

**Research Title** Preparation of *in-situ* forming gel system for controlled-release of periodontal antimicrobial agents  
**Researcher** Associate Professor Dr. Thawatchai Phaechamud and Assistant Professor Dr. Juree Charoenteeraboon  
**Office** Faculty of Pharmacy, Silpakorn University  
**Research Grants** Fiscal Budget of Year 2014  
Research and Development Institute, Silpakorn University  
**Year of Completion** 2015  
**Type of Research** Applied research  
**Subjects** Chemical Science and Pharmacy

### Abstract

This research was to investigate the parameters affecting the gel properties, drug release and antimicrobial activities of *in situ* forming gel systems to deliver antimicrobial agents in periodontitis treatment. The effect of variable parameters *i.e.* types and amount of polymer, drugs and hydrophilic/hydrophobic plasticizers on the gel properties were performed. The gel appearance, pH, viscosity, rheology, syringeability, gel formation, rate of water diffusion into the gels, degradation, drug release behavior and antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Streptococcus mutans* and *Porphyromonas gingivalis* were determined. An *in situ* forming gel system was prepared using *N*-methyl-2-pyrrolidone (NMP) as solvent. Each polymers, Ethocel, bleached shellac and Eudragit RS in different amounts were used to formulate the *in situ* forming gel systems. Increasing the amount of each polymer increased the viscosity and the work of syringeability. All *in situ* gel systems showed the Newtonian behavior. The gel formation of Ethocel and Eudragit RS systems after injected into PBS pH 6.8 showed the rigid gel, whereas the bleached shellac was elastic gel. *In situ* forming gel systems containing 5% w/w antimicrobial agents, doxycycline hyclate, metronidazole or benzoyl peroxide showed the antimicrobial activity against *P. gingivalis*. Increasing the amount of each polymer decreased the releasing of doxycycline hyclate. All systems showed the drug release by Fickian diffusion mechanism. For stability studies, the *in situ* gel system should be kept at 4°C since the gel appearance, gel properties and antimicrobial activity were unchanged as comparing to those of the initial. In the addition, a synergistic effect between doxycycline hyclate and metronidazole was obtained against *P. gingivalis*.

Keyword : *in situ* forming gel, antimicrobial agent, periodontitis

ชื่อโครงการ การเตรียมระบบเจลก่อตัวเองสำหรับควบคุมการปลดปล่อยยาที่ยับยั้งเชื้อโรคปริทันต์  
ชื่อผู้วิจัย รองศาสตราจารย์ ดร.รัชชชัย แพชมัด และ ผู้ช่วยศาสตราจารย์ ดร.จรัญ เจริญฐิรบูรณ์  
หน่วยงานที่สังกัด คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร  
แหล่งทุนอุดหนุนการวิจัย งบประมาณแผ่นดินประจำปี 2557  
สถาบันวิจัยและพัฒนา มหาวิทยาลัยศิลปากร

