การพัฒนาสูตรตำรับเจลพื้นสำหรับใช้ในร่องลึกปริทันต์ ชนิดเพื่อควบคุมการปลดปล่อยยาเมโทรนิดาโซล สำหรับผู้ป่วยโรคปริทันต์อักเสบ

นายอลงกต แสงจันทร์ฉาย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชอุตสาหกรรม ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

FORMULATION DEVELOPMENT OF PERIODONTAL GEL BASE TO CONTROL METRONIDAZOLE RELEASE FOR PERIODONTITIS PATIENTS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy
Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2008

Thesis Title	FORMULATION DEVELOPMENT OF PERIODONTAL GEL BASE TO CONTROL METRONIDAZOLE RELEASE FOR PERIODONTITIS PATIENTS
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อลงกต แสงจันทร์ฉาย : การพัฒนาสูตรตำรับเจลพื้นสำหรับใช้ในร่องลึกปริทันต์ ชนิดเพื่อควบคุมการปลดปล่อยยาเมโทรนิดาโซล สำหรับผู้ป่วยโรคปริทันต์อักเสบ. (FORMULATION DEVELOPMENT OF PERIODONTAL GEL BASE TO CONTROL METRONIDAZOLE RELEASE FOR PERIODONTITIS PATIENTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร.กาญจน์พิมล ฤทธิเดช, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ทพ. ดร.กิตติ ต.รุ่งเรือง, 187 หน้า.

โดยทั่วไป ผู้ป่วยที่เป็นโรคปริทันต์อักเสบจะรับประทานยาฆ่าเชื้อแบคทีเรีย โดยยาที่ใช้รักษา มีผลข้างเคียงต่างๆ ระดับความเข้มข้นยาต่ำในร่องลึกปริทันต์ มีโอกาสติดเชื้อแทรกซ้อนได้จากการรับประทานยาฆ่าเชื้อแบคทีเรียนานๆและการดื้อต่อยา ซึ่งวัตถุประสงค์ของการศึกษาวิจัยครั้งนี้ เพื่อพัฒนาระบบนำส่งยาเมโทรนิดาโซล ซึ่งเป็นยาฆ่าเชื้อแบคทีเรียชนิดที่ไม่ใช้ออกซิเจนตรงบริเวณร่องลึกปริทันต์ให้ออกฤทธิ์นาน โดยในการศึกษาเบื้องต้น ได้เตรียมสูตรตำรับเจลพื้นจากโพลิเมอร์ชนิดที่ชอบน้ำ ได้แก่ carbopol 940 hydroxylethyl cellulose hydroxylpropylmethyl cellulose polyvinyl alcohol polyvinylpyrrolidone sodiumcarboxymethyl cellulose และ poloxamer 407 โพลิเมอร์และสารประกอบอื่นที่ไม่ชอบน้ำ ได้แก่ ethylcellulose Eudragit® RS Eudragit® RL polyethylene Aerosil® white soft paraffin glyceryl monostearate stearic acid isopropyl myristate และ mineral oil และสูตรตำรับที่มีทั้งโพลิเมอร์และสารประกอบอื่นที่ชอบและไม่ชอบน้ำ จากนั้นนำแต่ละสูตรตำรับมาทดสอบลักษณะทางกายภาพ ได้แก่ ความหนืด ความสามารถในการฉีดผ่านเข็ม ความสามารถในการแผ่กระจายภายใต้แรงกด การทดสอบการหายไปของเจล เวลาและแรงที่ใช้ในการติดกับผิวลำไส้เล็กของหมู และการทดสอบในผู้ป่วย จากการศึกษาพบว่า การเพิ่มปริมาณของโพลิเมอร์ชนิดที่ชอบน้ำ ทำให้ความหนืดเพิ่มขึ้น ความสามารถในการฉีดผ่านเข็มและการแผ่กระจายลดลง การติดกับผิวลำไส้เล็กของสูตรตำรับที่มีโพลิเมอร์ที่ชอบน้ำมากกว่าสูตรตำรับที่มีโพลิเมอร์ที่ไม่ชอบน้ำผสมอยู่ การหายไปของเจลพื้นชนิดที่ชอบน้ำ เกิดจากการละลายของโพลิเมอร์ ซึ่งเกี่ยวเนื่องจากการพองตัวและกร่อนออกไปของเจล จากนั้นนำเจลไปทดสอบกับผู้ป่วยที่มีล่องลึกปริทันต์มากกว่า 3 มิลลิเมตร โดยพิจารณาจากสูตรตำรับที่มีลักษณะน่าใช้ เช่น ความหนืดต่ำและสามารถผ่านเข็มได้ดี เป็นต้น โดยในระหว่างใส่เจลที่ผสมตัวยาเมโทรนิดาโซล 5 เปอร์เซ็นต์ลงไปในบริเวณร่องลึกปริทันต์ พบว่ามีการไหลออกของเลือดตลอดเวลาทำให้เจลทุกสูตรตำรับหลุดออกจากร่องลึกปริทันต์ ยกเว้นสูตรตำรับยาเตรียมที่ผสมระหว่างโพลเมอร์ที่ชอบน้ำ และไม่ชอบน้ำกับสารประกอบไฮโดรคาร์บอน(ระบบ 3-3)สามารถคงอยู่ในร่องลึกปริทันต์ได้นานมากกว่า 24 ชั่วโมง จากนั้นนำระบบดังกล่าวนี้ ไปทำการทดสอบโดยการเพิ่มขึ้นของปริมาณยาในสูตรตำรับ, การทดสอบกับเชื้อจุลินทรีย์, การปลดปล่อยยาและการทดสอบความคงตัว หลังจากการทดสอบพบว่า การเพิ่มขึ้นของผลึกผงยาที่ละลายน้ำได้น้อยในตำรับ และอัดกันอย่างหนาแน่นของผลึกผงยาทำให้การปลดปล่อยยาลดลง โดยสูตรตำรับเจลพื้นที่เตรียมขึ้นทั้งหมด จะสูญเสียความคงตัวในการทดสอบการเก็บในอุณหภูมิห้อง, สภาวะการเก็บ Thai-FDA stability test (45°C และ 75%RH) และในสภาวะ 6 Freeze-thaw cycles แต่มีความคงตัวดีในการเก็บในอุณหภูมิตู้เย็น

ภาควิชา.....วิทยาการเภสัชกรรม..... ลายมือชื่อนิสิต.....

.....และเภสัชอุตสาหกรรม.....

สาขาวิชา.....เภสัชอุตสาหกรรม..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์.....

ปีการศึกษา....2551..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4976609833: MAJOR INDUSTRIAL PHARMACY
KEYWORDS: PERIODONTAL/ GEL BASE/ METRONIDAZOLE/ RELEASE PERIODONTITIS PATIENTS

ALONGKOT SANGCHANCHAI: FORMULATION DEVELOPMENT OF PERIODONTAL GEL BASE TO CONTROL METRONIDAZOLE RELEASE FOR PERIODONTITIS PATIENTS. ADVISOR: PROF. GARNPIMOL C. RITTHIDEJ, Ph.D., CO-ADVISOR: ASSIST. PROF. KITTI TORRUNGRUANG, Ph.D., 187 pp.

Generally, antimicrobial agents are orally administered to periodontitis patients. This could induce some side effects, poor local concentrations of drug, superinfections and bacterial resistance. The purpose of this study was to develop a localized drug delivery system that offered prolonged administration of metronidazole, an anaerobe antimicrobial agent. In preliminary study, various components were used to prepare gel base system such as hydrophilic gel base including carbopol, hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose, polyvinyl alcohol, polyvinylpyrrolidone, sodium carboxy methyl cellulose and poloxamer 407, hydrophobic gel base including insoluble polymers (ethylcellulose, Eudragit® RS, Eudragit® RL), polyethylene, Aerosil® , hydrocarbon compounds (white soft paraffin), glyceryl monostearate, stearic acid, isopropyl myristate, mineral oil and the combination of hydrophilic and hydrophobic component, hydrophilic-hydrophobic gel base. Each formulation was characterized in terms of physical appearances, viscosity, syringeability, spreadability, disappearance property test, *ex vivo* mucoadhesion time, *ex vivo* mucoadhesion force and *in vivo* evaluation in patient. The viscosity was closely related with syringeability and spreadability. Increasing concentration of components especially hydrophilic polymers could present to increasing viscosity, decreasing syringeability and spreadability. The mucoadhesion on porcine intestinal mucosa of hydrophilic gel base was more than those form hydrophobic and hydrophilic- hydrophobic gel base system. Disappearance of hydrophilic gel base was polymers dissolution due to swelling erosion. The selected of periodontal gels which showed good application such as low viscosity and high syringeability. When selected of periodontal gel base systems containing 5% metronidazole were evaluated in patients with periodontal pocket deeper than 3 mm, highly or extremely bleeding was occurred. All selected gels were then removed by bleeding, except hydrophilic-hydrophobic gel based on insoluble polymers and hydrocarbon compound (system 3-3) which was remained for more than 24 hours. Afterward, this system was evaluated by increasing of drug concentration, microbial sensitivity test, drug release and stability test. The release rate of drug was decreased due to the dense of poor crystal drug solubility in the matrix of gel structure. All selected gels could not tolerate room temperature, Thai-FDA stability test (45°C and 75% RH) and 6 freeze-thaw cycles and but remained intact when storage at refrigerated temperature.

Department:……Pharmaceutics and…… Student's Signature: ….……..…….
.....Industrial Pharmacy…..

Field of Study:….Industrial Pharmacy….. Advisor's Signature: ……..…...…….

Academic Year:..2008……..... Co-Advisor's Signature: …….

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my thesis advisor, Professor Dr. Garnpimol C. Rittidej for her invaluable advice, problem solving, encouragement and understanding. I could not succeed in my thesis and made it a valuable research work without her support, I truly feel grateful to her.

My deep appreciation is to Assistant Professor Dr. Kitti Torrungruang, my thesis co-advisor, Faculty of Dentistry, Chulalongkorn University for spending his time to support equipment and knowledge about periodontitis and microbial activity test.

Thankfulness goes to Associate Professor Dr. Poj Kulvanich, Assistant Professor Dr. Waree Tiyaboonchai and Dr. Narueporn Sutanthavibul for spending valuable time to be my thesis committee and for their suggestion and comments.

Special thanks go to the Siam Pharmaceutical Co., Ltd., Pharmasant Laboratories Co.,Ltd. and BASF (Thai) Ltd. for the support of active ingredient. Special thanks are also due to Graduate School of Chulalongkorn University for partially granting financial support.

My special thank also goes to Flight Lieutenant Governor Pakin Watin, graduate student, Faculty of Dentistry, Chulalongkorn University for technical assistance to insert the gel preparations to periodontitis patients.

I wish to thank all patients, my friends and colleagues in Department of Industrial Pharmacy and other persons whose names have not been mentioned here for their assistance and encouragement.

Finally, I would like to express my infinite gratitude and great appreciation to my parents and my sister who helped and encouraged me during my study. I felt really grateful to my family for the love, caring, understanding throughout my life.

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LIST OF ABBREVIATIONS

Ae	=	Aerosil®
Alc	=	Ethanol, 95%
CMC	=	Carboxy methyl cellulose
CP	=	Carbopol 940
EC	=	Ethylcellulose
et al	=	et alii (and other)
GCF	=	Gingival crevicular fluid
GMS	=	Glyceryl monostearate
HEC	=	Hydroxy ethyl cellulose 4000
HPLC	=	high performance liquid chromatography
HPMC	=	Hydroxy propyl methyl cellulose E15
hr	=	hour(s)
IPM	=	Isopropyl myristate
ml	=	milliliter
mm	=	millimeter
MN	=	Mineral oil
MTZ	=	Metronidazole
No	=	number of sample
PE	=	Polyethylene
pH	=	the negative logarithym of the hydrogen ion
Plo	=	Poloxamer® 407
PVA	=	Poly vinyl alcohol
PVP	=	Polyvinyl pyrrolidone K90
R^2	=	coefficient of determination
RL	=	Eudragit® RL-100
rpm	=	revolution per minute
RS	=	Eudragit® RS-100
SD	=	stand deviation
ST	=	stearic acid
W	=	ultrapure water

w/w	=	weight by weight
WS	=	White soft paraffin
%	=	percentage
<	=	less than
>	=	more than
^{o}C	=	degree Celsius
µg	=	microgram(s)
µl	=	microliter(s)

CHARPTER I

INTRODUCTION

Periodontal diseases are a collective term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingiva), supporting bone (alveolar bone), periodontal ligament and cementum [Smalley, 1994]. They are a group of conditions, including gingivitis and periodontitis, which affect the supporting structures of the teeth [Listgarten, 1987]. In the development of periodontal disease, there is an initial extension to and accumulation of plaque at the gingival margin that, in turn, induces an inflammatory response. Due to the direct actions of both plaque and the induced inflammatory response within the deeper tissues, a space (pocket) develops between the roots of the affected teeth and the soft tissues. In this protected environment, bacteria accumulate and flourish. If the disease is allowed to progress, increased tooth mobility and ultimately tooth loss results [Medlicott et al., 1994]. Consequently, given the importance of bacteria in the aetiology and progression of periodontal disease, treatment regimes frequently involve mechanical removal of plaque, usually in conjunction with topical antimicrobial chemotherapy.

The antibiotic therapy of periodontal diseases is mainly based on two different approaches: extensive oral rinses with solutions and systemic administration. On the other hand, both approaches can be unsuccessful and/or produce adverse problems. In fact, the first one could result in a failure of antibiotics to reach the deeper subgingival tissues, while the second one could present disadvantages such as (a) bacterial resistance to the administered antibiotic and (b) unpleasant or toxic side effects as a consequence of the systemic regimen [Okuda et al., 1992] Because of these considerations, a variety of specialized local delivery systems (i.e. intrapocket devices) were designed to maintain the antibiotic

in the gingival crevicular fluid at a concentration higher than that achieved by systemic administration [Kornman, 1993]

Bacterial flora in periodontal pockets play an important role in the etiology of periodontal disease. These pathogens include obligately anaerobic gram-negative species such as *Porphyromonas gingivalis, Prevotella intermedia. Bacteriodes forsyihus, Fusobacierium nudeatum, Selenomonas* and *Campylobacter* species and facultatively anaerobic gram-negative rods such as *Actinobacillus actinomycetemcomitans. Capnocytophaga* species and *Eikenella eorrodens* [Dzink et al. 1988]. The choice of the antibiotics in periodontal diseases must be based on the bacterial etiology of the infection (Slots and Rams 1990). Several antibiotics have been tested for their clinical and microbiological efficacy in periodontal diseases. Among the antibiotics that are being considered for periodontal treatment, metronidazole is particularly attractive due to its selective efficacy against obligate anaerobes. It has narrow spectrum and works specifically on the anaerobic flora associated with periodontitis, leaving the flora associated with health intact. In several studies, treatment with metronidazole has been found to improve clinical parameters and reduce the number of disease related bacteria [Loesche et al., 1996]. However, most results to date have been based on its systemic use. A recent development has resulted in a marketed 25% metronidazole dental gel, consisting of metronidazole crystals suspended in a lipid matrix [Stoltze and Stellfeld, 1992], for local application in the periodontal pocket. Now there has been an increased interest in its local application in order to provide higher concentration of the drug at the target site and the adequate time period required for effective treatment independent of patient compliance [Ainarno et al., 1992]. However, the drug of choice must be given in higher doses to maintain the effective concentration of the drug at periodontal pockets. Therefore, this type of treatment requires sufficient caution against occasional side-effects, e.g., gastrointestinal disorders, supperinfection and the development of resistant bacteria [Genco, 1981].

Topical applications with mouth washes and dentifrices follow an exponential concentration profile, while blood and gingiva crevicular fluid (GCF) levels remains all the time and fail to penetrate deep into periodontal pockets. Consequently, the high flow rate of GCF will cause a fast evacuation of the already released drug from the pocket to the mouth, thereby depleting the concentration of the drug occur in the pocket. Therefore, the rate of release should be higher at the initial stage of release, to achieve an immediate therapeutic level of drug in the pocket. The next stage should maintain therapeutic level, and moderate release profile is required. The average depth of a pocket is between 6 and 8 mm [Mastiholimath et al., 2006]. Therefore, the therapeutic drug device also cannot be large.

A variety of specialized local drug delivery systems were designed to maintain the concentration higher than that achieved by systemic administration [Binderman and Yaffe 2000]. To overcome the disadvantage mentioned above, a controlled release injectable dental gel has been investigated. Hydrophilic gels can be easily prepared and administered but a higher eliminated rapidly through saliva, bleeding, GCF and environment variable. Hydrophobic gels can be against aqueous environment but the type of oil-based drug delivery systems within the aqueous environment of the periodontal pocket showed poor retention [Jones et al., 1996].

An advance in pathological researches on periodontal disease and pharmaceutical technologies, the local drug treatment for periodontal disease by sustained delivery systems, has been recently developed [Goodson et al., 1985]. However, the techniques are still unsatisfactory for clinical use because they consist of insoluble polymers such as ethylene vinyl acetate, polyethylmethacrylate [Addy et al., 1985] and ethyl cellulose as a film or strip [Gobomb et al., 1984]. These substances must be removed from the periodontal pocket after the completion of drug release. This occasionally causes local mechanical irritation and disturbs periodontal repair.

To overcome the disadvantages mentioned above, Development a controlled-release injectable gel has been attempted. Bases on different type and amount of the materials will be formulated. Various mucoadhesive polymers (carbopol 940, hydroxypropyl methyl cellulose, polyvinyl alcohol, polyvinyllpyrolidone and carboxy methyl cellulose), thermo-reversible polymer (poloxamer 407), water insoluble polymer (Eudragit® RS, Eudragit® RL and ethylcellulose) and hydrocarbon compounds (mineral oil, Isopropyl myristate, polyethylene and white soft paraffin) were used as a main principle in each gel base formulations. Metronidazole was used as model drug. Various pharmaceutical parameters like physical appearance, viscosity, syringeability, spreadability, *ex vivo* adhesive property and *in vivo* periodontal adhesion, disappearance test, weight loss, erosion loss, morphology**,** stability, drug release characteristics, *in vitro* antibacterial activity on *P. gingivalis* of dental gel base and 24 hours after that periodontal gel could be remain inside patient's periodontal pocket for the delivery device were evaluated.

