73	20	107.13±0.13
	40	0.13	0.58	40	105.78±0.05
PBS pH 6.8	10	0.21	4.54	10	102.66±1.24
	20	0.09	4.21	20	105.52±0.24
	40	0.05	2.52	40	104.61±0.10
0.45% NaCl	10	0.31	3.50	10	104.17±0.43
	20	0.08	3.18	20	109.82±0.12
	40	0.17	1.81	40	107.38±0.05
0.9% NaCl	10	0.48	2.15	10	99.87±0.35
	20	0.17	1.86	20	105.81±0.21
	40	0.09	1.74	40	104.75±0.04
3% NaCl	10	0.06	2.87	10	106.14±0.24
	20	0.05	0.41	20	109.39±0.05
	40	0.04	0.19	40	106.61±0.05
SGF	10	0.06	0.15	10	106.07±0.09
	20	0.08	1.63	20	99.04±0.04
	40	0.02	0.87	40	97.93±0.04
SIF	10	0.02	0.19	10	97.23±0.86
	20	0.24	0.34	20	97.25±0.37
	40	0.12	0.08	40	94.46±0.51

Table A8 System suitability (20 µg/mL model drug, n=6)

Compounds	Solvents	Peak area		Retention time (min)		Theoretical plates (USP)		Asymmetry	
		Mean±SD	%RSD	Mean±SD	%RSD	Mean±SD	%RSD	Mean±SD	%RSD
DIL HCl	Water	1184782±9986	0.84	5.591±0.006	0.12	7843±73	0.93	1.103±0.022	2.00
	HCl pH 1.2	1228677±4638	0.38	5.428±0.002	0.05	7336±49	0.66	1.106±0.006	0.57
	PBS pH 6.8	1225285±4433	0.36	5.562±0.022	0.39	7706±263	3.41	1.108±0.009	0.70
	0.45% NaCl	1163348±3071	0.26	5.612±0.004	0.09	7161±47	0.66	1.101±0.010	0.91
	0.9% NaCl	1251504±9852	0.79	5.588±0.002	0.04	6978±32	0.46	1.100±0.010	0.93
	3% NaCl	1259663±7645	0.61	5.554±0.017	0.31	7134±180	2.52	1.098±0.014	1.26
	SGF	1178648±4991	0.42	5.275±0.004	0.07	8130±28	0.34	1.079±0.017	1.58
	SIF	1118856±4013	0.36	5.315±0.004	0.07	8399±33	0.39	1.078±0.016	1.45
PRO HCl	Water	450247±1597	0.35	3.467±0.004	0.12	9717±158	1.62	1.184±0.030	2.52
	HCl pH 1.2	429829±4000	0.93	3.428±0.005	0.14	10154±195	1.92	1.172±0.036	3.05
	PBS pH 6.8	431063±1195	0.28	3.422±0.004	0.11	10284±81	0.79	1.166±0.033	2.90
	0.45% NaCl	435348±1699	0.39	3.435±0.004	0.11	10197±78	0.77	1.172±0.033	0.26
	0.9% NaCl	422803±1731	0.41	3.429±0.008	0.23	10317±99	0.96	1.158±0.032	2.77
	3% NaCl	402352±1579	0.39	3.430±0.003	0.10	10259±36	0.35	1.164±0.032	2.76
AMB HCl	Water	570839±2427	0.43	7.378±0.083	1.12	10227±275	2.68	1.126±0.012	1.07
	HCl pH 1.2	589336±4246	0.72	7.413±0.099	1.34	10011±249	2.48	1.122±0.015	1.30
	PBS pH 6.8	576235±3977	0.69	7.341±0.017	0.23	10395±330	3.18	1.115±0.008	0.75
	0.45% NaCl	607103±3187	0.52	7.317±0.008	0.11	10385±92	0.88	1.119±0.007	0.63
	0.9% NaCl	609697±3134	0.51	7.372±0.005	0.07	10744±71	0.66	1.099±0.014	1.25
	3% NaCl	608644±4980	0.82	7.378±0.035	0.48	10549±146	1.39	1.106±0.019	1.69

Table A8 System suitability (20 µg/mL model drug, n=6) (cont.).