ปีที่เสร็จ พ.ศ. 2558

ประเภทการวิจัย การวิจัยประยุกต์  
สาขาวิชา วิทยาศาสตร์เคมีและเภสัช

#### บทคัดย่อ

การวิจัยนี้ศึกษาปัจจัยที่ส่งผลต่อคุณสมบัติของเจล การปลดปล่อยยา และฤทธิ์ในการยับยั้งเชื้อโรคของเจลก่อตัวเองสำหรับนำส่งยาที่ยับยั้งเชื้อโรคในการรักษาโรคปริทันต์อักเสบ โดยศึกษาผลของปัจจัยต่างๆ ได้แก่ ชนิดและปริมาณของพอลิเมอร์ ยา และสารเพิ่มความยืดหยุ่นชนิดที่ชอบน้ำและไม่ชอบน้ำ ต่อสมบัติต่างๆ ของเจลก่อตัวเอง ได้แก่ ลักษณะภายนอก ความเป็นกรดต่าง ความหนืด รูปแบบการไหล ความสามารถในการฉีด การเกิดเจล อัตราการแพร่ของน้ำสู่เจล การสลายตัว รูปแบบการปลดปล่อยยา และฤทธิ์ยับยั้งเชื้อจุลชีพต่อเชื้อสแตป-ไฟโลคอคคัสออเรียส เอสเชอริเชียโคไล แคนดิดาอัลบิแคนส์ สเตรปโตคอคคัสมิวแทนส์ และพอไฟโรโมแนสจิงจิวัลิส ระบบเจลก่อตัวเองเตรียมโดยใช้เอ็น-เมทิล-สอง-ไพโรลิโดน (เอ็นเอ็มพี) เป็นสารทำละลายพอลิเมอร์ที่ใช้เตรียมตำรับเจลก่อตัวเองคือ เอทโทเซล เซลแล็กฟอกขาว และยูตราจิต อาร์เอส จากผลการศึกษาพบว่าปริมาณของพอลิเมอร์แต่ละชนิดที่เพิ่มขึ้นทำให้ความหนืดและงานที่ใช้ในการฉีดเพิ่มขึ้น รูปแบบการไหลของตำรับเจลก่อตัวเองทั้งหมดเป็นแบบนิวโตเนียน เจลหลังการฉีดในสารละลายฟอสเฟตบัฟเฟอร์ พีเอช 6.8 ของเอทโทเซลและยูตราจิตมีลักษณะเป็นเจลที่แข็ง ส่วนเซลแล็ก ฟอกขาวเป็นเจลที่มีความยืดหยุ่น ตำรับเจลก่อตัวเองที่มียาที่ยับยั้งเชื้อโรคร้อยละ 5 โดยน้ำหนัก (ด็อกซีไซคลินไฮคลอต เมทโทรนิดาโซล และ เบนโซอิลเพอร์ออกไซด์) มีฤทธิ์ในการยับยั้งเชื้อก่อโรคปริทันต์อักเสบ (พอไฟโรโมแนสจิงจิวัลิส) ปริมาณของพอลิเมอร์แต่ละชนิดที่เพิ่มขึ้นทำให้การปลดปล่อยยาด็อกซีไซคลินไฮคลอตลดลง ซึ่งกลไกการปลดปล่อยยาเป็นการแพร่แบบ Fickian จากการศึกษาความคงตัวของตำรับได้ พบว่าตำรับเจลก่อตัวเองที่เก็บที่อุณหภูมิ 4°C เป็นเวลา 3 เดือน มีลักษณะภายนอก สมบัติของเจล และฤทธิ์ในการยับยั้งเชื้อโรคไม่แตกต่างจากก่อนการศึกษาความคงตัว ในการศึกษาเพิ่มเติมพบว่าการใช้ยาร่วมกันระหว่างยาด็อกซีไซคลินไฮคลอตและเมทโทรนิดาโซลให้ผลการเสริมฤทธิ์กันในการยับยั้งเชื้อพอไฟโรโมแนส จิงจิวัลิส

คำสำคัญ : ระบบเจลก่อตัวเอง/ยาที่ยับยั้งเชื้อโรค/โรคปริทันต์อักเสบ

## **ACKNOWLEDGEMENTS**

For this research work, the author wishes to express sincere appreciation to every people who contributed in diverse ways to the completion of this research. I would like to especially thank Assoc. Prof. Dr. Jintakorn Kuvatanasuchate, Department of Microbiology, Faculty of Dentistry, Chulalongkorn University for supporting the anaerobic bacteria in periodontitis diseases (*Porphyromonas gingivalis* ATCC 33277). I am grateful to Faculty of Pharmacy, Graduate School, and Research and Development Institute, Silpakorn University. A big thank you goes to Miss Jongjan Mahadlek, Miss Kotchamon Yodkhum and Miss Orn Setthajindalert for their helpful assistance.

## TABLE OF CONTENTS

	Page
English Abstract .....	iv
Thai Abstract .....	v
Acknowledgments .....	vi
List of Tables .....	viii
List of Figures.....	xiii
List of Abbreviations.....	xxii
Chapter	
1    Introduction .....	1
2    Literature Reviews.....	7
3    Materials and Methods .....	40
4    Results and Discussion.....	56
5    Conclusions .....	187
References .....	190
Appendices .....	207
Output.....	217
Biography .....	218