Objectives of the study

The aims of this study were the following:

1. To develop the formulations of periodontal gel to remain inside periodontal pocket and control the release of metronidazole.
2. To study the effect of type and amount of material on the physicochemical characteristics of the periodontal gel.
3. To study the drug release from periodontal gel and to determine the release kinetic.
4. To study the anti-microbial activity against *P. gingivalis* of selected metronidazole periodontal gel compared to periodontal gel base.

CHAPTER II

LITERATURE REVIEW

1. Description of Periodontitis

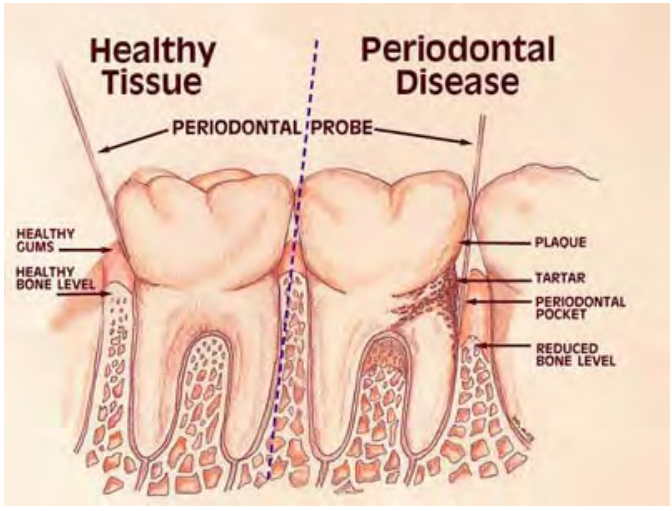

Figure 1 Comparing physical changes of healthy tissue progress to periodontal disease [Shailesh and Aarti, 1999]

Periodontal diseases (Figure 1) are recognized as a major public health problem throughout the world and common in all age groups, ethnicities, races, genders and socioeconomic level [Genco, Evans and Ellison, 1969]. They are a collective term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingiva), supporting bone (alveolar bone), periodontal ligament and cementum refers to gingivitis and periodontitis (Figure 2). Gingivitis is a common disease of the periodontium associated with alterations in the gingiva, such as redness and swelling. The most common disease is initiated by plaque accumulation in the gingivodental and area is basically inflammatory in character. When bacteria are allowed to accumulate at the gingival margin, a series of transformations takes place in the gingival tissues, leading from a healthy gingiva to the state of gingivitis**.** The accumulation of microorganisms and ensuing inflammation

makes the gum tissue painful to the touch and bleeding occurs during probing or tooth cleaning. When the gingivitis is not treated, it can advance to "**periodontitis**", an inflammation of the supporting tissue surrounding teeth caused by bacteria and bacterial toxins and the body's enzymes fighting the infection actually start to break down the bone and connective tissue that hold teeth in place . If not treated, the bones gums and the connective tissue that support the teeth are destroyed. The epithelium of the gingival migrates along the tooth surface forming "**periodontal pockets**" that provide an ideal environment for the growth and proliferation of microbes [Slots, 1979]. The teeth may eventually become loose and have to be removed.

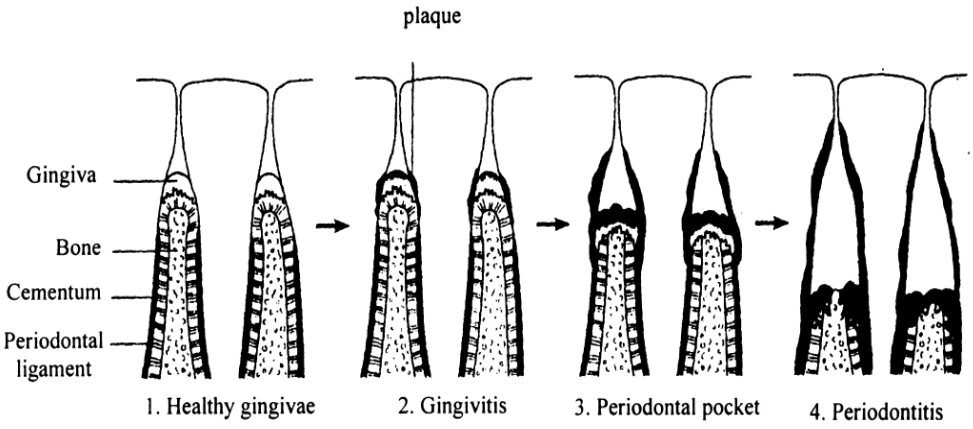

Figure 2 The stages of periodontal diseases [Vyas, Sihorkar and Mishra, 2000].

A healthy periodontium is characterized by a gingival sulcus of approximately 1 to 3 mm in depth around the crown of the tooth. Healthy gingiva appears light pink in color, with a firm stippled surface that does not bleed spontaneously or with mild instrumentation. As the disease progresses, there is an increase in pathogenic bacterial growth and gingival sulcus deepening (> 3-15 mm) to form a periodontal pocket. The periodontal pocket becomes filled profusely with fluid due to an increased gingival crevicular fluid (GCF) flow. The actual volume of fluid from a given gingival sulcus is difficult to measure, generally less than 0.5-1.0 µl/hour in an undisturbed sulcular location. The mean fluid volume in approximal spaces ranged from 0.24 to 1.56 µl per tooth (0.24- 0.43 µl/hour for anterior teeth and 0.43-1.56 µl/hour for molars). In subjects with crowns or periodontal disease, these values are higher. Goodson and others [1987] calculated that 0.5-2.5 ml of GCF is secreted into the oral cavity per

day. In addition, the rate of outflow of crevicular fluid in periodontal disease as 20 µl/h, indicating a pocket fluid replacement rate of 40 times/hour. This estimation is influenced by the extent of the infection and other underlying dental diseases [Goodson, 1983]. In general, estimated GCF flow at individual sites can be measured the amount of fluid collected by Periotron, an instrument designed to quantify submicroliter volumes of fluid sampled on a filter paper strip, a filter paper strip of 1.5 mm width, inserted 1.0 mm into a gingival sulcus of slightly inflamed gingiva, absorption of fluid in 3 minutes is measured.

It is unclear whether GCF results from physiological or pathogenic conditions since certain parameters (protein concentrations) resemble that of a physiological transudate, while others (Na^+/K^+ ratios) appear to be an inflammatory exudates [Alfano, 1974]. Two mechanisms have been put forth to explain the origins of GCF: 1) the generation of a standing osmotic gradient and/or 2) the initiation of classical inflammation. These mechanisms are described below:

1. The osmotic gradient may arise from macromolecular bacterial by-products residing in the subgingival plaque. These macromolecules accumulate at the basement membrane resulting in a localized increased solute concentration and establishment of an osmotic gradient. Solvent molecules drawn across the tissue, raise the intercellular hydrostatic pressure and cause the exudation of GCF into the periodontal pocket.

2. If the bacteria plaque is not removed, its macromolecular byproducts will eventually penetrate the basement membrane. Depending on the enzymatic and toxic properties of these molecules, a classical inflammatory exudation may occur. Thus, gingival fluid may progress, at different times, or in various areas of the dentition, from an initial osmotically modulated exudate to a secondary inflammatory exudate, with consequent alterations in its composition.

2. Bioenvironmental Considerations

a) Oral Cavity

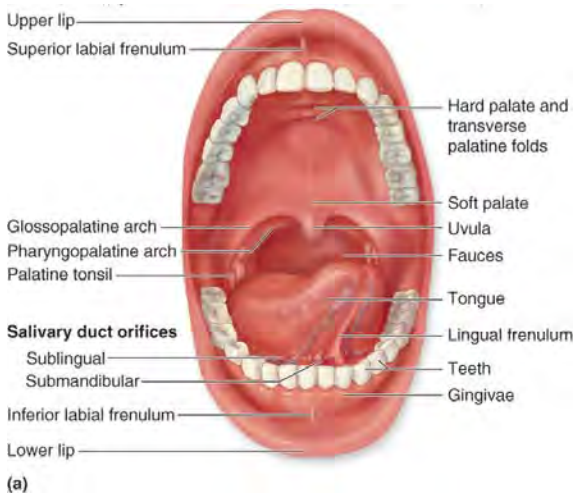

Figure 3 Structures of the oral cavity [Herbrandson, 1999]

The environment of the oral mucosa and its composition has been well studied, a complex and dynamic environment. [Collins and Dawes, 1987; Rathbone and Hadgraft, 1991]. Its main characteristics are the continued secretion of saliva from major and minor salivary glands. The daily output of saliva in human is between 750-1000 ml [Ghosh and Pfister, 2005]. Oral fluids can be considered the protective fluid for all tissues of the oral cavity. It acts as a buffer, maintaining a pH range from 5.75 to 7.05 [Pickel et al., 1965] and is mainly composed of water (99.5%), organic compounds (0.3%), inorganic and trace elements (0.2%) [Mandel, 1974]. Generally, the environmental conditions inside a human's mouth are harsh: the humidity is mostly 100%. The temperature, though generally around 37 °C, can vary between +5 and +55 °C for short times at least, for example, when eating or drinking cold or hot meals or beverages. Mastication can generate forces of up to 500 N and abrasion can occur on the teeth and on any item that resembles a chewing surface. Despite its buffering properties, salivary pH can drop as low as 2 when consuming acidic drinks.

Moreover, the healthy oral cavity is colonized by microorganisms like fungi, viruses and bacteria, of which more than 700 species or phylotypes have been detected in the oral cavity. However, relatively few species have been clearly associated with progressive periodontitis (Table 1) [Aas et al., 2005]. Special attention must, therefore, be paid to the hygiene requirements of an artificial device inside the mouth.

Table 1 Association between putative periodontal pathogens and periodontitis [American Academy of Periodontology, 2004]

Very Strong	Strong	Moderate	Early Stage of Investigation
Porphyromonas gingivalis *Actinobacillus actinomycetemcomitans* *Tannerella forsythensis*† Spirochetes of acute necrotizing gingivitis	*Prevotella intermedia* *Dialister pneumosintes/ Dialister invisus* *Eubacterium nodatum* *Treponema denticola*	*Campylobacter rectus* *Peptostreptococcus micros* *Fusobacterium nucleatum* *Selenomonas noxia* *Eikenella corrodens* Beta-hemolytic streptococci	Gram-negative enteric rods *Pseudomonas* species *Staphylococcus* species *Enterococcus faecalis* *Candida albicans*

Local factors cause inflammation, the principal pathologic manifestation of periodontal disease. However, the systemic factors alter tissue response and as a result the effect of local irritants may be aggravated. Local factors contributing to the pathogenesis include microorganisms, calculus (tartar), food impaction, mouth breathing, tooth malposition, faulty or irritating restorations or appliances and chemical or drug use (e.g. dilantin).

Virulence factors may also lead to the pathogenicity of the disease. Collagenase and other enzymes originating from bacteria can destroy the connective tissue and ligament of the periodontium. Toxins of the bacteria contribute to the progress of periodontal disease. Bacteria and their metabolites or by-products may act as chemotactic agents, leading to migration of polymorphonuclear cells, evoking an inflammatory response by activating the immunological system [Collins and Dawes, 1987].

b) Microbiology

Periodontal disease as an infection. Most forms of gingivitis and periodontitis are caused primarily by bacteria that colonize the gingival crevice and attach to intraperiodontal pockets [Mandel, 1974]. The omnipresence of many varieties of oral microorganisms growing as a film (bacterial biofilm) of plaque, for the most part on the non-self-cleansing areas of the teeth below the cervical convexity, has been recognized. Biofilms originate either from the normal gingival sulcus in case of marginal periodontitis, or from the gingival pocket in advanced periodontal disease (Goodson, Binder and Socransky, 1987; Aas et al., 2005; Carranza and Odont, 1990]. All reveal microorganisms of many different types. The composition of bacterial plaque associated with gingival health differs from that of plaque associated with the different periodontal diseases.

In general, gram negative, facultative, anaerobic microorganisms are the principal bacteria associated with the periodontal diseases [Aas et al., 2005]. Prominent among these are Bacteroids species, such as *Porphyromonas gingivalis, Prevotella intermedius, Fusiform* organisms, *Actinobacillus actinomycetemcomitans, Wolinella recta, Eikenella* species, various cocci and bacilli, sprirochetes , amoebas and trichomonads [Piovano, 1999]. Especially, *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedius* are the major periodontal bacteria species in most forms of progressive periodontitis [Lopez, 2000].

The normal oral flora is vast, however, making it impossible to prove conclusively that a particular type of microorganism is responsible for the pathogenesis of a specific periodontal disease. The flora is typically characterized by a predominance of gram-negative anaerobic rods. In juvenile periodontitis, gram-negative anaerobic rods increase in the areas of the deep pockets. A similar increase also occurs in the percent count of *Actinobacillus actinomycetemcomitans* and *Capnocytophaga sputigena* [Holen, 1975]. The periodontal pocket is a pathologically dependent gingival sulcus and is one of the important clinical features of periodontal

disease. Progressive pocket formation leads to destruction of the supporting periodontal tissues and loosening or exfoliation of the teeth. Microorganisms and their products that produce pathological tissue lead to the deepening of the gingival sulcus and create periodontal pockets. Pocket formation starts as an inflammatory process in the connective tissue wall of the gingival sulcus due to bacterial plaque (Figure 4) (Newman and Socransky, 1979; Listgarten and Hellden, 1978]. Changes involved in the transition from the normal gingival sulcus to the pathological periodontal pocket are associated with different proportions of bacterial cells in dental plaque. The cellular and fluid inflammatory exudes cause degeneration of the surrounding connective tissue, including gingival fibers. Two hypothesis have been proposed regarding the mechanism of collagen fiber loss from the local immune responses. Collagenase and other lysosomal enzymes from polymorphonuclear leucocytes and macrophages become extracellular and destroy gingival fibers or fibroblasts phagocytose collagen fibers by extending cytoplasmic processes to the ligament-cementum interface [Mandell, 1974]. Leukocytes and oedema from the inflamed connective tissue infiltrate the epithelium lining in the pocket, resulting in varying degrees of degeneration and necrosis.

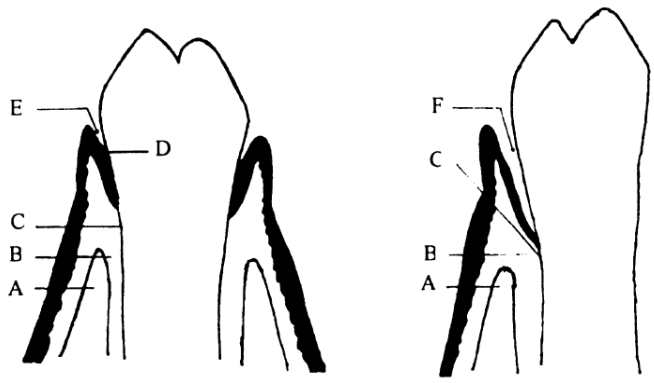

Figure 4 Diagrammatic presentation of pocket formation [Vyas et al., 2000] (A) healthy periodontium, (B) periodontal pocket. A = Alveolar bone, B = periodontal ligaments, C = cementum, D = cementum enamel junction, E = sulcus,and F = periodontal pocket

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Microbiology of periodontal disease, periodontal disease is now considered to be a group of diseases or infections. Each disease is associated with a different group of microorganisms. The resulting clinical signs and symptoms can be similar or unique by the mechanisms which subgingival bacteria and contribute to the pathogenesis of periodontal disease (Figure 5).

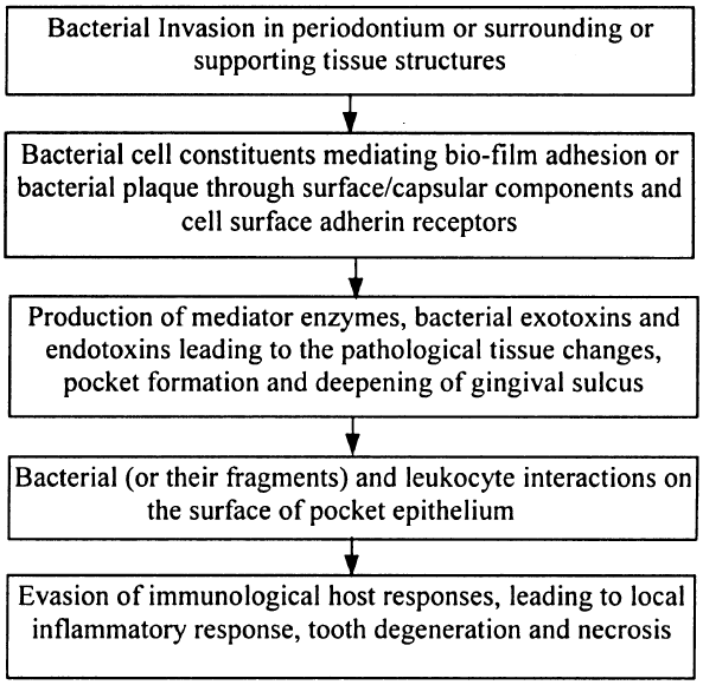

Figure 5 Pathogenesis of periodontal pocket diseases [Vyas et al., 2000]

c) Dental Plaque and Biofilm

Recognition of pocket bacteria as biofilm. There is great interest in the use of antimicrobial agents and/or antiseptics for the prevention and treatment of plaque-related oral diseases and many researcher have reported the results of studies in which the minimum inhibitory concentrations of agents for cariogenic and periodontopathogenic bacteria have been determined [Danser et al., 1996]. However, such data are relevant only to situations where the organisms of interest are in aqueous suspensions (fluid phase or planktonic), whereas in caries and inflammatory periodontal diseases the target organisms are in the form of biofilms, a form in which

they behave very differently. The bacteria in biofilms bind together in a sticky web of tangled polysaccharide fibers. These connect cells and anchor them to a surface and to each other. Within this microcosm, anaerobic and aerobic bacteria can thrive alongside each other, sharing water passageways and a complex structure. The polysaccharide coating is like a coat of armour. Different types of bacteria may collaborate to make a bacterial biofilm (Figure 6).