Compounds	Solvents	Peak area		Retention time (min)		Theoretical plates (USP)		Asymmetry	
		Mean±SD	%RSD	Mean±SD	%RSD	Mean±SD	%RSD	Mean±SD	%RSD
PAR	Water	1730752±923	0.05	3.536±0.005	0.15	12452±16	0.13	1.090±0.023	2.09
	HCl pH 1.2	1734462±633	0.04	3.530±0.003	0.09	12524±60	0.48	1.078±0.025	2.30
	PBS pH 6.8	1806843±2010	0.11	3.529±0.003	0.09	12183±55	0.45	1.030±0.010	0.94
	0.45% NaCl	1793591±6558	0.37	3.532±0.002	0.07	12382±42	0.34	1.069±0.022	2.08
	0.9% NaCl	1811818±2652	0.15	3.532±0.002	0.07	12240±57	0.46	1.040±0.024	2.33
	3% NaCl	1696373±958	0.06	3.530±0.003	0.09	12429±40	0.33	1.089±0.021	1.92
	SGF	1739366±1159	0.07	3.486±0.005	0.14	12443±56	0.45	1.072±0.029	2.72
	SIF	1796299±4522	0.25	3.488±0.005	0.14	12307±79	0.64	1.061±0.025	2.32

APPENDIX B

STABILITY OF DILTIAZEM HYDROCHLORIDE UNDER ACIDIC CONDITION

Stability of DIL HCl under acidic condition

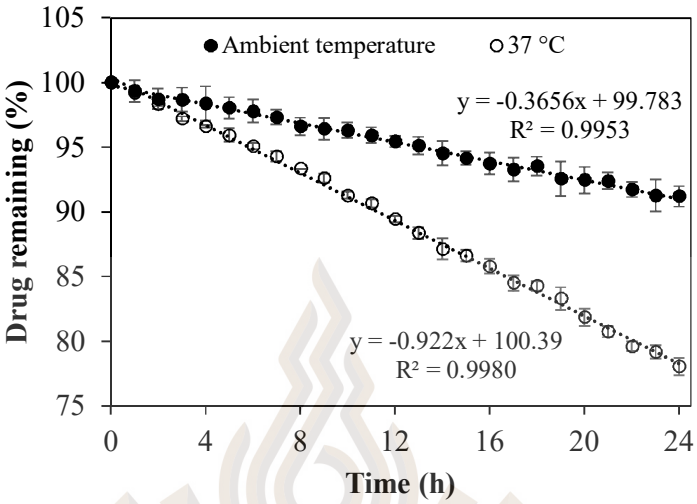


Figure C1 Stability of DIL HCl (40 µg/mL) under acidic condition.

APPENDIX C

PREPARATION OF TEST SOLUTIONS AND BUFFERS

Preparation of HCl pH 1.2

7 mL of 37% hydrochloric acid was transferred to 1000 mL volumetric flask and adjusted to the volume using water.

Preparation of PBS pH 6.8

0.2 M potassium dihydrogen phosphate was prepared by adding 6.81 g of potassium dihydrogen phosphate in a 250-mL volumetric flask, diluted with water and adjusted to the volume.

0.2 M sodium hydroxide was prepared by adding 2 g of sodium hydroxide in a 250-mL volumetric flask, diluted with water and adjusted to the volume.

PBS pH 6.8 was prepared by mixing 250 mL of 0.2 M potassium dihydrogen phosphate and 112 mL of 0.2 M sodium hydroxide. The approximately 630 mL water was added and mixed. The obtained solution was adjusted to pH 6.8 using 0.5 M hydrochloric acid. The solution was adjusted with water to 1000 mL.

Preparation of acetate buffer pH 4.5

2 M acetic acid was prepared by adding 11.4 mL glacial acetic acid in a 100-mL volumetric flask and adjusted with water to the volume.

The 2.99 g sodium acetate trihydrate was added to the 1000-mL volume metric flask. Water was added and mixed until sodium acetate trihydrate dissolved. The 14 mL 2 M acetic acid was added to the obtained solution and mixed. The mixture was adjusted to pH 4.5 using 0.5 M hydrochloric acid. The solution was adjusted with water to 1000 mL.

Preparation of SGF

2 g of sodium chloride and 3.2 g of purified pepsin derived from porcine stomach mucosa (with an activity of 800-2,500 units per mg of protein) were dissolved in 7 mL of hydrochloric acid. Then water was added and adjusted into 1000 mL. The solution has a pH of about 1.2.

Preparation of SIF

6.8 g of potassium dihydrogen phosphate was added into 250 mL of water and mixed. The 77 mL of 0.2 N sodium hydroxide and 500 mL of water were added into the obtain solution. Then, 10 g of pancreatin was added and mixed. The pH was adjusted to 6.8±0.1 using 0.2 N sodium hydroxide or 0.2 N hydrochloric acid. Finally, the volume was adjusted to 1000 mL.

BIOGRAPHY

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