## LIST OF TABLES

Tables	Page
1 Summary of some investigated intra-pocket delivery systems for antimicrobial agents.....	15
2 Interpretation of diffusional release mechanism from drug release data from thin polymer film.....	36
3 Diffusional exponent and mechanisms of diffusional release from various non-swellable controlled release systems.....	37
4 Diffusional exponent and mechanisms of drug from various swellable controlled release systems.....	37
5 Model files used with Scientist <sup>®</sup> .....	39
6 Composition formula of the gels containing different types and amounts of polymer in <i>N</i> -methyl-2-pyrrolidone.....	46
7 Composition formula of the gels containing different types of drugs (5% w/w).....	50
8 Composition formula of Eudragit RS systems containing different amounts of plasticizers (polyethylene glycol 1500 and peppermint oil) without and with doxycycline hyclate (5% w/w)...	54
9 Minimal inhibitory concentrations (MICs) of antimicrobial agents against <i>S. aureus</i> ATCC 6538P, <i>E. coli</i> ATCC 25922, <i>C. albicans</i> ATCC 17110, <i>S. mutans</i> ATCC 27175 and <i>P. gingivalis</i> ATCC 33277 determined by broth microdilution method.....	58
10 Fractional inhibitory concentration (FIC) index values of the combinations.....	58
11 Gel appearance and pH value of various formula (Ethocel, bleached shellac and Eudragit RS).....	59

Tables	Page
12 Effect of polymers concentrations and temperature on flow parameters	68
13 Effect of the concentration of polymers (Ethocel, bleached shellac and Eudragit RS) on the work of syringeability.....	69
14 Effect of polymer amounts in the systems on rate of water diffusion into gels.....	73
15 pH values of Ethocel, bleached shellac and Eudragit RS systems containing various drugs (5% w/w) .....	74
16 Flow parameters of Ethocel formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) at 25°C and 37°C.....	95
17 Flow parameters of bleached shellac formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) at 25°C and 37°C.....	95
18 Flow parameters of Eudragit RS formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) at 25°C and 37°C.....	96
19 Syringeability of Ethocel, bleached shellac and Eudragit RS formula containing different drugs (doxycycline hyclate, metronidazole and benzoyl peroxide).....	97
20 Effect of polymer amounts in the formula containing different drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) on rate of water diffusion into gels.....	107
21 Comparison of degree of goodness-of-fit from curve fitting of the release profiles of doxycycline hyclate in phosphate buffer pH 6.8 using dialysis membrane method to different release models.....	118
22 Estimate parameter from curve fitting of doxycycline hyclate release in phosphate buffer pH 6.8 using dialysis membrane method to power law expression.....	118

Tables	Page
23 Comparison of degree of goodness-of-fit from curve fitting of the release profiles of doxycycline hyclate in phosphate buffer pH 6.8 using membrane-less method to different release models.....	119
24 Estimate parameter from curve fitting of doxycycline hyclate release in phosphate buffer pH 6.8 using membrane-less method to power law expression.....	119
25 The percentage of weight loss of formula containing different drugs (doxycycline hyclate, metronidazole and benzoyl peroxide).....	123
26 Gel appearance and pH value of 35% w/w Eudragit RS formula containing different amounts of polyethylene glycol 1500 and without and with doxycycline hyclate (5% w/w).....	130
27 Flow parameters of Eudragit RS (35% w/w) formula containing different amount of polyethylene glycol 1500 without and with doxycycline hyclate (5% w/w) at 25°C and 37°C.....	136
28 Syringeability of Eudragit RS (35% w/w) formula without and with 5% w/w doxycycline hyclate containing different amount of polyethylene glycol 1500.....	137
29 Effect of polyethylene glycol 1500 amount in the 35% w/w Eudragit RS formula without and with doxycycline hyclate (5% w/w) on rate of water diffusion into gels.....	137
30 Comparison of degree of goodness-of-fit from curve fitting of the release profiles of doxycycline hyclate from 35% w/w Eudragit RS formula containing different amounts of polyethylene glycol 1500 (PEG1500) (2.5-10% w/w) in phosphate buffer pH 6.8 using dialysis membrane and membrane-less method to different release models.....	143