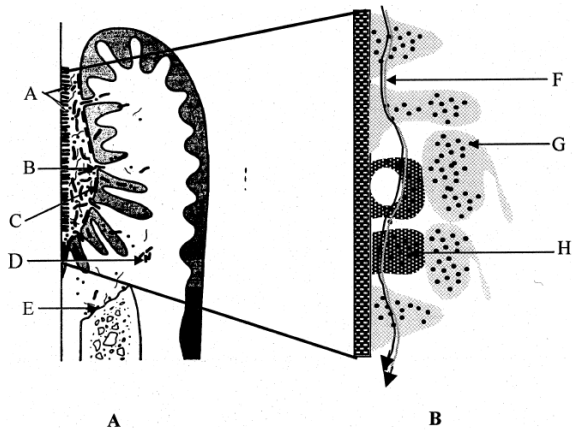

Figure 6 Structure of biofilm [Vyas et al., 2000] (A) Plaque bacteria associated as a biofilm with periodontal tissues. A . = tooth attached plaque, B . = unattached plaque, C . = epithelial associated plaque, D .= bacteria with connective tissue, and E . = bacteria on bone surface. (B) Biofilm formation (hypothetical) in the form of bacterial plaque. F . = microcosm and discrete microcolonies of bacteria, G . = open water channels, H . = dense polysaccharide and epoxypolysaccharide matrix.

By 1990, researchers confirmed that biofilm bacteria are morphologically and metabolically distinct from free-flowing ones, and that any bacterium can form a biofilm, once it finds a place to stick, as mostly provided by the mucosal layers underlining different peripheral organs. Adhesion to the bio-surface sets off a genetic cascade that turns on specific genes to make polysaccharides and/or to express surface receptors needed to establish the biofilm [Vyas, 2000].

The oral cavity, like other parts of the gastrointestinal tract, possesses natural microflora. It has a number of features that makes it a unique microbial habitat. Teeth characteristically provide hard non-shedding surfaces that allow accumulation of large masses of microorganisms (dental plaque), especially in stagnant areas. This accumulation is restricted on mucosal surfaces due to continuous epithelial desquamation; the only exception is the dorsum of the tongue that is highly papillated and thus supports higher densities of microbes [Marsh, 2000]. Another important feature is that the oral cavity is continuously bathed with saliva, which has a profound effect on the ecology of the mouth. Saliva has a pH range (6.75-7.25) that favors growth of many microorganisms. Salivary components influence oral microbes by one of four mechanisms: aggregating microbes to facilitate their clearance from the mouth, adsorbing to teeth surface to form an acquired pellicle to which microorganisms can attach, serving as a primary source of nutrients, and mediating microbial inhibition or killing [Scannapieco, 1994]. In addition to saliva, the gingival crevicular fluid (GCF), a plasma derived fluid that flows through the junctional epithelium, provides microbes in the gingival crevice with nutrients and carries host immune components that play an important role in regulating the microflora therein [Marsh, 2000]. The oral cavity is not a homogenous environment. There are differences among sites in key ecological factors like adhesion ligands, pH, nutrients, redox potential, oxygen and temperature. Thus the lips, palate, cheek, tongue and the different teeth surfaces are distinct habitats, each supports a characteristic microbial community. Which species occupy a particular habitat depends on the habitat properties; however, metabolism of these species may modify the surrounding environment, making it suitable for other species to colonize. The general bacteria is found in the oral cavity is presented in Table 2.

Clinically, dental plaque is the soft, tenacious deposit that forms on tooth surfaces and which is not readily removed by rinsing with water [Bowen, 1976]. Microbiologically, it can be defined as the diverse community of microorganisms found on the tooth surface as a biofilm, embedded in an extracelluar matrix of polymers of host and microbial origin. Since it is now recognized that dental plaque behaves as a typical microbial biofilm, the new definition of a biofilm by Marsh

[2003] can be adopted to redefine dental plaque as a microbially derived sessile community characterized by cells that are irreversibly attached to the tooth surface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription.

Table 2 Bacterial genera found in the oral cavity [Marsh, 2000]

	Gram-positive	Gram-negative	
Cocci	*Abiotrophia* *Enterococcus* *Peptostreptococcus* *Streptococcus* *Staphylococcus* *Stomatococcus*	*Moraxella* *Neisseria* *Veillonella*	
Rods	*Actinomyces* *Bifidobacterium* *Corynebacterium* *Eubacterium* *Lactobacillus* *Propionibacterium* *Pseudoramibacter* *Rothia*	*Actinobacillus* *Bacteroids* *Campylobacter* *Cantonella* *Capnocytophaga* *Cantipedia* *Desulphovibro* *Desulphobacter* *Eikenella* *Fusobacterium*	*Haemophilus* *Johnsonii* *Leptotrichia* *Prophyromonas* *Prevotella* *Selenomonas* *Simonsiella* *Tannarella** *Treponema* *Wolinella*

* A new genus; not present in the original source.

Dental plaque has the general properties of a biofilm that make the involved microorganisms dramatically different from their planktonic counterparts. Such properties include open architecture, protection from host defenses, enhanced resistance to antimicrobial agents, neutralization of inhibitors, novel gene expression, coordinated gene responses, spatial and environmental heterogeneity, broader habitat range and more efficient metabolism [Marsh, 2000]. Dental biofilm is primarily composed of microorganisms; one gram of wet plaque contains approximately 2×10^{11} bacteria. The intercellular matrix account for 20-30% of the biofilm mass, and is principally made up of polysaccharides of microbial origin (glucans and fructans) [Carranza and Newman, 1996].

Of all oral microbial ecosystems, dental plaque has been the major focus of oral microbiological research probably because of its characteristic features as a complex polymicrobial biofilm and its association with dental caries and periodontal diseases. According to its location, dental biofilm can be classified into fissure, smooth surface, approximal, supragingival, and subgingival. Composition of dental biofilm varies among these sites due to differences in their biological properties.

d) **Microbial Virulence Factors**

Virulence factors can be broadly separated on the basis of their target cell or tissue component and whether they operate by directly causing tissue damage or indirectly by perturbing host defenses and fibroblast repair mechanisms, or exacerbating destructive events by promoting the inflammatory response. Although elevated levels of specific antibodies and mediators of inflammation, *e.g.*, prostaglandins and interleukins, can be detected in gingival crevicular fluid (GCF), tissues, and cultured cells taken from diseased sites, it is difficult to establish the functional presence of microbial enzymes and cytotoxins or their relative contributions to the tissue destructive processes.

e) Inflammation and Fluid Stasis

In the diseased periodontium, chronic inflammation is accompanied by reduced blood flow, and in tissues which appear hyperemic, there can be relative fluid stasis and hypoxia. This may have a number of consequences. For example, fibroblastic turnover of gingival and periodontal ligament collagen is higher than in any other collagenous tissue, with 20% of gingival collagen turnover occurring per day. This requires an adequate supply of ascorbic acid, an essential co-factor in the hydroxylation of prolyl and lysyl residues, a factor which also governs both the secretion and extracellular cross-linking of collagen. There is conflicting evidence regarding the efficacy of ascorbic acid supplementation in resolution of gingivitis and periodontal lesions in patients other than those with vitamin C deficience. Although

there appears to be no appreciable loss of interstitial collagen, perivascular collagen is reduced. Repair of collagen fiber networks at inflammatory foci may be compromised by lack of ascorbate and other essential metabolites. Increased flow of gingival crevicular fluid Since microbes are not believed to invade tissues during gingivitis, the changes occurring to the connective tissues are thought to be mediated by noxious microbial products. These are thought to include hyaluronidase, chondroitinase, proteases, LPS, LTA, peptidoglycan, and toxic metabolic by-products which infiltrate the epithelium. These disrupt epithelium and underlying connective tissues, increasing vascular permeability and allowing for a greater infiltration of noxious materials. The resulting inflammation and increased fluid flow into the gingival sulcus promotes further accumulation of a wide range of bacteria by providing both nutrients and a suitable physical environment, creating a positive feedback.

f) Hypoxia

Mettraux et al. [1984] have shown that the oxygen tension (pO_2) in moderate (5-6 mm) and deep (7-10 mm) periodontal pockets (range, 5-27 mm Hg) is significantly lower than even venous pO_2 (20-40 mm Hg). Although neutrophils can phagocytose in an anaerobic environment, hypoxia can inhibit, but not abolish, antimicrobial activity [Mandell, 1974]. In the hypoxic periodontal pocket environment and inflamed gingival tissues, oxygen-dependent killing mechanisms may be impaired, since O_2 consumption is necessary for the generation of toxic reactive oxidant species.

g) Elevated pH

The pH distribution in the gingival sulci adjacent to inflamed sites and in diseased pockets is skewed toward the alkaline side of neutrality, with pH as high as 9.06 being recorded. Such conditions favor *P. gingivalis*, which has an optimum growth pH between 7.5 and 8.

During growth in the chemostat between pH 6.7 and 7.0 (values corresponding to the healthy gingival sulcus), hyaluronidase and collagenase activities (those enzymes which are capable of lysing connective tissues and which might contribute to the initiation and progression of a lesion) are maximal. When the pH was raised to 8.0, a value at which the inflammatory response is greatest, the "trypsin-like" activity rose, while the collagenolytic activity decreased. This shift in protease expression may thus enable *P. gingivalis* to affect a local paralysis of the immune system by proteolyticallly degrading immunoglobulins and inactivating complement.

h) Bleeding

Spontaneous bleeding of the gingiva and bleeding on probing are indicative of inflammation and are among the criteria used to diagnose chronic gingivitis and chronic periodontitis. Blood represents a plentiful nutrient source to a wide range of microorganisms, and changes in its composition have been linked with alterations in microflora.

3. Controlling Periodontal Infections

The resistance of these biofilms to antimicrobial agents is phenomenal, researchers are investigating creative ways to conquer biofilms. Preventive and therapeutic regimens for biofilm control and elimination based on antimicrobial agents formulated in various conventional and local drug delivery devices are being evaluated.

Three strategies have been proposed for reducing the risk of periodontal diseases. Each attempts to intercept the disease process at critical points in its development.

a) Reduce Supragingival Plaque

Supragingival plaque reduction by home care and professional cleaning is the most universally practiced periodontal treatment available; it is considered essential in the treatment of periodontal diseases.

b) Control Pathogen Transmission

Introduction of an antibacterial mouthwash and toothpaste may insulate sites from infected pathogen reservoirs elsewhere in the mouth. Hujoel et al. tested rinsing once per week and observed a 45 percent reduction in tooth loss after 1 year. Quirynen et al. [2000] examining one-stage, full- mouth disinfection, observed a parallel significant reduction in periodontal pathogens and improvement in clinical health following chlorhexidine rinses.

c) Disinfect Pathogen Reservoirs

Many investigators have recognized disease reservoirs as seeding sources for intraoral spread of disease and as an important consideration in determining therapeutic outcome. Of the infection sources in the oral cavity,untreated sites elsewhere in the mouth represent the most obvious potential source of re- infection. At least three mechanisms are used to address this threat: SRP; local drug delivery; and systemic antibiotics

4. Administration of Drugs for Periodontal Disease Patients

The concept of the antibiotics or antimicrobials periodontal therapy centers upon the pathogenic microorganism, the patient and the drug. The concept is based on the premise or etiology that specific microorganisms cause destructive periodontal disease and that the antimicrobial agents in *vivo* can exceed concentrations necessary to kill or inhibit the pathogens. Antimicrobial may be prescribed for periodontal patients who do not respond to conventional mechanical therapy, for patients with

acute periodontal infections associated with systemic manifestations, for prophylaxis in medically compromised patients and as a adjunct to surgical and non-surgical periodontal therapy[American Academy of Periodontology, 2004].

The pharmacological characteristics of drugs are critical in deciding their use, dosage and routes, and frequency of administration. Important pharmacological determinants include body weight, degree of absorption, rate of metabolism, and duration of effective antimicrobial levels at the site of infection. The efficacy of periodontal antibiotic therapy is determined by the antimicrobial spectrum and the pharmacokinetic characteristics of the drug and by local environmental factors including:

1) Drug binding to tissues
2) Protection of pathogens through binding, consumption, or degradation of the drug by non-target microorganisms
3) Subgingival plaque biofilm protecting the pathogens
4) Total bacterial load relative to the maximum achievable antibiotic concentration
5) Effectiveness of the host defenses
6) Pathogens in periodontal tissues, root surfaces, and extra-dental oral sites not affected by the therapy. The unique therapeutic difficulties imposed by dental biofilms are highlighted elsewhere

Selection of Antibiotics

Strategies for choosing the type of drugs required for the treatment of periodontitis are primarily aimed at suppression or elimination of specific periopathogens from the periodontal pocket, thus resulting in a shift of bacterial strains towards those associated with normal physiological flora. Since 1988, the drugs most frequently prescribed by periodontitis include tetracycline, doxycycline, metronidazole, penicillin, amoxicillin and chlorhexidine (CHX).

In clinical study, metronidazole (MTZ) has been investigated and used in a drug delivery system. It was found to be the most effective in the treatment of chronic periodontal disease. Other reasons for selecting metronidazole include its low solubility (in the free base form) when dissolved in physiological fluids, and the ease of incorporation of this drug into the gel base delivery system.

MTZ is soluble 1 and 1.5 in 100 of water and ethanol, respectively. It is a white to pale yellow crystalline powder and oderless. Melting point range between 159-163°C. Hydrolysis and photo oxidation was responsible for the degradation of MTZ. The reaction order for MTZ in these aqueous and solvent systems followed pseoso-first order degradation kinetics. Maximum stability of MTZ was at pH 4.7. Metronidazole has been reported to be sensitive to light [Moor et al., 2000]. Previous work [Raynold et al, 1996] has indicated that the photodecomposition reaction of MTZ appeared to followed pseudo-first order kinetic. The presence or absence of oxygen was found to exert very little effect on the photodecomposition rate of MTZ. The degradation of MTZ in different solvent decreased in the order: chloroform > isopropanol > methanol > water.

Aqueous solution of MTZ 0.5% in citrate: phosphate buffer at pH 5. It is proposed that the initial yellow degradation product is an “excimer ion-pair” formed by the stabilization of MTZ in its first electronic excited state by the citrate molecule [Stoltze et al, 1992]

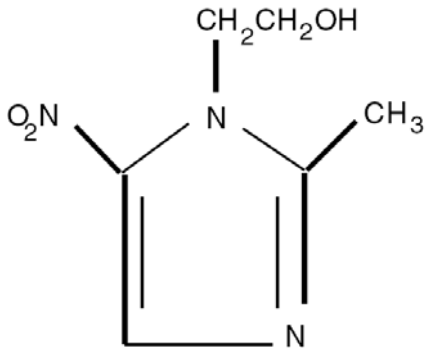

Figure 7 Structure of metronidazole

Metronidazole, a 5-nitroimidazole compound (Figure 7), specifically targets anaerobic microorganisms in the deeper 3 mm of periodontal pocket. Initially, metronidazole was thought to interact with biochemical pathways present only in obligate anaerobes. It is now known that cytotoxic metabolites of metronidazole directly interact with bacterial DNA, and possibly other macromolecules, resulting in cell death. Upon entry into an anaerobic organism, metronidazole is reduced at the 5-nitro position by electron transport proteins that are part of anaerobic metabolic energy-yielding pathways. Alteration of the metronidazole molecule creates a continuous concentration gradient favoring diffusion of additional metronidazole into the cell. Reduction of the parent compound yields many short-lived cytotoxic free radicals. These free radicals react with macromolecules, particularly DNA, resulting in cell death. Although resistance to metronidazole occurs in some anaerobic bacteria, e.g. *Fusobacterium* species, it is relatively rare and appears to be due to a decrease in the ability of the bacterium to actively reduce the 5-nitro position.

The most common adverse reactions associated with metronidazole involve the gastrointestinal tract. About 12% of the patients experience nausea which may be accompanied by headache, anorexia, and vomiting. Drowsiness, depression, skin rashes, and vaginal and⁄or urethral burning have been reported. Metronidazole affects the activity of hepatic enzymes involved with the metabolism of ethanol, producing unpleasant symptoms due to the accumulation of acetaldehyde in the blood. Alcohol ingestion is strictly contraindicated in patients receiving metronidazole. Metronidazole crosses the placenta barrier, entering the fetal circulation system. It is also secreted in breast milk. Because of the association of metronidazole with tumorigenicity in some animals, the drug is contraindicated in pregnant women or nursing mothers.

The fact that metronidazole specifically targets anaerobic bacteria makes it an attractive antibiotic for use as an adjunct to periodontal therapy. Metronidazole readily penetrates into the gingival crevicular fluid and achieves concentrations in excess of the MICs established in vitro for most putative periodontal pathogens. It is known to be quite active against gram-negative anaerobes such as *Bacteroides*

fragilis, Prevotella melaninogenica, Prevotella disiens, Prevotella oralis, Prevotella intermedia and *Fusobacterium spp*. The anaerobic grampositive sporing bacilli such as *Clostridium perfringens, Clostridium tetani, Clostridium sordelli* and *Clostridium* septicum are nearly always susceptible. To be successful in the treatment of periodontitis, local delivery regimens must provide therapeutic levels of the antimicrobial agent in the subgingival area over a prolonged period of time. Minimum inhibitory concentration (MIC) for susceptible anaerobic bacteria generally ranges from 0.1 to 8 µg/ml. A dental gel consisting of a semisolid suspension of 25% metronidazole benzoate in a mixture of glycerol monooleate and sesame oil will, in contact with gingival crevicular fluid, form reversed hexagonal liquid crystals. This will prevent the gel from being easily washed out of the periodontal pocket and thereby provide the subgingival area with therapeutic levels of metronidazole over a prolonged period of time. The MIC of metronidazole needed (MIC_{50}) to affect strains relevant to periodontal pathology is <1 µg/ ml [Baker et al, 1985]. Mean gingival crevicular fluid concentration after a single application of 25% metronidazole dental gel has been found to be 461 µg/ml. [Stoltze, 1992] Metronidazole administered systemically will give gingival crevicular fluid concentrations of ~ 8 to 10 µg/ml. Thus, it is possible to obtain considerably higher therapeutic doses in periodontal sites using locally applied antimicrobial drugs than by means of systemic application. In addition, local antimicrobial therapy reduces the risk for side effects along with being independent of patient compliance.

The adjunctive use of metronidazole in conjunction with thorough mechanical debridement results in reduction in spirochetes and Gram-negative anaerobic rods, including *P. intermedia*, *P. gingivalis*, and *T. forsythia*. Relative to either baseline or mechanical debridement alone, some improvement is generally obtained in probing pocket depth and in clinical attachment level. Generally, deeper sites (> 5 mm probing pocket depth) tend to respond better than moderate sites (4–5 mm probing pocket depth). Thus, metronidazole therapy seems to be more effective when treating adult periodontitis patients exhibiting deeper pockets that contain a susceptible gram-negative anaerobic microbiota [Clay et al., 2004].