Tables	Page
31 Estimate parameter from curve fitting of doxycycline hyclate release from 35%w/w Eudragit RS formula containing different amounts of polyethylene glycol 1500 (PEG1500) (2.5-10%w/w) in phosphate buffer pH 6.8 using dialysis membrane and membrane-less method to power law expression.....	142
32 The percentage of weight loss of 35%w/w Eudragit RS formula containing different amounts of polyethylene glycol 1500 without and with 5%w/w doxycycline hyclate.....	145
33 Gel appearance and pH value of 35%w/w Eudragit RS formula containing different amounts of peppermint oil and without and with doxycycline hyclate (5%w/w).....	149
34 Flow parameters of Eudragit RS (35% w/w) formula containing different amount of peppermint oil without and with doxycycline hyclate (5%w/w) at 25°C and 37°C.....	154
35 Syringeability of Eudragit RS (35% w/w) formula with and without doxycycline hyclate containing different amounts of peppermint oil (2.5%, 5%, 7.5% and 10%w/w).....	156
36 Effect of peppermint oil amount in the 35%w/w Eudragit RS formula without and with doxycycline hyclate (5%w/w) on rate of water diffusion into gels.....	157
37 Comparison of degree of goodness-of-fit from curve fitting of the release profiles of doxycycline hyclate from 35%w/w Eudragit RS formula containing different amounts of peppermint oil (PO) (2.5-10%w/w) in phosphate buffer pH 6.8 using dialysis membrane and membrane-less method to different release models.....	162
38 Estimate parameter from curve fitting of doxycycline hyclate release from 35%w/w Eudragit RS formula containing different amounts of peppermint oil (PO) (2.5-10%w/w) in phosphate buffer pH 6.8 using dialysis membrane and membrane-less method to power law expression.....	162

Tables	Page
39 The percentage of weight loss of 35%w/w Eudragit RS formula containing different amounts of peppermint oil without and with 5% w/w doxycycline hyclate.....	164
40 Flow parameters, syringeability and antimicrobial activity against <i>S. mutans</i> of of Eudragit RS (35%w/w) formula containing different amount of peppermint oil and polyethylene glycol 1500 without and with benzoyl peroxide (5%w/w).....	168
41 Comparison of degree of goodness-of-fit from curve fitting of drug release from various systems.....	169
42 The pH values of various systems after stability test (3 months) at 4°C, 25°C and 45°C.....	171
43 Flow parameters of Eudragit RS (35%w/w) formula containing polyethylene glycol 1500 and peppermint oil and with and without doxycycline hyclate after stability test (3 months) at 4°C.....	178
44 Flow parameters of Eudragit RS (35%w/w) formula containing polyethylene glycol 1500 and peppermint oil and with and without doxycycline hyclate after stability test (3 months) at 25°C.....	178
45 Flow parameters of Eudragit RS (35%w/w) formula containing polyethylene glycol 1500 and peppermint oil and with and without doxycycline hyclate after stability test (3 months) at 45°C.....	179
46 Comparison of degree of goodness-of-fit from curve fitting of the release profiles of doxycycline hyclate in phosphate buffer pH 6.8 after stability test (3 months) at 4°C, 25°C and 45°C using dialysis membrane method to different release models.....	184
47 Estimate parameter from curve fitting of doxycycline hyclate release in phosphate buffer pH 6.8 after stability test (3 months) at 4°C, 25°C and 45°C using dialysis membrane method to different release models.....	185

## LIST OF FIGURES

Figure		Page
1	Chemical structure of ethylcellulose.....	19
2	Chemical structure of Eudragit RS.....	20
3	Chemical structure of <i>N</i> -methyl-2-pyrrolidone.....	20
4	Chemical structure of doxycycline hyclate.....	22
5	Chemical structure of metronidazole.....	23
6	Chemical structure of benzoyl peroxide. ....	24
7	Chemical structure of polyethylene glycol.....	26
8	Checkerboard techniques.....	29
9	Results of checkerboard represented as isobolograms.....	30
10	Flow behaviors.....	31
11	Viscosity and time curves of thixotropy and rheopexy.....	32
12	Zero-order, first-order and square-root time release patterns from devices containing the same initial active agent content.....	33
13	Viscosity curves of Ethocel formula at (A) 25°C and (B) 37°C.....	61
14	Viscosity curves of bleached shellac formula at (A) 25°C and (B) 37°C	62
15	Viscosity curves of Eudragit RS formula at (A) 25°C and (B) 37°C.....	63
16	Flow curve of Ethocel formula containing different amount of Ethocel at (A) 25°C and (B) 37°C.....	65
17	Flow curve of bleached shellac formula containing different amount of bleached shellac at (A) 25°C and (B) 37°C.....	66
18	Flow curve of Eudragit RS formula containing different amount of Eudragit RS at (A) 25°C and (B) 37°C.....	67