5. Systemic Administration of Antimicrobial Agents

Antimicrobial agents have been used systemically in periodontal therapy for over 25 years based on reports that systemically administered antibiotics are excreted via the saliva and/or gingival fluid. In combination with treatment of the infected pockets, systemic antimicrobial agents may be of importance in one or the following ways:

1. Reaching and killing bacteria that cannot be removed by scaling, root planing and curettage, e.g. bacteria that have penetrated into the tissues in advanced periodontitis or localized juvenile periodontitis.

2. Conjugation with non-surgical therapy, reduction or elimination of the need for periodontal therapy. Once bacteria have been removed from the pocket, aggressive plaque repopulates the pocket within a few weeks if hygienic conditions are not maintained.

3. Enhancing new attachment and bone regeneration. The re-infection of the pocket area is probably one of the major factors working against new attachment. The maintenance of a noninfected area may favour the new attachment of tissues and is also likely to improve the chances for success [Saito et al., 1994].

When providing pharmacological therapy to the periodontal pocket, the factors which must be considered are effective therapy, predictable clinical results, low incidence of drug side effects or interactions, decreased costs and patient acceptance of the drug. For a drug to be useful: 1) the periopathogens must be susceptible to the drug; 2) they must not develop resistance to the drug; and 3) they must be exposed to effective inhibitory concentrations of the drug for an adequate time period. Early discontinuation of the systemic therapy may result in return of periopathogens, leading to reinfection. Risks associated with long term use of systemic antibiotics include the development of resistant bacterial strains, reduced bacterial sensitivity, superimposed infections and changes in gastrointestinal drug

absorption [Goodson, 1985]. The administration of systemic antibiotics may initially result in therapeutic drug levels at the gingival site, which decline to subtherapeutic levels over time.

In order to maintain bactericidal drug concentrations in the periodontal pocket, higher systemic drug concentrations may be required. Due to the often poor biodistribution of some systemic antibiotics to the periodontal pocket, high serum drug concentrations are needed to produce effective therapy. This may lead to undesirabIe side effects. Metronidazole has been shown, in a limited number of cases, to produce side effects of diarrhea, dizziness, headaches, and nausea, as well as having drug interactions with alcohol [Loesche et al., 2002].

6. Local Administration of Antibiotics and Antimicrobial agents

Reaching the entire periodontal pocket is difficult because of its very small entrance (150 µm for a 4 mm deep pocket**.** Mouth rinsing and supragingival irrigation do not reach subgingival areas. Only subgingival irrigation can overcome this obstacle. Furthermore, the constant outflow of crevicular fluid explains the extremely fast clearance of any topically applied product. The expected half-life of a pharmaceutical agent in the gingival pocket is about 1 minute [Oosterwaal, 1990]. Furthermore, periodontal pathogens in the subgingival environment reside in a biofilm adhering to the exposed root cementum or to the soft tissue, or even invading the pocket epithelium, the underlying connective tissue and/or the root dentine. The aggregation of bacteria in a biofilm impairs the diffusion or may even inactivate antimicrobial agents. High concentrations of the active ingredient are needed before a beneficial effect can be expected. Biofilm experiments indicate that the necessary minimum inhibitory concentrations of antimicrobial agents are at least 50 times (or even 210,000 times) higher than for bacteria growing under planktonic conditions (Kleinfelder, 1999). Moreover, the minimum contact time for an antimicrobial agent to be active depends on the mechanism by which the agent inhibits or destroys target bacteria. Chlorhexidine (which kills microorganisms by compromising the integrity of the cell membrane) and/ or povidone-iodine (which kills bacteria on contact) require a

shorter exposure time than, for example, a bacteriostatic agent, such as tetracycline, which inhibits protein synthesis. Even the shorter killing time, however, is not reached after a single drug application, but may be obtained after repeated subgingival irrigation within a short period of time [Oosterwaal, 1991]. An antimicrobial drug can be used in conjunction with power-driven scalers as a coolant instead of water. As such, a prolonged contact time can be established.

With the above-mentioned half-life data for subgingivally applied drugs, the minimal contact time needed for antibiotics cannot be reached in practice even with repeat subgingival irrigation. However, the substantivity of a topically applied agent may increase spontaneously if it remains or binds to the soft and/or hard tissue surfaces within the pocket or retain inside with special mechanisms. This establishes a drug reservoir from which the antimicrobial agent can be slowly released. Another means to increase the subgingival substantivity of an agent is by its incorporation in a slow-release device, a reservoir with a limiting element that controls the rate of drug release (Figure 8).

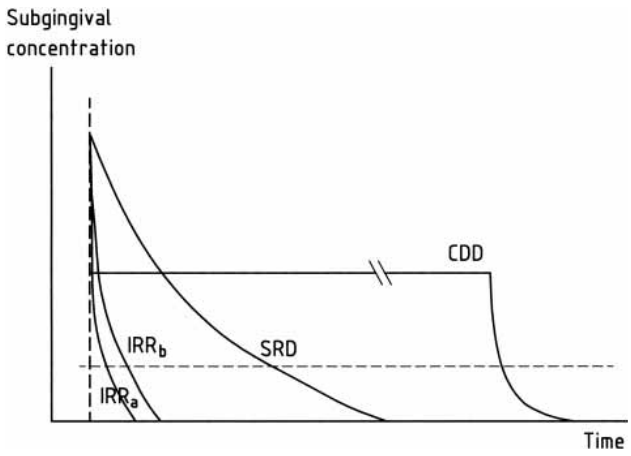

Figure 8 Subgingival concentration of an antimicrobial agent after subgingival irrigation (IRR_a without substantivity, IRR_b with substantivity), or incorporated in a device with sustained release (SRD: drug delivery for less than 24 hours) or with controlled delivery (CDD: drug delivery over a longer period) [Quirynen, 2002]

In order to be effective, a pharmaceutical agent should reach the entire periodontal pocket (such as the bottom) and should be maintained long enough at a sufficient concentration for the intended pharmaceutical effect to occur. Periodontal pockets, however, possess complicating anatomic characteristics [Greenstein, 2000].

7. An on Site Drug Delivery Approach

The anatomical structure of the periodontal pocket lends itself well to the insertion of a localized controlled drug delivery system. Such a device, in general, should have a reservoir of less than 1.0 milligrams (mg) of drug and a release rate of a few micrograms per hour. This rate should be sufficient to maintain the GCF drug concentration at therapeutic levels. Several drug delivery devices have been developed over the last ten to fifteen years to try to meet these goals. The following criteria were selected for comparison of the different drug delivery systems: application technique, range of pocket size application, patient acceptance, drug delivery time, choice of therapeutic agent, composition and safety, and device degradation time. Each of these criteria is described below.

a) Application Technique

The drug delivery systems should be easy to place in the periodontal pocket. As substantial operator differences may be encountered, a learning curve is required as proper placement is important to the overall clinical success of the device [Konunan, 1993]. The device should be easy to handle, and relatively quick and simple to apply, to ensure a high success of proper placement.

b) Range of Pocket Size Application

The delivery system should work equally well in all sites and all patients. The optimal use conditions have not yet been defined by investigators with experience in particular techniques, but have great variability from site to site.

c) Patient Acceptance

Survival times of drug bearing delivery devices in the periodontal pockets play a key role in determining the outcome of the treatment. In addition, such devices have to be aesthetically acceptable to the patient. In view of the vanity most people exhibit regarding their mouth, such devices should not extend above the gingival margin, must not be bulky or interfere with normal daily oral hygiene, including tooth brushing and dental flossing, and should not require the patient to change their dietary patterns. Since the inflamed periodontium is very sensitive, the device should be amenable to rapid insertion into the pocket, and pain and discomfort to the patient during the treatment period should be minimized [Friedman and Steinberg, 1990].

d) Drug delivery time

Delivery systems differ in several ways. It is uncertain at this time what other factors besides drug release kinetics, choice of drug and its carrier, and physical device placement and distention of the pocket are factors influencing clinical outcome and how long the device should remain in the pocket and release its drug. The singular aim of using antimicrobials as part of a treatment scheme is to achieve within the periodontal pocket a concentration of drug that is sufficient to kill (bactericidal) or arrest the growth (bacteriostatic) of pathogenic microorganisms over the required period of time. A poorly absorbed drug that has a low penetration through the mucosal tissues would enable the drug level to build up to a high concentration, and prolonged duration in the pocket. Ultimately, the concentration of the drug in the gingival crevicular fluid and the time that the drug concentration is maintained above the MIC in the pocket depends upon the drug's substantiveness and antibacterial potency. For instance, the placement of acrylic resin strips [Addy and Langeroudi, 1985] containing 40% (w/w) metronidazole into the periodontal pocket for two to three days reduced total microbial counts by more than 75% and achieved a shift in microbial patterns to a Gram-positive cocci dominance, which is generally considered associated with good health. It should also be noted that in most controlled release local delivery systems the level of antimicrobial release into the periodontal pocket far

exceeds levels involved in normal antimicrobial mechanisms. It is very likely that these agents in such initial high concentrations exert multiple effects on the local environment, only one of which may be antimicrobial in the traditional sense [Kornuman, 1993].

e) Choice of Therapeutic Agent

Since the average depth of a periodontal pocket is between 6 and 8 mm, the therapeutic drug delivery device cannot be large. Thus, it is necessary that a small dosage of the active agent in the device should be highly effective as a therapeutic agent. Antibacterial drugs should be highly specific against the pathogenic bacteria in the pocket. The development of resistant strains of bacteria might occur due to the long duration of the antibacterial agent in the periodontal pocket. The drug choice should then be effective for a particular type of periodontal disease it is being used to treat, and should not lead to the development of resistance bacterial strains. Using different drug delivery devices may be influenced by drug bioavailability, concentration, duration and spectrum of activity, resulting in differences in treatment results [Drisco, 1996].

f) Composition and Safety

A common approach to insuring the safety of a device is to establish its biocompatibility. Initially, when using biomaterials, the biocompatibility question was focused singularly on adverse effects, not on the performance of the device. Presently, the concerns involve both the aspects of performance and device adverse effects. The issue of biocompatibility is not whether there are adverse reactions associated with the biomaterial, but whether that material performs satisfactorily (i.e., in the intended fashion) in the application under consideration [Williams et al., 1981]. All implantable medical devices should meet two general criteria. They should be (1) biofunctional and (2) biocompatible during the period of implantation. If either riteria is not met, the device may present a threat to the patient and generally requires removal and replacement. Biofunctionability relates to the intended performance of

the item, throughout the entire implantation period. Biocompatibility, as mentioned previously, refers to the absence of any adverse effects of the device during the implantation period, and is often associated with the broad toxicological aspects of the device or material.

g) Device Degradation Time

A degradable controlled release device would have several advantages over a nondegradable device. For example, elimination of a return visit to the periodontist to extract the device from the periodontal pocket would represent great time and cost savings. A degradable device should not be an obstacle during reattachment of the periodontal tissues to the tooth, thereby offering minimal interference in the reduction of pocket depth. A factor that needs to be taken into account when considering degradable devices is the problem of toxicity. The degradable components of the device have to be dissolved or absorbed from the site without causing any tissue irritation [Friedman and Steinberg, 1990]. It is desirable to have the release system degrade during the time of drug release.

Soskolne et al [1983] produced ethylcellulose strips containing chlorhexidine, which provided sustained drug release for up to seven days. They were able to show a persistent reduction of periopathogens for at least ten days using tetracycline loaded monolithic fibers composed of ethylene vinyl acetate co-polymer. Both of these devices and the fibers used by Goodson et al. [1991] are neither biodegradable nor bioerodible, and once depleted, the patients are required to return to the practitioner for removal of the implants. Fiber devices of this type require elaborate manufacturing methods and take longer to apply than the strips.

Addy et al. [1985] demonstrated the effectiveness of acryIic strips which delivered chlorhexidine, metronidazole and tetracycline. These systems produced high and sustained druglevels for seven days leading to significant reductions in the count of pathogenic bacteria for up to three months. However, these strips did not degrade over time.

Williams et al. [1984] described the cross-linking agents (such as gluteraldehyde or formaldehyde) may also cause biocompatibility problems in the periodontal pocket. In addition, phagocytosis of nonbiodegradable particles by macrophages induces release of lysosomal enzymes and other mediators of inflammation which results in tissue irritation.

Although the 25% (w/w) tetracycline poly (lactide-co-glycolide) system [Maze et al., 1995] seems to have a reasonably desirable drug release time of 10 days, the device degradation time is slow. There is concern over its detailed application technique such as the loss of strips during treatment.

The Elyzol® dental gel (25% (wlw) metronidazole benzoate in a mixture of mono- and triglycerides used in the studies of Ainarno et al., [1992] initially looked like a desirable, exciting, new, sustained release periodontal drug delivery system. UnfortunateIy, it has several undesirable drawbacks. These include: a variable dose of drug in each periodontal pocket; bitter taste; gingival tenderness and pressure as the gel liquefies and then hardens (with some swelling) in the periodontal pocket over 20 minutes; a residence time is 24 to 36 hours [Stoltzel, 1992] in the pocket; polymer degradation time of 12 hours, and a large potential for drug loss both due to gel overflow and during crystal formation due to swallowing the gel. In fact, up to 70% of the dose is available to be swallowed. This may lead to a detectable systemic drug concentration, thus causing this **“localized”** delivery system to now have some of the drawbacks associated with systemic metronidazole delivery.

There are two possible approaches to improve the drug action: (i) sustained and controlled drug release to reduce or eliminate side effects by improving the therapeutic index; (ii) site-specific drug delivery to minimize systemic effects. These two strategies have been explored by the association of drugs with different vehicles, either naturals or synthetics. However, most of these systems failed to realize their potential in clinical phase studies. In this respect, it is critical not to under-estimate problems such as weak therapeutic activity resulting from a limited accessibility to the tissue to be treated or toxicity and/or immunogenicity of the delivery system.

Synthetic polymers have proved to be extremely interesting because they can be tailor-made to meet pharmacological or biological requirements.

Drug delivery systems can be classified according to the mechanism controlling drug release. We distinguish three categories: (i) "**solvent controlled**" matrix systems based on macromolecular matrix permeability to small molecules after matrix swelling into hydrated medium; (ii) "**reservoir systems**" controlled by drug diffusion across a polymeric membrane; (iii) "**chemically controlled systems**" where the rate of drug release is controlled by the rate and extent of degradation of chemical bonds and the erosion of the polymeric matrix. For all these systems, the basic polymer can be of natural origin such as proteins or collagen, semi-synthetic such as cellulose derivatives [Minabe, 1989] or synthetic, all of which must preferably degrade during use. Natural polymers have been considered as biodegradable carriers.

Many polymer based systems for antibiotic delivery in the treatment of periodontal diseases have been studied and evaluated in *vitro* and/or in *vivo*. Unfortunately, the majority of the studies provide little indication of the effect of the preparation on the progression of periodontitis. In addition, few clinical data were reported and therefore no association between changes in the flora and changes in disease patterns could be established. Some of these systems are not resorbable, while most are biodegradable. Nonbiodegradable systems have to be removed after complete drug release, which may cause irritation and inflammation of the treated site. Conversely, a biodegradable sustained release drug delivery system which can be placed into the periodontal pocket and maintain therapeutic concentrations for prolonged periods of time would be advantageous. Indeed, in addition to improving compliance over systemic antibiotics, biodegradable devices are cost effective as they will not require a second visit to the periodontist for removal.

8. Drug Delivery Device Considerations

Various types of local drug delivery system have been proposed for gingivitis, e.g. injectable devices, gels and ointments and films, etc. They represent the initial

steps that have been taken in the field of topical sustained release chemotherapy for the treatment and control of gingivitis. Intra-pocket, sustained release, drug delivery devices have been shown to be clinically effective in the treatment of periodontitis. Intra-pocket devices can be divided into two broad categories depending on whether they are biodegradable or not. Non-degradable devices have the advantage that the therapist controls the removal of the device and therefore has greater control over the time of exposure of the pocket to the drug. However, a non-degradable device left in situ beyond its period of therapeutic efficacy is a potential hazard in that it could result in a foreign body response or immune response. Conversely, a biodegradable, sustained release, drug delivery system which can be placed into the periodontal pocket to maintain therapeutic concentrations for prolonged periods would be advantageous. This is because in addition to improving patient compliance over systemic antibiotics, patients are not required to visit the periodontist to have these devices removed at the completion of therapy, thereby making them more cost effective. Several degradable and non-degradable devices are under investigation for the delivery of antimicrobial agents into the periodontal pocket [Medlicott,1994], for example, fibers (non-biodegradable), films (biodegradable and non-biodegradable), bioabsorbable dental materials, biodegradable gels/ointments, injectables and microcapsules showed in Figure 9.

A) Reservoir delivery systems

The use of fibers, or thread-like devices, for the sustained release of drugs into the periodontal pocket was the first reservoir device introduced by Goodson et al. [1983] using cellulose acetate dialysis tubing (diameter 250 μm) to deliver solid tetracycline hydrochloride into the periodontal pocket. An appropriate length of tubing was administered by placement at the opening of the periodontal pocket and application of gentle pressure to insert it below the gingival margin. The system showed some clinical effects but was unable to sustain therapeutic levels of the drug for sufficient time to be clinically useful [Goodson et al., 1985].

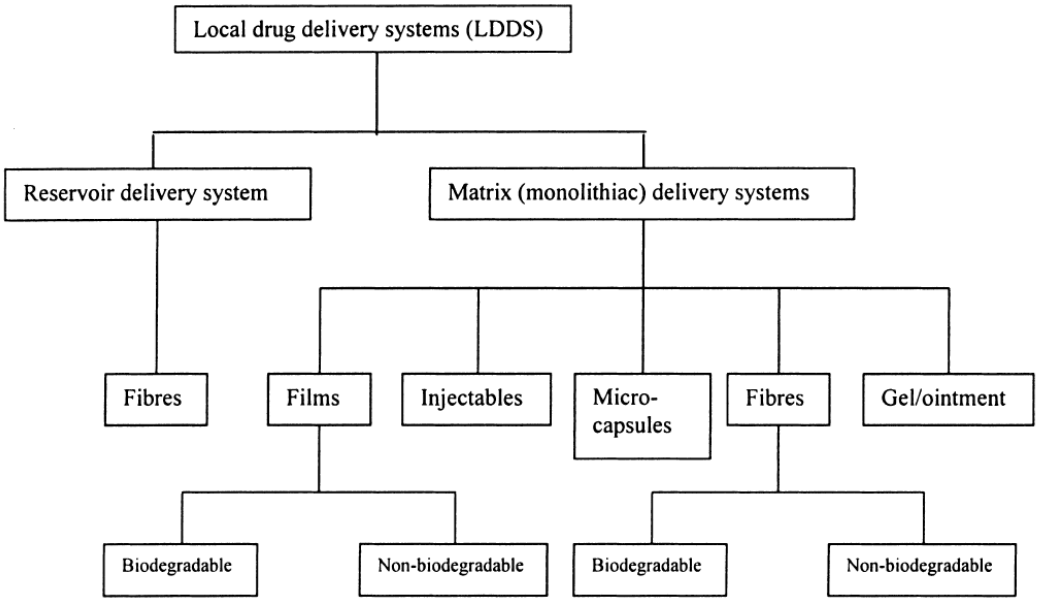

Figure 9 Local sustained delivery systems (LSDS) for the treatment of periodontal pocket diseases.

1) Fibers

1-1) Matrix delivery systems

A number of polymers were investigated as matrices (monolithic) for the delivery of tetracycline to periodontal pockets. Fibers were prepared by heat extrusion of 25% w/w tetracycline hydrochloride in polyethylene, polypropylene, poly(e-caprolactone), polyurethane, ethyl vinyl acetate or cellulose acetate propionate. Rapid release was observed from polyethylene and polyurethane fibers, with most of drug released within 24 hours. Polypropylene, poly(e-caprolactone) and cellulose acetate propionate fibers released only a small fraction of their drug load rapidly, and could not provide an extended release profile. Ethylene vinyl acetate fibers produced a sustained in vitro release over 9 days and was proposed as a suitable carrier for tetracyclin delivery in periodontal pocket diseases.

These initial studies were followed by others, notably those of Goodson and coworkers [Goodson, 1979], which have led to the development of a

commercial delivery system (Actisite, Alza Corporation, Palo Alto, CA) (Figure 10). Two multicenter studies [Newman et al., 1979] show that the treatment of periodontal pocket with this system resulted in significant reductions in pocket probing depths and bleeding on probing and significant increases in attachment levels compared to the other treatments tested.

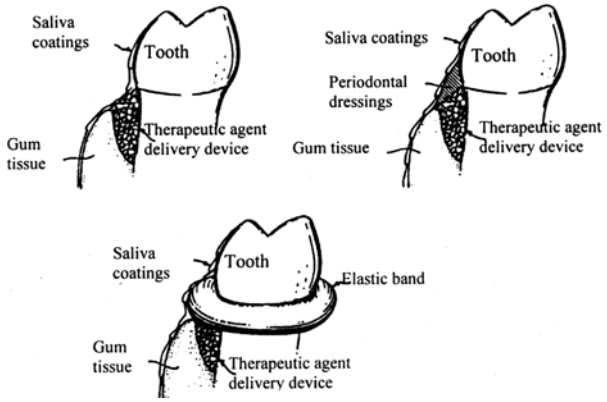

Figure 10 Drug dispensing nonbiodegradable fibers for the treatment of periodontal diseases

2) Films

Films are matrix delivery system in which drug is distributed throughout the polymer and release occurs by drug diffusion and/or matrix dissolution or erosion. This dosage form has several advantageous physical properties for intra-pocket use. The dimensions and shape of the film can be easily controlled to correspond to the dimensions of the pocket to be treated. It can be rapidly inserted into the pocket with minimal discomfort to the patient. It can be inserted to the base of the pocket and be totally submerged. If the thickness of the film does 5not exceed approximately 400 lm, and its physical properties provide it with sufficient adhesiveness, it will remain submerged without any noticeable interference to the patient's oral hygiene habits. Both degradable and non-degradable forms of films have been developed. Those that release drug by diffusion alone are prepared using water insoluble non-degradable polymers, whereas those that release by diffusion and matrix erosion or dissolution use soluble or biodegradable polymers in the matrix.

2-1) Non-Degradable Films

The first descriptions of an intra-pocket, non-biodegradable matrix delivery device appeared in 1982. Addy et al [1985] described the use of matrix films of polymethylmethacrylate for the intra-pocket delivery of tetracycline, metronidazole and chlorhexidine. Self-polymerizing mixtures of the polymer, monomer, and the appropriate drug were cured, as sheets, under high pressure and then cut into suitable sized films. Studies showed that in vitro release profile and duration of release of drugs from acrylic films (10×1 ×0.5 mm) was dependent on the drug payload in the delivery system. The extent of in vitro release also depended on the nature of drug incorporated, with films containing 30%w/w chlorhexidine, tetracycline or metronidazole releasing 57.0, 40.0 and 96.6% of their drug load. They further described formulations delivering in vitro therapeutic levels of all three drugs over a 14 days period. Clinical and microbiological assessment of films containing 30% w/w drug have shown metronidazole containing strips to be more effective, but there has been no evaluation of in vivo release rates achieved in the gingival crevicular fluid [Polson et al., 1996].

In later studies they showed various degrees of clinical efficacy in vitro but these systems were found to be associated with a slower rate of relapse of clinical parameters and have not been developed for clinical use. Ethylcellulose matrix films for intra-pocket drug delivery has been described [Friedman and Golomb, 1982]. These films were made by casting ethanol or chloroform solutions of the polymer into molds and allowing the solvent to evaporate. The appropriate drug and plasticizing agent were incorporated into the solution prior to casting. The dried films (200 - 300 µm thick) were then cut into the required shapes. Films containing chlorhexidine, metronidazole , minocycline and tetracycline have been developed and tested to varying degrees. The release of the therapeutic agent from these filmsis dependent on the solvent used, the presence of a plasticizer, the nature and concentration of the drug in the film and on the physical dimensions of the film. Films cast from ethanol solutions containing 5% w/w chlorhexidine released 95% of the drug load over 10 days, whereas chloroform-cast films released 20% of drug load over a 205 day period

[Golomb et al., 1984]. This could be ascribed to the differential solubility of the drug in the casting solvents. Drug release from chloroform-cast films was modified by the addition of polyethylene glycol to the formulation. Golomb et al. [1984] described metronidazole-bearing films casted with PEG 3000 and concluded that the amount of crystalline water bound to the surface of the films increased with the inclusion of PEG. It was further suggested that enhanced release of drug was due to improved water binding to the surface of matrix films containing PEG. They also assessed the efficacy of periodic treatment with chlorhexidine-containing films in a 2-year study of maintenance of periodontal pocket and its bacterial load. Treatment was shown to produce significantly lower incidence of bleeding on probing, pocket depths and attachment levels when compared to the conventional maintenance treatment. The limitations of such delivery devices include the need for removal and replacement, as they did not degrade. Moreover, the drug load is released over 3 days. This meant that patients require repeated dental visits to complete treatment. On the other hand, less expertise is required than for scaling and plaque removal.

2-2) Degradable Matrix Films

Degradable delivery systems erode or dissolve in the gingival crevice so that removal after treatment is not required. Drug release occurs by erosion or dissolution and drug diffusion through the matrix. The contribution of each of these mechanisms to the overall rate of release can be varied. Sustained release profile can be engineered by appropriate manipulation of one or more release mechanisms. A number of biodegradable polymers have been investigated for the delivery of antimicrobial agents in the treatment of periodontal diseases, including hydroxypropyl cellulose, polyesters [Medlicott et al., 1992] and cross-linked collagens and protein films. Hydroxypropyl cellulose films containing chlorhexidine and tetracycline for intra-pocket drug delivery have been described. Release of the drug and dissolution of the polymer were found to occur over different time intervals. Device erosion is not the major mechanism responsible for initial drug release (nearly 80% in initial 2 hours), but probably accounts for the more gradual release seen from the device from 2 to 24 hours. Tetracycline levels of between 0.5 and 3.5 μg/ml were achieved in the

gingival crevicular fluid 24 hours after insertion of films containing 1% w/w tetracycline in hydroxypropyl cellulose. Reduction in probing depth, plaque index, gingival index, gingival index rate of bleeding and Bacteroids asaccharolyticus were reported with use of chlorhexidine (1% w/w) containing strips. A prolonged release of ofloxacin was obtained by incorporation of slowly soluble methacrylic acid copolymer S particles into hydroxypropyl cellulose films.

Collins et al. [1989] developed a slowly biodegradable compact using polyhydroxybutyric acid to deliver tetracycline in the treatment of pocket diseases. A pseudo-zero order release profile of tetracycline in vitro was recorded over a 9 days period with nearly 50% of the drug load being delivered over that period. Deasy et al. [1989] studied the effects of tetracycline hydrochloride and metronidazole released from 0.5 mm thick films formed by compacting a 15 mg mixture of the drug and polyhydroxybutyric acid in an infrared press. The in-vitro release rate of drug was found to be dependent on the drug load and the drug used. The films, although intact after 5 days in a buffer solution, became progressively more fragile with loss of mechanical strength. Clinically, filmsvcontaining 25% of either drug were placed into pockets at 4 days intervals of 16 days and their effect compared to untreated control pockets. In general, improvement in both clinical and microbiological parameters was noted over the 16 days of treatment, with a return to control levels on cessation of treatment. No information was provided on the in *vivo* survival time of the film. Amorphous poly(DL) lactic acid compacts of tetracycline were used for supergingival delivery in the treatment of gingivitis. Salivary tetracycline levels were maintained at greater than 1 µg/ml for 4 days and 0.5 µg/ml in the next 6days period. However, the clinical parameters could not be maintained upon the completion of the therapy.

The biodegradable polyester poly(e-caprolactone) has been tested in vitro as a matrix for sustained release delivery both as a fiber for the delivery of tetracycline and as a film for the delivery of chlorhexidine. Clinically the fibers released their tetracycline content very rapidly with a half life of 11 hours. In a further study Dunn and coworkers [1982] used poly (e-caprolactone) to coat fibers produced with poly(e-caprolactone), hydroxypropyl cellulose and polyethylene glycol and

found zero order release in vitro. They suggested that poly(e-caprolactone) and hydroxypropyl cellulose were most suitable for use as inner core material as these fibers were flexible and offered the greatest potential for effective drug delivery.

Different types of collagen-based membranes have also been tested as degradable devices for local drug delivery. Cross-linked atelocollagenbound protein (Byco™) has been investigated as possible carrier material for antibacterial agents in the management of periodontal pocket diseases [Minabe et a., 1989]. A degradable controlled release device based on a formaldehyde cross-linked Byco protein matrix containing chlorhexidine has been described. Byco™ protein is a hydrolysed gelatin of bovine origin. The release of chlorhexidine from this device and its dissolution in vitro were shown to be dependent on the degree of protein crosslinking. The nature of the chlorhexidine salt used also affected the release rate. Based on this study the Perio Chip (Perio Products Ltd, Jerusalem, Israel) has been developed for the controlled delivery of chlorhexidine subgingivally [Minabe et al., 1989]. This is a 5 mm ×4 mm × 0.3 mm film containing 2.5 mg of chlorhexidine gluconate. The crosslinked collagen films were shown to produce significantly higher improvements in the gingival index, pocket depth, incidence of bleeding on probing, density of subgingival microorganisms and spirochaetes for a period of 7 weeks with the maximum effects seen in the first 2 weeks. A collagen film containing 5% metronidazole was evaluated as an adjunct to scaling and root planning in a 3-month clinical trial. Apart from the dimension of the device (5 mm × 5 mm), no information was provided about the nature of the matrix, the release kinetics of the device or its degradability. These authors reported a significant adjunctive effect for the local metronidazole therapy on gingival index, bleeding on probing, probing pocket depth and attachment level when compared with scaling and root planing alone. Diplen-Denta biopolymer adhesive film with chlorhexidine has been used in for treating periodontal inflammation.

B) Microcapsules

Microcapsules are being used for the delivery of encapsulated antibacterial agents in treating periodontal disease. These are dissolution-controlled polymeric

reservoir devices, which may deliver their contents with a prolonged release profile in the salivary or crevicular fluid. Microcapsules prepared from lactic acid/glycolic acid copolymers have been proposed for delivery of tetracycline and minocycline. Baker et al. [1998] suspended tetracycline-containing microcapsules in a Pluronic F 127 gel. This material forms a gel at body temperature to hold the microcapsules in the periodontal pocket for the duration of the treatment. They showed that after administration of the gel containing microcapsules to periodontal pockets in monkeys, the concentrations in the gingival crevicular fluid could be maintained at effective levels for 3-4 days. On the other hand, administered minocycline microcapsules in a dry state to periodontal pockets of beagle dogs, and showed that an effective minocycline concentration was maintained for nearly 2 weeks.

C) Injectable Devices

Injecting a delivery system into the pocket has a number of advantages. It is a relatively simple procedure with little or no associated discomfort. The initially fluid formulation, which is necessary for its use with a syringe, allows the formulation to gain access to the entire pocket. In order to be retained in the pocket the formulation would need to change into a sticky semi-solid or solid so as to prevent it from removal by the GCF flow. Two different systems are commercially available. The first, a 2% Minocycline ointment (Dentomycin®, Cyanamid International, Lederle Division, Wayne, NJ and SunStar, Osajam, Japan), does not appear to have any sustained release properties. In one study this ointment was applied as an adjunct to scaling and root planing.

The second system (Elysol®, Dumex, Copenhagen, Denmark) is a controlled release delivery system. The liquid phase of this formulation consists of a water-free mixture of melted glycerol mono-oleate and metronidazole benzoate to which a triglyceride, sesame oil, has been added to lower the melting point in order to improve the flow properties of the gel in the syringe. When the mixture comes into contact with water it sets in a liquid crystalline state. The formulation contains 25% metronidazole as 40% w/w metronidazole benzoate. The solubility of the drug and its

concentration in the formulation influence its release profile. The matrix is degraded by neutrophils and bacterial lipases present in the GCF.

Concentrations of 103-1297 µg/ml of metronidazole were recorded with in inflamed pockets treated with this device, with effective doses being maintained for 24-36 h. Systemic levels of metronidazole between 0.2 and 1.3 µm/ml were measured after the administration of 29-103 mg of the gel. The recommended therapy is two separate applications into each pocket, one week apart [Tinanoff, Hock, and Hellden, 1980]. Clinical studies comparing this therapeutic approach alone, to scaling and root planing, indicate that the metronidazole gel results in reduction in probing pocket depth and bleeding on probing which are not significantly different from the results obtained with scaling and root planning. Some examples of products on the world market are presented in Table 3.

Table 3 List of commercial subgingival delivery systems

Trade name	Manufacturer	Candidate drug	Matrix	Biodegradability profile
Perio Chip®	Perio Products, Jerusalam, Israel	Chlorhexidine	Film	Biodegradable, dissolution time: 7–10 days
Actisite®	Alza Corp. Palo Alto, CA, USA	Tetracycline	Gel	Non-biodegradable
OnSite®	Alza Corp. Palo Alto, CA, USA	Antibiotic	Fibre	Non-biodegradable
Elysol®	Dumex, Copenhagan, Denmark	Metronidazole	Gel	Biodegradable
Dentomycin®	Lederle Div. Wayne, NJ, USA	Minocycline	Ointment	Formulation does not contain solid phases

9. Dental gel materials of local drug delivery system

Local delivery dental material should be release of drug achieves higher concentrations in periodontal pocket using a lower dosage with an associated reduction in side and toxic effects, non-allergenic and non-irritant. It should be widely used in oral controlled release pharmaceutical formulation. In addition, the remains of vehicles (e.g. suspension, solid or semisolid) in periodontal pocket are most importance. They are should be first considered while the drug is showed release properties. The vehicles with the best retention, ease of use, and lowest cost can be

used to deliver with any agent in periodontal pocket and this is an interesting concept that the nature of the implies vehicle determines clinical success.

Using biodegradable or non-biodegradable polymers could be controlled drug release delivery systems and produce concentration profiles that are constant and sustained release in the periodontal pocket during therapeutic periods. In addition, controlled delivery of antimicrobial agents can alter the periodontal flora with a decrease in total bacterial mass and pathogenic species. While future research will concentrate on developing more ideal and bio-friendly polymers and introducing novel agents, controlled delivery offers clinicians a potential adjunct or alternative to traditional treatments.

An advance in pathological researches on periodontal disease and pharmaceutical technologies, the local drug treatment for periodontal disease by sustained delivery systems, has been recently developed. However, the techniques are still unsatisfactory for clinical use because they consist of insoluble polymers such as ethylene vinyl acetate, polyethylmethacrylate. [Addy et al., 1985] These substances must be removed from the periodontal pocket after the completion of drug release. This occasionally causes local mechanical irritation and disturbs periodontal repair. To be useful for periodontal therapy, it is desirable to have a bioerodible drug delivery system that can maintain an effective drug release rate in the periodontal pocket while simultaneously eroding throughout the duration of treatment up to several days.

Recently, a thermosensitive polymer, Poloxamer 407, has been introduced as a gelling polymer in dental gel base because it exhibits the sol-gel transition temperature [Maheswari et al., 2006]. However, poloxamer is not biodegradable and the formed gel is dissolved in a few hours [Kellyb et al., 2004]. Moreover, toxicity of poloxamer has been reported when administered systemically into rat.

Generally, semi-solid (gel) formulations can indeed have some advantages (see Table 4). In fact, in spite of relatively faster release of the incorporated drug (with respect to fibers or microparticles), gels can be more easily prepared and

administered. Moreover, they possess a higher biocompatibility and mucoadhesivity, allowing adhesion to the mucosa in the dental pocket and, finally, they can be rapidly eliminated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site, including ease of application, good spreadability, appropriate hardness, and prolonged residence time in the periodontal pockets [Jones et al. 1997].