Figure	Page
19 <i>In vitro</i> gel formation of Ethocel formula (5-20% w/w) at various times (0, 1, 5 and 30 min).....	70
20 <i>In vitro</i> gel formation of bleached shellac formula (15-30% w/w) at various times (0, 1, 5 and 30 min).....	71
21 <i>In vitro</i> gel formation of Eudragit RS formula (15-35% w/w) at various times (0, 1, 5 and 30 min).....	72
22 Viscosity curves of Ethocel-5% w/w doxycycline hyclate formula at (A) 25°C and (B) 37°C.....	76
23 Viscosity curves of Ethocel-5% w/w metronidazole formula at (A) 25°C and (B) 37°C.....	77
24 Viscosity curves of Ethocel-5% w/w benzoyl peroxide formula at (A) 25°C and (B) 37°C.....	78
25 Viscosity curves of bleached shellac-5% w/w doxycycline hyclate formula at (A) 25°C and (B) 37°C.....	79
26 Viscosity curves of bleached shellac-5% w/w metronidazole formula at (A) 25°C and (B) 37°C.....	80
27 Viscosity curves of bleached shellac-5% w/w benzoyl peroxide formula at (A) 25°C and (B) 37°C.....	81
28 Viscosity curves of Eudragit RS-5% w/w doxycycline hyclate formula at (A) 25°C and (B) 37°C.....	82
29 Viscosity curves of Eudragit RS-5% w/w metronidazole formula at (A) 25°C and (B) 37°C.....	83
30 Viscosity curves of Eudragit RS-5% w/w benzoyl peroxide formula at (A) 25°C and (B) 37°C.....	84
31 Flow curve of Ethocel formula containing 5% w/w doxycycline hyclate at (A) 25°C and (B) 37°C.....	86
32 Flow curve of Ethocel formula containing 5% w/w metronidazole at (A) 25°C and (B) 37°C.....	87

Figure	Page
33 Flow curve of Ethocel formula containing 5% w/w benzoyl peroxide (A) 25°C and (B) 37°C.....	88
34 Flow curve of bleached shellac formula containing 5% w/w doxycycline hyclate at (A) 25°C and (B) 37°C.....	89
35 Flow curve of bleached shellac formula containing 5% w/w metronidazole at (A) 25°C and (B) 37°C.....	90
36 Flow curve of bleached shellac formula containing 5% w/w benzoyl peroxide at (A) 25°C and (B) 37°C.....	91
37 Flow curve of Eudragit RS formula containing 5% w/w doxycycline hyclate at (A) 25°C and (B) 37°C.....	92
38 Flow curve of Eudragit RS formula containing 5% w/w metronidazole at (A) 25°C and (B) 37°C.....	93
39 Flow curve of Eudragit RS formula containing 5% w/w benzoyl peroxide at (A) 25°C and (B) 37°C.....	94
40 <i>In vitro</i> gel formation of Ethocel formula (5-20% w/w) containing 5% w/w doxycycline hyclate at various times (0, 1, 5 and 30 min).....	98
41 <i>In vitro</i> gel formation of Ethocel formula (5-20% w/w) containing 5% w/w metronidazole at various times (0, 1, 5 and 30 min).....	99
42 <i>In vitro</i> gel formation of Ethocel formula (5-20% w/w) containing 5% w/w benzoyl peroxide at various times (0, 1, 5 and 30 min).....	100
43 <i>In vitro</i> gel formation of bleached shellac formula (15-30% w/w) containing 5% w/w doxycycline hyclate at various times (0, 1, 5 and 30 min).....	101
44 <i>In vitro</i> gel formation of bleached shellac formula (15-30% w/w) containing 5% w/w metronidazole at various times (0, 1, 5 and 30 min).....	102