Table 4 Pharmaceutical characteristics of drug delivery systems for the treatment of periodontal diseases [Esposito et al., 1996]

Characteristic	**Gels**	**Solid devices (fibers or microparticles)**
Preparation method	Easy	Complex (instruments needed)
Bioadhesivity	Yes	No
Release period	Days, weeks	Weeks, months
Biodegradability	Yes	Yes[a]
Biocompatibility	Yes	Yes (risk of inflammatory or adverse reactions)
Application modality	Easily administrable by appropriate syringe and needles	Special syringe needed (microspheres) or application and removal by specialist (fibers)

a = Only by using biodegradable polymers

Appropriate materials for mucoadhesion are mainly hydrogel-forming polymers which are called wet adhesives because they require moisture to exhibit adhesive property [Jones et al., 1997]. Examples of bioadhesive polymers are cellulose derivatives, natural gums, sodium alginate, polyoxyethylenes and polyacrylates. One class of mucoadhesive systems used for controlled release of drugs in many pharmaceutical applications is represented by the poloxamers, which are nonionic poly (ethylene oxide)-poly(propylene oxide)- poly(ethylene oxide) triblock copolymers. Poloxamer 407 has been one of the most extensively used copolymers. It

has low toxicity, is compatible with other chemicals and can solubilize drugs with different physicochemical properties. Additionally, aqueous solutions of poloxamer 407, at concentrations of 20% and above, demonstrate a thermoreversible gelation behaviour, characterized by a critical temperature[Escobar-Chávez et al., 2006]. At temperatures under the critical one, the poloxamer solution is in the form of a low-viscosity, while above it, when approaching body temperature, a viscous transparent gel is formed.

A number of charged and neutral polymers have been classified as biomucoadhesives, since they are known to bind very strongly to mucus via non-covalent bonds. Carbomer or carbopol is a polyacrylic acid polymer, crosslinked with allyl sucrose. As a mucoadhesive polymer, carbomer has been investigated extensively by the pharmaceutical researchers because of its high viscosity at low concentration and low toxicity. In vitro experiment has proved that carbomer have good bioadhesion with the gastrointestinal mucus. Recently, drug delivery system in periodontal pocket widely used mainly carbopol and other mucoadhesive polymers aimed to remain inside the periodontal pocket and sustained the drug release property [Varshosaz et al., 2002].

Elyzol®, 25% dental gel contains metronidazole in the form of metronidazole benzoate as the active substance. It consists of a semi-solid suspension of metronidazole benzoate 411 mg in a mixture of glyceryl mono-oleate (GMO) 518 mg and triglyceride (sesame oil) 71 mg. It will flow freely when applied to the pockets. In contact with the gingival crevicular fluid (GCF) highly viscous liquid crystals are spontaneously formed in the gel. This prevents the gel from being easily expelled from the pockets. It is designed for application into gingival pockets. After application, the preparation acquires greater flowability and fills the pocket. On contact with the gingival fluid, it forms a highly viscous gel. This is slowly broken down and metronidazole is released gradually form the gel.

In this study, we developed a new periodontal gel base using mixtures of hydrophilic polymer such as carbopol, hydroxylpropylmethyl cellulose (HPMC),

hydroxylethyl cellulose(HEC), polyvinylpyrrolidone (PVP), polyvinyl alcohol(PVA), and Sodiumcarboxymethyl cellulose(NaCMC) and hydrophobic part such as hydrophobic polymer (ethylcellulose, Eudragit® RS, Eudragit® RL, polyethylene) and hydrocarbon compound (mineral oil, , glycerol monostearate(GMS), stearic acid, isopropyl myristate(IPM) and white soft paraffin (WS). Furthermore, we also used poloxamer, a nonionic triblock copolymers composed of a central hydrophobic chain and two hydrophilic chains, in development and formulation of periodontal gel base to controlled drugs delivery system.

CHARPTER III

EXPERIMENTAL

Materials

All materials employed in this study were obtained from commercial sources and as received.

- Aerosil® (Lot No. Zb55869, Maxway, Germany)
- Carbopol 940 (Lot No. 182654, distributed by Rama Production Co., Ltd., Thailand)
- Ethanol, 95% (Lot No. 04579, Sappasamitr, Thailand)
- Ethylcellulose (Lot No. 9004-57-3, distributed by Rama Production Co., Ltd., Thailand)
- Eudragit® RS-100 (Lot No. 06-90261, Rohm Pharma, Germany)
- Eudragit®RL-100 (Lot No. 0860408267, Rohm Pharma, Germany)
- Fetal bovine serum (Life Technology, Paisley, Scotland)
- Glyceryl monostearate (Lot No. 20609, distributed by Srichand United Dispensary Co., Ltd., Thailand)
- Hemin (Sigma Chemical Co., St. Louis, MO, USA)
- Hydroxypropylmethyl cellulose, Methocel E15 (Lot No. 122635, distributed by Rama Production Co., Ltd., Thailand)
- Hydroxyethyl cellulose 4000 (Lot No. H1324, distributed by Aek Trong, Thailand)
- Isopropyl myristate (Lot No. 405657/1, Fluka Chemical, USA)
- Methanol, HPLC grade (Burdick & Jackson, USA)
- Metronidazole (Batch No. 07120701, Siam Pharmaceutical Co.,Ltd., Thailand)

- Mineral oil (Lot No. 532504, distributed by Srichand United Dispensary Co., Ltd., Thailand)
- Poloxamer 407 (Lutrol® micro 127 MP) (Lot no. w029851, BASF, USA)
- Polyethylene, MW = 1500 (Lot No. 562878, distributed by Rama Production Co., Ltd., Thailand)
- Polyvinylpyrrolidone K_{90} (Lot No. P6738, distributed by Rama Production Co., Ltd., Thailand)
- Polyvinyl alcohol, MW = 125000 (Lot No. K33974902.VWR International Ltd.,UK)
- Potassium dihydrogen ortho-phosphate (Lot No. AF501339, Ajax Finechem, Australia)
- Sodium carboxymethyl cellulose (Lot No.7532C6, distributed by Aek Trong, Thailand)
- Sodium hydroxide (Lot No. 64271, Darmstadt, Germany)
- Stearic acid (Lot No. 459043, distributed by Srichand United Dispensary Co., Ltd., Thailand)
- Tinidazole (Lot No.TNZ/70615, Pharmaland (1982) Co., Ltd.,Thailand)
- Triethanolamine (Lot No.SF19730201, distributed by Srichand United Dispensary Co., Ltd., Thailand)
- Trypticase soy broth and Trypticase soy blood agar (BBL Microbiology Systems)
- Vitamin K (Alantic Laboratories, Corp., Ltd., Thailand)
- White soft paraffin (Lot No. 407622, Government Pharmaceutical Organization, Thailand)
- Ultrapure water equipped with filter system (Balson, Balson Inc., USA)

Equipment

- Analytical balance (Satorius, A200S, Germany)
- GasPak system (BBl Microbiology System, Cockeysville, MD, USA
- High performance liquid chromatography (HPLC) (Model SCL-10A VP, Shimadzu, Japan)
  - Degasser (Model DGU-14A, Shimadzu, Japan)
  - Pumb A, B liquid chromatography (Model LC-10AD vp, Shimadzu, Japan)
  - Auto injector (Model SIL-10A vp, Shimadzu, Japan)
  - Column oven (Model CTO-10AS, Shimadzu, Japan)
  - UV-VIS detector (Model SPD-M10A, Shimadzu, Japan)
  - System controller (Model SLL-10A vp, Shimadzu, Japan)
- Hot air oven (Model B7600, Mammert, USA)
- Instron® universal testing machine (Model 5565 H124, UK)
- Magnetic stirrer (Variomay multipoint, Komet, Taiwan)
- pH meter (Model 210A+, Thermo Orion, Germany)
- Stereo Microscope (Model ML 9300, Meiji, Japan)
- Viscometer (Rotovisco1, Germany)
- Vortex mixer (Model G 560E,Vortex-genie, USA)
- Water bath (Model 010T2 , Hetotherm®, Birkeroed, Denmark)
- Ultrasonic bath (Transsonic digitals, Elma®, Germany)

Glassware and Miscellaneous

- Dessicator Cabinet
- 0.45 nylon membrane filter (Waters, USA)
- Aluminium foil (MMP Packing, Thailand)
- Beaker (Pyrex, USA)
- Cylinder (Pyrex, USA)

- Lock tip syringe (3 ml) and needle (21G×1′) (Terumo, Thailand)
- Micropipette and disposable pipette tip (Socorex, Switzerland)
- Parafilm (American National Can., USA)
- Petei dish (10 cm diameter)
- Transfering pipette (Witeg, Germany)
- Volumetric flask (Pyrex, USA)
- *Porphyomonas gingivalis* ATCC 53978 (W50)
- Porcine intestinal mucosa

Methods

1. Formulation study design for appropriate periodontal gel base system

1.1 Preparation of periodontal gel bases

Since, there are various approaches for the production of periodontal gel bases, this study was to investigate the requirements and to development drug delivery system to be inserted into the periodontal pocket. According to polymers and excipients used; the investigated periodontal gel base systems would be classified to 3 categories; hydrophilic, hydrophobic and hydrophobic-hydrophilic periodontal gel bases. The compositions in each system are shown in Table 1- 3, respectively**.**

In addition, all preparations of this study were packed in the aluminum tubes and stored in desiccators at room and refrigerated temperature before further studies.

1.1.1 Preparation of hydrophilic gel base (System 1)

a) System 1-1 (Hydrophilic polymers)

In this system, all formulations contained carbopol with or without other hydrophilic polymer. Weighed quantity of carbopol 940 was taken and added to distilled water. The dispersion was stirred gradually and carbopol 940 was allowed to soaked for 2 hr. Triethanolamine was added to neutralize the carbopol solution and to form the gel. Sodium carboxymethyl cellulose (NaCMC) and polyvinylpyrrolidone (PVP) were mixed after carbopol was swollen with distilled water. Hydroxyethyl cellulose (HEC), polyvinyl alcohol (PVA) and hydroxylpropylmethyl cellulose (HPMC) were added to 75°C distilled water until clear solution and then were mixed with carbopol. Finally, the pH was adjusted to 6.8 with triethanolamine for further studies.

b) System 1-2 (Thermoreversible polymer)

In this system, poloxamer was used as thermoreversible polymer. The Thermoreversible gel or thermosetting gel base was prepared according to the "cold technique" [Sagrado et al., 1995]. A weighed amount of poloxamer® 407 was gradually added to cold water (5-10°C) under magnetic stirring up to a final concentration of poloxamer. Each dispersion in the containers were sealed and left in a refrigerator overnight at 4-6°C. Silicon dioxide (Aerosil®) was added to the gel and homogenously mixed.

c) System 1-3 (Hydrophilic-thermoreversible polymers)

Hydrophilic gel (System 1-1) was prepared in the total of concentration of the polymer and homogenously mixed with the preformed thermoreversible gel (System 1-2) before further studies.

1.1.2 Preparation of hydrophobic gel base

a) System 2-1 (Polyethylene gel)

In this system, a thermoplastic polymer, polyethylene which is generally used in oral base, is formulated. Polyethylene bead and mineral oil were mixed and heated to about 90–95°C to form a viscous liquid base. Silicon dioxide was pulverized and only the portion with particle size < 63 mm was dispersed throughout the viscous liquid base. The mixture was slowly cooled with constant stirring until congealed.

b) System 2-2 (EC-R gel)

Ethylcellulose and acrylate polymers which are two hydrophobic commonly used polymers for pharmaceutical products were combined in this system. Each ingredient, Ethylcellulose, Eudragit® RS and Eudragit® RL, were dissolved in 95% ethyl alcohol to form a viscous liquid base under magnetic stirring up to a final concentration. The formulated gel bases were stored in desiccators at room temperature and refrigerated temperature before studies.

1.1.3 Preparation of hydrophobic-hydrophilic gel base

a) System 3-1 (EC-R-PVP gel)

PVP, hydrophilic polymer, was added and homogenously mixed in the ethylcellulose-Eudragit gel base preparation (System 2-2).

b) System 3-2 (EC-R-PVP-Plo gel)

Poloxamer®407 (micronized form) was added and homogenously mixed in the ethylcellulose-Eudragit gel base (System 2-2) with or without PVP.

c) System 3-3 (EC-R-WS-PVP gel)

White soft paraffin or vaseline, a semi-solid hydrocarbon was added and mixed with ethylcellulose-Eudragit-PVP gel base (System 3-1) until homogeneous dispersion were formed.

d) System 3-4 (EC-R-PVP-Plo-WS gel)

Poloxamer was added and homogenously mixed with ethylcellulose-Eudragit-PVP gel base (System 3-1). White soft paraffin was added and mixed until homogeneously dispersion.

e) System 3-5 (Emulsion gel)

Carbopol 940 was dispersed in 75°C distilled water as water phase by using a mechanical stirrer. Glyceryl monostearate, steric acid, isopropyl myristate and mineral oil as oil phase were heated to 70°C in casserole. Thereafter, the oil phase was transferred into the water phase and stirred until the formulated gel base was congealed.

Table 5 Composition in hydrophilic periodontal gel bases (System 1)

Group	Formulation code	Composition (%w/w)								
		HEC	**HPMC**	**PVA**	**PVP**	**CMC**	**CP**	**Plo**	**Ae**	**W (q.s. to)**
Hydrophilic gel (System1-1)	C_1						1			100
	C_5						5			100
	C_{11}						11			100
	$HEC_{10}C_1$	10					1			100
	$HPMC_{10}C_1$		10				1			100
	$PVA_{10}C_1$			10			1			100
	$PVP_{10}C_1$				10		1			100
	$CMC_{10}C_1$					10	1			100
Thermoreversible gel (System1-2)	P_{L20}							20	0.5	100
	P_{L40}							40	0.5	100
	$P_{L20}A_2$							20	2	100
	$P_{L20}A_5$							20	5	100
Hydrophilic-thermoreversible gel (System 1-3)	$P_{L20}C_1$						1	20	0.5	100
	$P_{L20}C_5$						5	20	0.5	100
	$P_{L\ 20}HEC_5$	5						20	0.5	100
	$P_{L20}HPMC_5$		5					20	0.5	100
	$P_{L20}PVP_5$			5				20	0.5	100
	$P_{L20}PVA_5$				5			20	0.5	100
	$P_{L20}CMC_5$					5		20	0.5	100

where CP= Carbopol 940, HEC= Hydroxy ethyl cellulose 4000, HPMC= Hydroxy propyl methyl cellulose E15, PVA= Poly vinyl alcohol, PVP= Polyvinyl pyrrolidone K90, CMC=Carboxy methyl cellulose, Plo = Poloxamer® 407, Ae = Aerosil® , W = ultrapure water

Table 6 Composition in hydrophobic periodontal gel bases (System 2)

Group	Formulation code	Composition (%w/w)						
		PE	**Ae**	**EC**	**RS**	**RL**	**MN (q.s. to)**	**Alc (q.s. to)**
Polyethylene gel base (system 2-1)	P_{E5}	5					100	
	P_{E10}	10					100	
	$P_{E5}A_{1.5}$	5	1.5				100	
	$P_{E5}A_{3}$	5	3				100	
EC-R gel base (system 2-2)	ER_S-1			12.5	25			100
	ER_S-2			17.5	25			100
	ER_{SL}-3			12.5	22.5	2.5		100
	ER_{SL}-4			12.5	20	5		100

where PE = Polyethylene, Ae = Aerosil® , EC = Ethylcellulose, RS = Eudragit® RS-100, RL = Eudragit® RL-100, MN = Mineral oil, Alc = Ethanol, 95%

Table 7 Composition in hydrophobic- hydrophilic periodontal gel bases (System 3-1 to system 3-4)

Group	Formulation code	Composition (%w/w)						
		EC	**RS**	**RL**	**Plo**	**WS**	**PVP**	**Alc (q.s. to)**
EC-R-PVP gel base system 3-1	ER_SP_v	12.5	25				2	100
EC-R-PVP-Plo gel base system 3-2	ER_SP_L	12.5	25		20			100
	$ER_SP_LP_v$	12.5	25		20		0.75	100
EC-R-WS-PVP gel base system 3-3	ER_SP_vW-1	12.5	25			20	0.75	100
	ER_SP_vW-2	12.5	25			20	2	100
	ER_SP_vW-3	12.5	25			25	2	100
	ER_SP_vW-4	15	25			25	2	100
	$ER_{SL}P_vW$-1	12.5	22.5	2.5		25	2	100
	$ER_{SL}P_vW$-2	12.5	20	5		25	2	100
	$ER_{SL}P_vW$-3	12.5	20			25	2	100
EC-R-PVP-Plo-WS gel base system 3-4	$ER_{SL}P_LP_vW$-1	12.5	22.5	2.5	20	5	2	100
	$ER_{SL}P_LP_vW$-2	12.5	22.5	2.5	5	20	2	100

where EC = Ethylcellulose, RS = Eudragit® RS-100, RL = Eudragit® RL-100, Plo = Poloxamer 407, WS = White soft paraffin, PVP = Polyvinylpyrrolidone**,** Alc = Ethanol, 95%

Table 8 Composition in hydrophobic- hydrophilic periodontal gel bases (System 3-5)

Group	**Formulation code**	**Composition (%w/w)**				
		CP	GMS	ST	IPM	MN
Emlsion gel base system 3-5	EG-1	1	1	1.25	1.5	2.5
	EG-2	1	2	2.5	3	5
	EG-3	5	2	2.5	3	5

where CP = Carbopol 940, GMS = Glyceryl monostearate, ST = Stearic acid, IPM = Isopropyl myristate**,** MN = Mineral oil

1.2 Physicochemical characterization

1.2.1 Physical appearance

The visual observation of each periodontal gel was initially recorded and general properties of each system such as color (transparent, clear or turbid gel), viscosity (stiffness, viscous, homogeneous or syneresis), appearance (lamellar gel, liquid, semisolid, jelly-like, rigid gel or ringing gel) were described. After preparation for one week, selected formulations were reevaluated.

1.2.2 Viscosity measurement

The viscosity of selected periodontal base gel as monitored by a HAAKE RotoVisco 1 using at least two different spindle number (No. 35 and 60) depending on sample viscosity. All formulations were measured at 25°C. The thermoreversible gel was particularly measured again at 37°C. Measurement was performed by interval of start until 100 seconds. Then the relationship between time and viscosity was plotted to show the rheological property of samples. Triplicate measurements of each sample were performed.

1.2.3 Syringeability

Syringeability was tested to measure the force required (in terms of force profile) to evaluate the periodontal gels from periodontal syringes using Instron® universal testing machine. Each formulation (1 ml) was packed into the periodontal syringe (syringe volume as 3 ml) and locked with clamp. The speed at 10 mm/min was used toward the plunger of periodontal syringe. Then the relationship between distances that the entire periodontal gel passed through a 21-gauge needle of diameter 0.3 mm and force was plotted to show the resistance property of sample. Triplicate measurements of each sample were performed.

1.2.4 Spreadability

Spreadability was tested to measure the gel movement under the force or pressure. One g of all gel preparations was pressed between two circular plates of 20 cm^2, of which 200 g of weight was placed over plate at room temperature (26-31 °C) and then measure the spreading distances after 5 minutes. Thermoreversible gel base in poloxamer were especially reevaluate at 37°C. Each measurement was repeated at least 6 times.

1.2.5 *Ex vivo* mucoadhesion time

The study was approved by Chulalongkorn Ethical Animal Care and Use Committee and conducted in accordance with the Declaration of Chulalongkorn University. The *ex vivo* adhesion time was performed in triplicate after application of periodontal gel on fresh cut porcine intestinal mucosa (size: 1.5×1.5 cm). The porcine intestinal tissues were fixed on the microscope slide (slanted to 45°) with plastic clamp. The mucosal side was flushed with 6.66 ml/min and 20 ml/min of 37±0.5 °C phosphate buffer pH 6.8 until periodontal gel base was removed. During the experiment, the time was recorded.

1.2.6 *Ex vivo* mucoadhesion force

The determination of the adhesive force of the prepared periodontal gel base was performed using an Instron® universal testing machine equipped with a 5-kN load cell. The ex vivo adhesion force was performed after. Afterward, A circular of internal side of fresh cut porcine intestinal mucosa was attached to the lower and upper support using aluminium clamp. The surface of porcine intestinal mucosa was wetted with PBS pH 6.8, then apply of 0.2 g periodontal gel on porcine mucosa and was brought immediately into contact with an initial force, the gap was set at 0.5 mm, for 1 minute. The whole experiment was performed at room temperature. The upper support was withdrawn at a speed of 10 mm/minute. During the experiment, the force was recorded. Each measurement was repeated at least 6 times.

1.2.7 *In Vivo* periodontal adhesion

Selected periodontal gel bases were incorporated with 5% metronidazole and tested in this experiment. The formulated periodontal gel bases were selected from good appearance, high syringeability, low viscosity, good of spreadability. In this study, the periodontal gel bases were selected in all groups of system, hydrophilic, hydrophobic and hydrophilic-hydrophobic were evaluated.

For each preparation, three healthy adult volunteers, aged between 35 and 60 years old. All subjects presented chronic periodontal disease and good systemic health conditions. They also presented at least 3 periodontal sites with probing depth $\geq$ 4 mm. All participants were informed about the nature of the study and gave their consent by signing an informed consent form. The study was approved by Chulalongkorn Ethical Committee and conducted in accordance with the Declaration of Chulalongkorn University. The volunteers were required to rinse their mouth with water before selected periodontal gel was inserted to periodontal pocket. The periodontal gel was packed into 3 ml lock tip syringe approximately 0.5 ml with a blunt curve needle. Top of the needle was inserted at the bottom of the periodontal pocket, then the periodontal gel was pressed by plunger and the needle was moved up carefully. Especially for hydrophobic base, the volunteers had to keep 15-30 ml of drinking water in their mouth for 3 minutes after finished. The volunteers were not allowed to drink, chew and eat anything for 5 hours. Thereafter, they could drink, chew and eat normally but teeth brushing had to be soft and smooth. All experimental design was noticed and tested by a periodontist at the Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. Duration that periodontal gels could remained inside periodontal pocket were evaluated at 5 and 24 hours.

1.2.8 *In Vitro* Release Studies

Suitable Periodontal gel base that required to periodontal gel had to remained inside periodontal pocket more than 24 hours with, good appearance, high syringeability, low viscosity and good of spreadability. The selected formula was performed by varying concentration of metronidazole incorporate into selected periodontal gel base system from 5%, 10%, 20% and 40%w/w MTZ. The dissolution of selected periodontal gel base was studied using a thermostated horizontal shaker (Hetotherm®) containing 15 ml of phosphate buffer (pH 4.7) as a medium in 100 ml beakers and maintained the temperature at 37 ± 0.5 °C with 16 rpm stirring. After the medium had equilibrated at the experimental temperature. The sample was weighed to about 0.2 g and inserted to a gap of double screen (sieve #40; size of each screen: 1.5×1.5 cm) and then placed in the medium. Samples of dissolution fluid (1 ml) were collected periodically and replaced with a fresh dissolution medium. After filtration through 0.45µm filter paper, metronidazole concentration was determined spectrophotometrically at 275 nm. The experiments were done in triplicate.

1.2.9 Disappearance property

This experiment was set and designed to investigate the tolerant property of periodontal gel base in rich of water and dynamic movement condition. This model easily to set and uncomplicated, but it could be exhibited the appreciate periodontal gel base system and planned to treatment in the suitable time. Formulated periodontal gel base of 0.5 g was put to the center of bottom of 150 ml-beaker. The periodontal gel was spread to 1 cm in a diameter. Phosphate buffer pH 6.8 of 100 ml was added. The beaker was shaken the rate at of 80 rpm by Hetotherm®. The temperature of s ystem was 37 ± 0.5 °C. The time was recorded when the periodontal gel base was disappeared.

1.2.10 Weight Loss Study

Weight loss is a most importance to seriously considered the possibility of system degradation. It was due to drug release property, drug remain in periodontal pocket site, erosion time and drug efficiency. Especially in the formulation that have to contained a higher amount of drug dispersion, it might be affected and destroyed by weight loss due to erosion of its component.

The selected hydrophobic periodontal gel had to remain inside periodoantal pocket more than 24 hours with, good appearance, high syringeability, low viscosity and good of spreadability. The selected hydrophobic periodontal gel was accurately weighed (Wi) to about 0.2 g and inserted to a gap of double screen (sieve #40; Pieces of screen: 1.5×1.5 cm) and immersed in 30 ml phosphate buffer pH 6.8 at 37 ± 0.5°C with a thermostated horizontal shaker (Hetotherm®) at 80 rpm. After 24 hours, the piece of hydrophobic periodontal gel was removed and kept in a desiccators over anhydrous calcium chloride for 7 days prior to being reweighed (Wf). The amount of drug released in the medium after 24 hours (Wr) was analyzed spectrophotometrically at 275 nm. Each experiment was performed in triplicate. The weight loss was calculated according to the following Equation 1 [Srinatha et al., 2006]:

$$\text{Weight loss \%} = \frac{(Wi - Wr) - Wf}{Wi} \times 100 \quad \text{……..(1)}$$

1.2.11 Anti-microbial activity of selected periodontal gel with or without 5% metronidazole

P. gingivalis ATCC 53978 (W50) was cultured anaerobically in a GasPak system at 37°C. Bacteria were grown overnight in trypticase soy broth supplemented with 5% fetal bovine serum, 5 mg/l hemin and 0.1 mg/l vitamin K. Turbidity of bacterial suspension was adjusted to a 0.5 McFarland standard. Sterile cotton swabs were used to streak the bacteria on trypticase soy blood agar

supplemented with 10% human whole blood, 5 mg/l hemin and 0.1 mg/l vitamin K. Two holes (2 mm diameter) were punched into the agar. 0.02 g of selected periodontal gel base or selected periodontal gel base containing 5% MTZ was placed into each hole. The bacteria were then incubated at 37°C for 24 h. The inhibition zones were measured in millimeters. The experiment was performed in duplicate.

1.2.12 Stability testing

Both selected periodontal gel base and selected periodontal gel base containing 5% metronidazole were observed at room temperature (26-31°C), refrigerated temperature (4-6°C), 45°C and 75% RH (Thai FDA stability), and accelerated condition (heating and cooling) at 4ºC for 48 hours and 45ºC for 48 hours for 6 cycles) [Prince, 1977]. The physical properties of selected formulations were studied and compared with those before stability testing. Stable systems were identified as those free of any physical change such as phase separation, syneresis, precipitation, discoloration, viscosity change and appearance. The stability testing was assessed after storage for 1 week, 1 month and 6 months.

1.2.13 Morphology observation

The surface and cross-sectional morphology of the hydrophobic periodontal gel bases prepared from ethylcellulose, Eudragit® RS and Eudragit® RL were observed using a stereo microscope. Prior to observation, samples were wetted by 37±0.5°C phosphate buffer pH 6.8 and pressed between two circular agar plate, of which 200g weight was placed over plate at room temperature for 8 hours. Thereafter, the samples were cut into two halves with blades and washed out with distilled-water before examination.

1.2.14 Assay of Metronidazole Content of the Gels

Weighed quantity of Gels as 0.2 g of preparations was transferred to 10 ml of 60°C water and mixed with alcohol 15 ml for 5 minutes. After filtration, 0.5 ml of the solution was diluted to 5 ml with water and mixed for 10 minutes, and the absorbance of the solution was measured at 275 nm by HPLC

High Performance Liquid Chromatographic (HPLC)

HPLC conditions

Column:	Hypersil® BDS(C18) column(150x4.6mm) 5 µm (Thermohypersil, UK) equipped with guard column packed with BDS(C18), 5 µm set at an ambient temperature
Detector:	UV detector at 275 nm
Injection volume:	20 µl
Flow rate:	1 ml/min
Mobile phase:	Ultrapure water :Methanol (80 : 20)

Mobile phase was filtrated through a membrane filter with a pore size of 0.45µm and degassed for at least 30 minutes prior to use.

Validation of HPLC method

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

Specificity

The specificity of the active constituent peak was determined by the resolution and tailing factor. The well resolved from the other peaks and symmetry of the peaks should be obtained. The standard solution of metronidazole/tinidazole in phosphate buffer pH 4.7 at the concentration 63.6 µg/ml was prepared and evaluated using chromatographic condition as describe above.

Linearity

Triplicate injections of solutions containing drug in various concentrations from 0 to 106 µg/ml in phosphate buffer pH 4.7 was prepared and analyzed. The linear equation of curve obtained by plotting the peak area at each level prepared versus the concentration of each standard was calculated using the least square method.

Precision

a) Within run precision

The within run precision was determined by analyzing three sets of five standard solutions of metronidazole and tinidazole in the same day. The coefficient of variation of the peak area response (%CV) for each concentration was determined.

b) Between run precision

The between run precision was determined by comparing each concentration of metronidazole/tinidazole standard solutions prepared and injected on different days. The percentage coefficient of variation (%CV) of metronidazole/tinidazole of peak area response from three sets of standard solutions on different days was calculated.

Accuracy and recovery

The recoveries of metronidazole/tinidazole from placebo were assessed by spiking placebo (periodontal gel base containing all the components except the drug) with metronidazole/tinidazole and following the extraction procedures described earlier. Placebo was spiked in triplicate at three level spanning 80-120% of the amount of metronidazole/tinidazole in dosage form. The average recovery and the coefficient of variance were calculated.

System suitability

System suitability tests were used to verify that the resolution and reproducibility of the chromatographic system were adequate for analysis to be done.

1.3 Statistical Analysis

The rate of release of metronidazole (k), the dissolution efficiency [Umesh, 1992], the correlation coefficient of different kinetic models of release data, and mechanical properties were evaluated statistically using t test, one -way analysis of variance (ANOVA). Post-hoc statistical analysis of the means of individual groups was performed using Fischer's least significant difference test ($P < 0.05$ denoting significance) using SPSS (Version 13) computer software.

CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation study design for appropriate periodontal gel base system

1.1 Preparation of drug-free for formulated periodontal gel

Physical appearance

The physical appearance of drug-free periodontal gel by visual inspection is listed in table 9. Initially, all preparations were homogeneously viscous to semisolid gels. After storage of all formulations at room and refrigerated temperatures for 1 week. The physical appearance was affected by storage condition and phase separation occurred especially viscosity property and system compatibility. Furthermore, more pronounced effect was seen in long term stability of 1 month and 6 months.

The appearance of each formulation depended on the characteristic of component used in formulation. The characteristics of periodontal gel base were classified according to 3 characters; clarity, viscosity and homogeneity. The results of all systems revealed that the clarity of the product was depended on the main composition of gel. The periodontal gel base systems from carbopol (CP), hydroxylethyl cellulose (HEC), hydroxylpropylmethyl cellulose (HPMC) and poloxamer (Plo) were colorless and clear transparent gel while systems from carboxy methyl cellulose (CMC), polyvinylpyrrolidone (PVP) and the combination of ethylcellulose (E), Eudragit®RS-100 (RS) and Eudragit® RL-100 (RL) had colourless to clear yellowish color. The systems containing polyvinyl alcohol (PVA) showed white to cream-colored appearance of powder. Polyethylene (PE) with mineral oil was turbid gel as solid dispersion might be presented. Addition of white soft paraffin (WS), a white translucent jelly from semisolid mixture of hydrocarbon compound,

resulted in translucent gel. Aerosil (Ae), a fine powder, water insoluble polymer, could obtain turbid gel at concentration of Aerosil > 0.5 % . In addition, all emulsion gels were opaque due to the size of oil droplet.

Furthermore, the amounts of component also affect the characteristic or appearance of final product especially the viscosity property. The system that composed higher percentage of Aerosil had the final appearance of viscous or highly viscous gel. Different type of polymer also showed different viscosity. Moreover, the viscosity property was affected by temperature in formulation that composed of poloxamer and white soft paraffin. In this study, the viscosity property showed to depend on the concentration of adhesive polymers, amount of each component and storage temperature condition.

The viscosity of poloxamer gels (Figure 11) was affected by storage temperature. The characteristic of poloxamer or Lutrol® that formed liquid-viscous gel at refrigerated temperature and changed to viscous gel when approaching to room temperature could be seen. In this study, poloxamer was used at the concentration above 20% and could form thermoreversible gels at the critical temperature (T_c). Esposito et al [1996] described that Tc of average 20% poloxamer in gel preparation was about 15.7°C. At the temperature below Tc, system of poloxamer showed low viscosity while at above Tc, transparent viscous gel could be formed. The sol-gel transition is a reversible process depended on the environmental showed an unstable temperature.

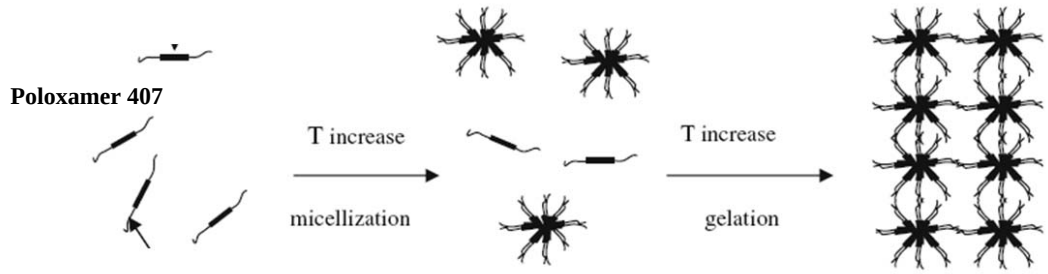

Figure 11 Schematic representation of the association mechanism of poloxamer 407 in water [Dumortier, 2006].

The stability of the formulated hydrophobic gel base prepared from white soft paraffin (WS) containing water insoluble polymer such as EC, RS and RL could show phase separation. The motion caused to phase separation due to thermal energy and motion energy. This might related the melting point of WS as ranges from 38 to 60 °C [Sean, 1993] that sensitive to room temperature. The system could produce high kinetic energy at room temperature and produced a property of a moving of two phases depended on heat energy environment. In addition, white soft paraffin is practically insoluble in water and ethanol [Raymond, 2003]. Therefore, the combination of white soft paraffin and water or ethanol could obtain immiscible system according to increasing of motion energy. Consequently, the suitable storage condition of these hydrophobic gel formulations was to retain the system at refrigerated temperature.

Phase separation was also occurred after 1 week at both storage temperature in **ER_SW** (12.5% E, 25% RS and 20% WS) , **ER_SP_vW-1** (12.5% E, 25% RS, 20% WS and 0.75% PVP), **ER_SP_vW-2** (12.5% E, 25% RS, 20% WS and 2% PVP) , **$ER_{SL}P_vW$-3** (7.5% E, 25% RS, 25% WS and 2% PVP), **$ER_{SL}P_vW$-4** (12.5% E, 20% RS, 25% WS and 2% PVP), **$ER_{SL}P_vW$-5** (12.5% E, 20% RS, 15% WS and 2% PVP) and **$ER_{SL}P_LP_vW$-3** (6.25% E, 11.25% RS, 1.25% RL, 20% Plo, 20% WS and 2% PVP). The phase separation of **ER_SW** could be produced by phase differential between EC and RS as a mixture of liquid-viscous phase and WS as a semi-solid phase.

According to phase separation of **ER_SW**, the system of **ER_SW** was then incorporated with 0.75 % PVP (**ER_SP_vW-1**). Although the viscosity was increased, this system still show separation at room and refrigerated temperature after 1 week. This revealed that the viscosity promotion of whole system might not be enough. As the concentration of the PVP increased from 0.75% to 2%, phase separation did not occur. The system showed stability property after 1week at refrigerated temperature, except at room temperature, separation was still occurred. This study suggested that this system had to have high or extremely high in viscosity, but syringeability had to be also considered. The increasing of physical stability was possible to enhance by static action that achieved by increasing the concentration of adhesive polymer or its

component and/or to keep the preparation in static condition in refrigerated temperature.

For **$ER_{SL}P_vW$-3**, decreasing concentration of RL from 5% to 0%, decreased the viscosity to liquid-viscous or slightly viscous gel. Finally, phase separation of this formulation occurred at room and refrigerated temperatures. The result evidently supported that the viscosity property was an important factor to increase stability in this system.

Viscosity test

In this study, most preparations showed viscous to highly viscous appearance. The viscosity property of polymeric gel was increased when increasing the concentration of polymers [David, 1997]. According to Varshosaz, Tavakoli, and Saidian [2002], increasing the concentration of hydrophilic polymers could control drug release while the viscosity was also increased. In addition, *in vitro release* study by diffusion cell method , the formulation containing the combination of 20% (w/w) CMC and 10% (w/w) methylcellulose (MC) was shown higher viscosity at 26×10^3 cps and obtained 50% of metronidazole (MTZ) release at 40.76 ± 2.04 hours more than the formulation containing the combination of 10% (w/w) CMC and 10% (w/w) MC at 16.07 $\times10^3$ cps and 22.77 ± 0.51 hours, respectively.

In preliminary study, the suitable viscosity values was evaluated though the recommended syringeability by periodontists. The appropriate formulations should be low viscous gel having newtonian flow and exhibited lower syringeability force [Vashosaz et al, 2002]. In general, the viscosity and syringeability force is closely similar behavior that shows resistance of gel movement. When increasing the viscosity, the syringeability force was also increased. All preparations of hydrophilic gel (System 1-1 to 1-3) were tested. The appropriate viscosity value was concluded to have newtonian or non-newtonian flow with lower yield value approximately ≤ 30 $\times10^3$ cps.