Figure	Page
45 <i>In vitro</i> gel formation of bleached shellac formula (15-30% w/w) containing 5% w/w benzoyl peroxide at various times (0, 1, 5 and 30 min).....	103
46 <i>In vitro</i> gel formation of Eudragit RS formula (15-35% w/w) containing 5% w/w doxycycline hyclate at various times (0, 1, 5 and 30 min).....	104
47 <i>In vitro</i> gel formation of Eudragit RS formula (15-35% w/w) containing 5% w/w metronidazole at various times (0, 1, 5 and 30 min).....	105
48 <i>In vitro</i> gel formation of Eudragit RS formula (15-35% w/w) containing 5% w/w benzoyl peroxide at various times (0, 1, 5 and 30 min).....	106
49 Effect of Ethocel amount on release of doxycycline hyclate using dialysis method.....	110
50 Effect of bleached shellac amount on release of doxycycline hyclate using dialysis method.....	110
51 Effect of Eudragit RS amount on release of doxycycline hyclate using dialysis method.....	111
52 Effect of Eudragit RS amount on release of (A) metronidazole and (B) benzoyl peroxide using dialysis method.....	112
53 Effect of Ethocel amount on release of doxycycline hyclate using membrane-less method.....	114
54 Effect of bleached shellac amount on release of doxycycline hyclate using membrane-less method.....	114
55 Effect of Eudragit RS amount on release of doxycycline hyclate using membrane-less method.....	115

Figure	Page
56 SEM micrograph of the dried gel systems; Doxycycline hyclate (5%w/w)-Ethocel systems containing 5%w/w Ethocel (A); 10%w/w Ethocel (B); 15%w/w Ethocel (C) and 20%w/w Ethocel (D) with different magnifications.....	121
57 SEM micrograph of the dried gel systems; Doxycycline hyclate (5%w/w)-Eudragit RS systems containing 15%w/w Eudragit RS (A); 25%w/w Eudragit RS (B); 30%w/w Eudragit RS (C) and 35%w/w Eudragit RS (D) with different magnifications.....	122
58 Inhibition zone diameter of the Ethocel formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) against <i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>S. mutans</i> and <i>P. gingivalis</i> .....	126
59 Inhibition zone diameter of the bleached shellac formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) against <i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>S. mutans</i> and <i>P. gingivalis</i> .....	127
60 Inhibition zone diameter of the Eudragit RS formula containing different types of drugs (doxycycline hyclate, metronidazole and benzoyl peroxide) against <i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>S. mutans</i> and <i>P. gingivalis</i> .....	128
61 Viscosity curves of Eudragit RS formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w) at (A) 25°C and (B) 37°C.....	131
62 Viscosity curves of Eudragit RS (35%w/w)-doxycycline hyclate (5%w/w) formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w) at (A) 25°C and (B) 37°C.....	132

Figure	Page	
63	Flow curve of Eudragit RS formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w) at (A) 25°C and (B) 37°C.....	134
64	Flow curve of Eudragit RS (35%w/w)-doxycycline hyclate (5%w/w) formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w).....	135
65	<i>In vitro</i> gel formation of Eudragit RS (35%w/w) formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w) at various times (0, 1, 5 and 30 min).....	138
66	<i>In vitro</i> gel formation of Eudragit RS (35%w/w) -doxycycline hyclate (5%w/w) formula containing different amount of polyethylene glycol 1500 (2.5%, 5%, 7.5% and 10%w/w) at various times (0, 1, 5 and 30 min).....	139
67	Effect of polyethylene glycol 1500 amount in 35%w/w Eudragit RS formula on release of doxycycline hyclate using dialysis method....	142
68	Effect of polyethylene glycol 1500 amount in 35%w/w Eudragit RS formula on release of doxycycline hyclate using membrane-less method.....	142
69	SEM micrograph of the dried gel systems; Doxycycline hyclate (5%w/w)-Eudragit RS (35%w/w) systems containing 2.5%w/w PEG1500 (A); 5%w/w PEG1500 (B); 7.5%w/w PEG1500 (C) and 10%w/w PEG1500 (D) with different magnifications.....	146
70	Inhibition zone diameter of the Eudragit RS (35%w/w)-doxycycline hyclate (5%w/w) formula containing different amounts of polyethylene glycol 1500 against <i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>S. mutans</i> and <i>P. gingivalis</i> .....	147
71	Viscosity curves of Eudragit RS (35%w/w) formula containing different amount of peppermint oil (2.5%, 5%, 7.5% and 10%w/w) at (A) 25°C and (B) 37°C.....	150

Figure	Page
72	151
73	152
74	153
75	156
76	157
77	160
78	160
79	163
80	165

Figure	Page
81 Release profiles of benzoyl peroxide in the Eudragit RS systems containing different amounts of (A) peppermint oil and (B) polyethylene glycol 1500.....	167
82 Appearance of gel formed in phosphate buffer pH 6.8; (A) 35 %w/w ERS gels, (B) 35 %w/w ERS gels containing BP (5 %w/w), (C) 35 %w/w ERS gels containing BP (5 %w/w) and PO (5 %w/w), and (D) 35 %w/w ERS gels containing BP (5 %w/w) and PEG1500 (2.5 %w/w).....	169
83 Viscosity curves of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 4°C at (A) 25°C and (B) 37°C.....	172
84 Viscosity curves of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 25°C at (A) 25°C and (B) 37°C.....	173
85 Viscosity curves of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 45°C at (A) 25°C and (B) 37°C.....	174
86 Flow curve of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 4°C at (A) 25°C and (B) 37°C.....	175
87 Flow curve of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 25°C at (A) 25°C and (B) 37°C.....	176
88 Flow curve of 35%w/w Eudragit RS formula containing polyethylene glycol, peppermint oil and doxycycline hyclate after stability test (3 months) at 45°C at (A) 25°C and (B) 37°C.....	177
89 Release profiles of the 35%w/w Eudragit RS - doxycycline hyclate (5%w/w) systems after stability test (3 months) at 4°C, 25°C and 45°C.....	182

Figure	Page	
90	Release profiles of the 35%w/w Eudragit RS - doxycycline hyclate (5%w/w) systems containing polyethylene glycol 1500 (5%w/w) after stability test (3 months) at 4°C, 25°C and 45°C.....	183
91	Release profiles of the 35%w/w Eudragit RS - doxycycline hyclate (5%w/w) systems containing peppermint oil (5%w/w) after stability test (3 months) at 4°C, 25°C and 45°C.....	183
92	Inhibition zone diameter of the Eudragit RS formula containing polyethylene glycol 1500 and peppermint oil against <i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>S. mutans</i> and <i>P. gingivalis</i> after stability test (3months) at 4°C, 25°C and 45°C.....	186
93	Standard curve of doxycycline hyclate in phosphate buffer pH 6.8 for the <i>in vitro</i> release study.....	209
94	Standard curve of metronidazole in phosphate buffer pH 6.8 for the <i>in vitro</i> release study.....	210
95	Standard curve of benzoyl peroxide in phosphate buffer pH 6.8: ethanol (5:2) for the <i>in vitro</i> release study.....	211
96	Calibration curve of <i>Staphylococcus aureus</i> ATCC 6853P.....	212
97	Calibration curve of <i>Escherichia coli</i> ATCC 25922.....	213
98	Calibration curve of <i>Candida albicans</i> ATCC 17110.....	214
99	Calibration curve of <i>Streptococcus mutans</i> ATCC 27175.....	215
100	Calibration curve of <i>Porphyrommonas gingivalis</i> ATCC 33277.....	216

## LIST OF ABBREVIATIONS

% w/w	percent weight by weight
°C	degree Celsius
µg	microgram(s)
µL	microliter(s)
µm	micrometer(s)
cfu/mL	colony forming unit/milliliter
cm	centimeter(s)
Conc.	concentration
D/cm <sup>2</sup>	Dyne/square centimeter
e.g.	(Latin); for example
Eq.	equation
<i>et al.</i>	and others
etc.	et cetera (Latin); and other things/ and so forth
G	(needle) gauge
g	gram(s)
h	hour(s)
L	liter(s)
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
msc	model selection criterion
MW	molecular weight
N	newton
nm	nanometer(s)
PBS	phosphate buffer solution
pH	potentia hydrogenii (Latin); power of hydrogen
R <sup>2</sup>	coefficient of determination
rpm	revolutions per minute
s	sec(s)
SEM	scanning electron microscope
S.D.	standard deviation