Table 9 Appearance and stability test after 7-days storage for equilibrium at room (25-31°C) and refrigerated temperature (4-6 °C)

Group	Formulation code	Physical appearance					
		Room temperature			Refrigerated temperature		
		Clarity	Viscosity	Homogeneity	Clarity	Viscosity	Homogeneity
System 1-1	C_1	1	++	H	1	+++	H
	C_5	1	+++	H	1	++++	H
	C_{11}	1	++++	H	1	S	H
	$HEC_{10}C$	1	S	H	1	R	H
	$HPMC_{10}C$	1	++++	H	1	S	H
	$PVA_{10}C$	1	+++	H	1	++++	H
	$PVP_{10}C$	1	+++	H	1	++++	H
	$CMC_{10}C$	1	++++	H	1	S	H
System 1-2	P_{L20}	1	+++	H	1	++	H
	P_{L40}	1	++++	H	1	+++	H
	$P_{L20}A_2$	2	+++	H	2	++	H
	$P_{L20}A_5$	2	++++	H	2	+++	H
	$P_{L20}C_1$	1	++++	H	1	+++	H
	$P_{L20}C_5$	1	S	H	1	++++	H
	$P_{L20}HEC_5$	1	++++	H	1	+++	H
	$P_{L20}HPMC_5$	1	++++	H	1	+++	H
	$P_{L20}PVA_5$	2	++++	H	2	+++	H
	$P_{L20}PVP_5$	1	+++	H	1	++	H
	$P_{L20}CMC_5$	1	++++	H	1	+++	H
System 2-1	P_{E5}	2	++	H	2	+++	H
	P_{E10}	2	++++	H	2	S	H
	$P_{E5}A_{1.5}$	2	+++	HD	2	++++	HD
	$P_{E5}A_3$	2	+++	HD	2	++++	HD

Table 9 Appearance and stability test after 7-days storage for equilibrium at room (25-31°C) and refrigerated temperature (4-6 °C) (to be continued)

Group	Formulation code	Physical appearance					
		Room temperature			Refrigerated temperature		
		Clarity	Viscosity	Homogeneity	Clarity	Viscosity	Homogeneity
System 2-2	ER_S-1	1	+	H	1	+	H
	ER_S-2	1	+	H	1	+	H
	ER_S-3	1	+	H	1	+	H
	ER_S-4	1	+	H	1	+	H
System 3-1	ER_SP_v	1	+	H	1	+	H
System 3-2	ER_SP_L	2	+++	HD	2	+++	HD
	$ER_SP_LP_v$	2	+++	HD	2	+++	HD
System 3-3	ER_SP_vW-1	NA	NA	S	NA	NA	S
	ER_SP_vW-2	NA	NA	S	2	xx	HD
	ER_SP_vW-3	2	xx	HD	2	xxx	HD
	ER_SP_vW-4	2	xx	HD	2	xxx	HD
	ER_{SL}PvW-1	2	xx	HD	2	xxx	HD
	$ER_{SL}P_vW$-2	2	xx	HD	2	xxx	HD
	$ER_{SL}P_vW$-3	NA	NA	S	NA	NA	S
System 3-4	$ER_{SL}P_LP_vW$-1	NA	NA	S	2	x	HD
	$ER_{SL}P_LP_vW$-2	NA	NA	S	2	x	HD
system 3-5	EG-1	3	++	H	3	+++	H
	EG-2	3	+++	H	3	++++	H
	EG-3	3	++++	H	3	S	H

Where **Clarity**; 1 = transparent, 2 = turbid or translucent, 3 = opaque or white to cream-colored, **Viscosity**; + = liquid- viscous gel, ++ = liquid- highly viscous gel, +++ = viscous gel, ++++ = highly viscous gel, S= stiffness gel, R = rigid gel (ringing gel), x = light-viscous hydrocarbon gel, xx = loose hydrocarbon gel, xxx = dense hydrocarbon gel, **Homogeneity**; H = homogeneous (clear or transparent gel) , HD = homogeneous (dispersed gel), S = phase separation and NA = no available data

In this viscosity study, error bars in all Figures were not reported for graph clarity as they may be confusing since many profiles were overlaped.

The viscosity profile of carbopol gel was shown in Figure 12. The preparation of 1% carbopol (CP) showed a good viscosity as newtonian flow. This formulation is commonly used in pharmaceutical products such as topical, oral and rectal preparations [Raymond, 2003]. In this study, the viscosity of preparation was increased when increased the concentration of polymer. The viscosity profile showed sharply increased then maintained constant representing by newtonian flow behavior. Increasing the concentrations of CP from 1% to 5%, would increase the viscosity level and the viscosity profile was also exhibited similar profile as newtonian flow. The viscosity profile was sharply increased and then reduced when the concentration of CP increased and approached to 11 %, the profile showed non-newtonian flow and pseudoplastic fluid flow, low viscosity at high shear rates and high viscosity at low shear rates. This type of fluid initially resists deformation, until a yield stress is reached which affects to decrease the viscosity. The system of 11% carbopol flowed like a very viscous substance and could revert to its original shape when the pressure was removed.

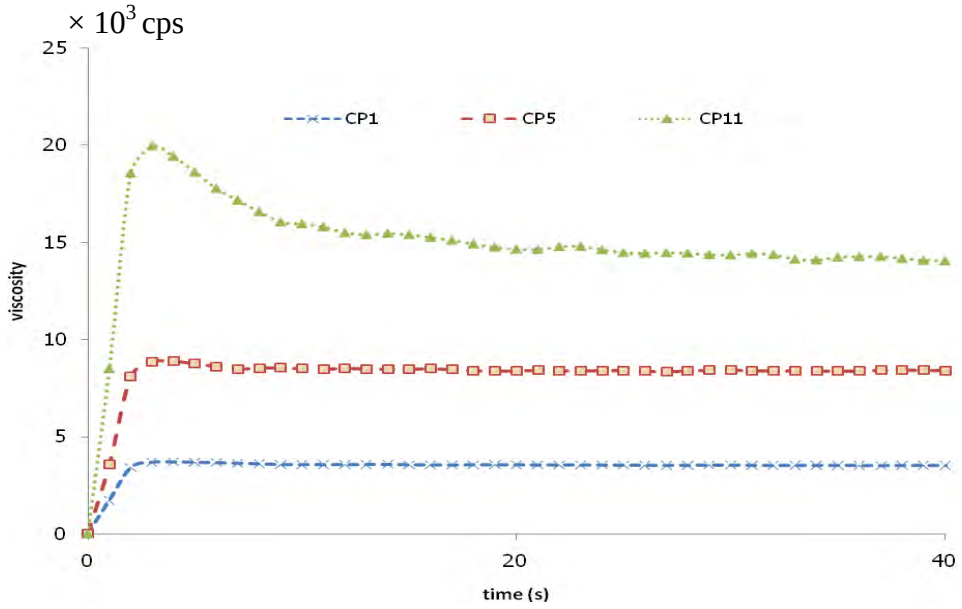

Figure 12 The viscosity profile of carbopol gel (hydrophilic gel system 1-1) with different concentration of carbopol

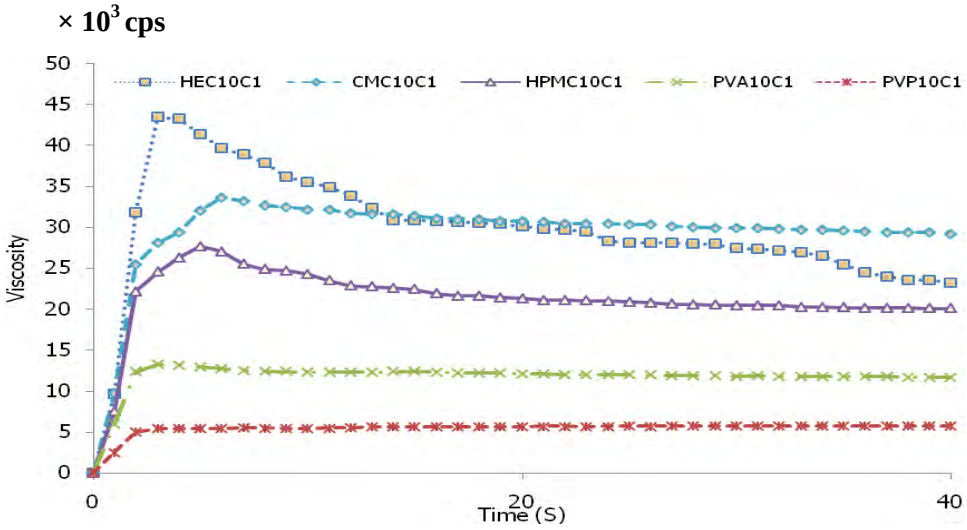

Figure 13 The viscosity profile of hydrophilic gel (system 1-1) with different type of polymer

When the system of CP was incorporated with 10% of a hydrophilic polymer such as HEC, HPMC and CMC in $\mathbf{C_1}$, non-newtonian flows, as pseudoplastic flows, were shown in Figure 13. The viscosity profile showed clearly and indicated that the systems were highly viscous gel when PVP and PVA were added in $\mathbf{C_1}$, the viscosity profile exhibited lower viscous gel as newtonian flow.

Varshosaz et al. [2002] found that most preparations of periodontal gels prepared from 10-20% (%w/w) CMC, 10-20% (%w/w) HEC and 10% (%w/w) CP showed a higher yield value which was affected from the increasing polymer concentration. All formulations showed pseudoplastic and plastic flow at higher concentration of hydrophilic polymer with higher yield value, except 10% (%w/w) PVP which showed the newtonian flow similarly in this result.

The rheology behavior of all formulations correlated with viscosity property. When the polymers gel showed fluid behavior, newtonian flow was obtained, whereas behavior of highly viscous to rigid body, non-newtonian was described. $\mathbf{HEC_{10}C_1}$ showed the highest viscosity and then reduced with the yield stress at 43.48 ± 1.67 ($\times 10^3$) cps. Deformation in this formulation occurred and the system could return to lower viscous gel as newtonian. Furthermore, although both having pseudoplastic

flow behavior **$CMC_{10}C_1$** (yield stress = 33.58 ± 2.87 $\times 10^3$ cps) showed higher viscosity than **$HPMC_{10}C_1$** (yield stress = 27.71 ± 3.32 10^3 cps) formulation.

Figure 14 shows the viscosity profile of thermoreversible gels (system 1-2). These formulations showed results similar to the previous hydrophilic gel as non-newtonion flow and pseudoplastic behavior when the system composed of poloxamer up to 40% (yield stress = 17.75 ± 1.13 $\times 10^3$ cps). The elevating of poloxamer concentration results in an essential increase of the gel viscosity, in which case the gelling temperature of the formulation is decreased (↓Tc) and may gelatinize already under the temperature [Kramaric et al., 1993], whereas all formulations containing 20% poloxamer exhibited newtonian flow as similar result of Yun-Seok Rhee [2005].

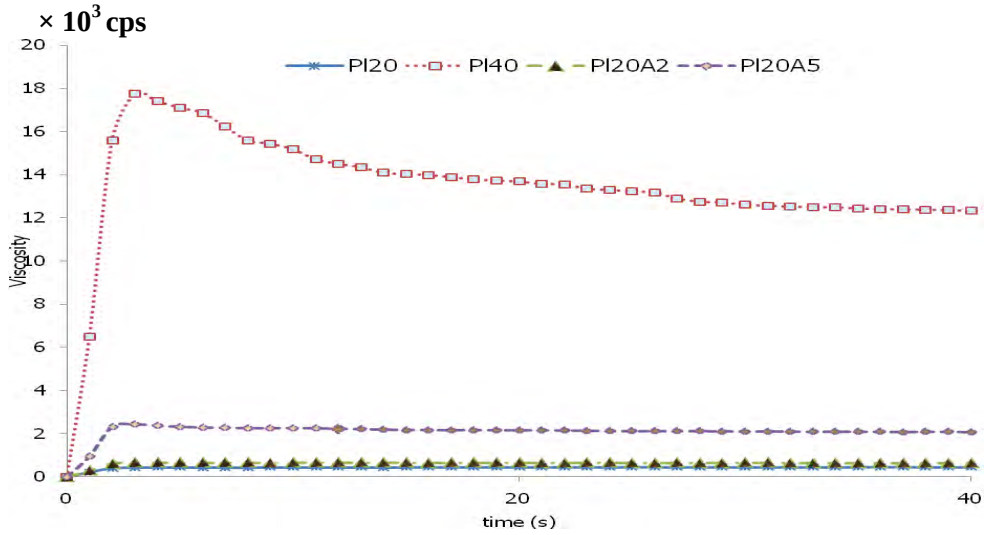

Figure 14 The viscosity profile of poloxamer gel at room temperature (system 1-2)

Since an effective increase in the gel viscosity (consistency) is the prior art solutions may only be provided by increasing the poloxamer concentration, in which case the gelling temperature of the formulation is decreased. Only a polymer concentration just providing for the gelling temperature above 25°C may be used [Kramaric et al.,1993]. Kelly et al. [2004] compared two concentration at 20% and 25% of poloxamer. They found that the 25% system showed a gelling temperature of approximately 15°C, which was considered too low. It was therefore decided to develop a formulation based on the 20% concentration, which showed a sol-gel transition temperature of just below 20°C. Therefore, the viscosity property is

incresed when sol-gel transition temperature is decreased due to the increasing concentration of thermoreversible polymer.

When increased concentration of Aerosil from 0.5% to 2% and 5% respectively, the viscosity profiles were also increased and exhibited newtonian flow. These systems with different amount of Aerosil showed statistically significant difference in viscosity profile of poloxamer gels (*p-value* < 0.05). And the highest viscosity was from formulation that had 5% Aerosil. Aerosil or colloidal silicon dioxide is a popular gelling agent that has been shown to gel in a wide range of solvent [Raymond, 2003]. Silicon dioxide may cause significant changes in the liquid crystalline phases and modify the rheological properties to increasing viscosity.

In addition, The effect of Aerosil concentration on sol-gel and gel-sol transition is shown in Figure 15. Incorporatio of Aerosil shifted sol-gel transition to a lower temperature but gel-sol to a higher temperature. Thus, the gelation range broadens with the concentration of the Aerosil. Block copolymer poloxamer gel is thought to be formed by H-bonding in the aqueous system, caused by attraction of the poloxamer etheroxygen atom to a proton of water. when the hydrogen bonding is supplemented by adding compounds with hydroxyl group from Aerosil produced the geletion point decreases [Malmsten and Lindman, 1992]

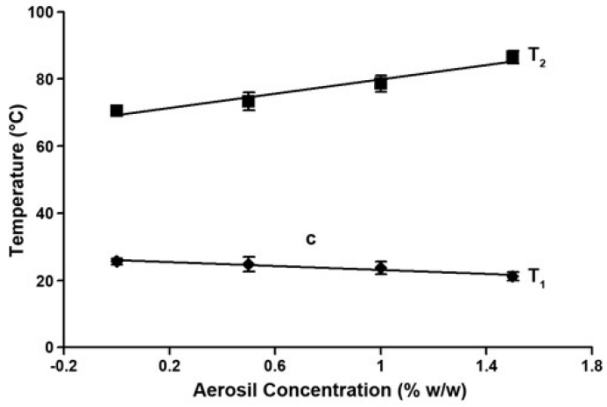

Figure 15 Effect of Aerosil concentration on gelation point (T1) and gel melting point (T2) [Maheshwari et al., 2006]

Shah and Paradkar [2006] reported the use of Aerosil for modification to increase viscosity and drug release from glyceryl monooleate liquid crystal phases. The major limitation of poloxamer gels is irritation at high concentration. This requires to obtain the optimum viscosity form and achieves the desired pharmaceutical performance. Therefore, attempts had been made to reduce the poloxamer concentration by adding different amount of hydrophilic polymers or Aerosil.

Figure 16 shows the system that combined hydrophilic polymers with thermoreversible polymer. All formulations exhibited newtonian flow behavior, except $\mathbf{P_{L20}C_5}$ that showed the highest viscosity and pseudoplastic flow with yield stress at 36.65 ± 4.12 ($\times 10^3$) cps. Since $\mathbf{P_{L20}C_1}$ at 1% carbopol exhibited newtonian flow, this result indicated that the system containing 20% of poloxamer could change the viscosity profile upon the concentration of carbopol (CP). In addition, for all formulations of newtonian profile, the viscosity was ranked, $\mathbf{P_{L20}C_1} > \mathbf{P_{L20}HEC_5} > \mathbf{P_{L20}CMC_5} > \mathbf{P_{L20}HPMC_5} > \mathbf{P_{L20}PVA_5} > \mathbf{P_{L20}PVP_5}$, respectively. There were no significant increased in viscosity profile of each formulation at 5% CMC, 5% HPMC and 5% PVA (*p-value* > 0.05). These results were affected by critical micellar concentration or gelling temperature according to decreasing gelling temperature. The effect of addition of some polymers might be described on the thermo-rheological properties of 20% poloxamer gels and composition. On the other hand, the addition of 0.5% (w/w) carbopol 934P to a 20% aqueous poloxamer 407 dispersion results in a maximum viscosity at a reduction of the sol-gel transition temperature [Kramaric et al., 1993]. In previous result of hydrophilic gel from Figure 13, the data from each system with only 10% of CMC, HPMC and PVA clearly showed difference in viscosity profile while each system of 5% CMC, HPMC and PVA incorporated with 20% of poloxamer exibited no significant difference in viscosity. These might be possible that the viscosity profile of each system was also promoted by thermoreversible effect of poloxamer gel on gelling temperature.

Kramaric et al., [1993] explained that the combination of hydrophilic polymers and poloxamer could decrease the critical micelle concentrations (Tc) or gel

transition temperature, especially the formulation composed of carbopol 934P and poloxamer results in a synergistic increase of gel viscosity since the gel viscosity is much higher than the sum of viscosities of the individual gelling components and was a result of specific physico-chemical interactions between a carbomer and a poloxamer. The hydrogen bonding between carbopol and poloxamer played an important role in reducing Tc but increasing viscosity property. In this way a simple and effective regulation of the thermo-rheological properties of such carrier in the sol state as well as in the gel state is attained, achieving a reduction of sol-viscosity, an increase of gel-viscosity and a rapid transition from sol to gel.

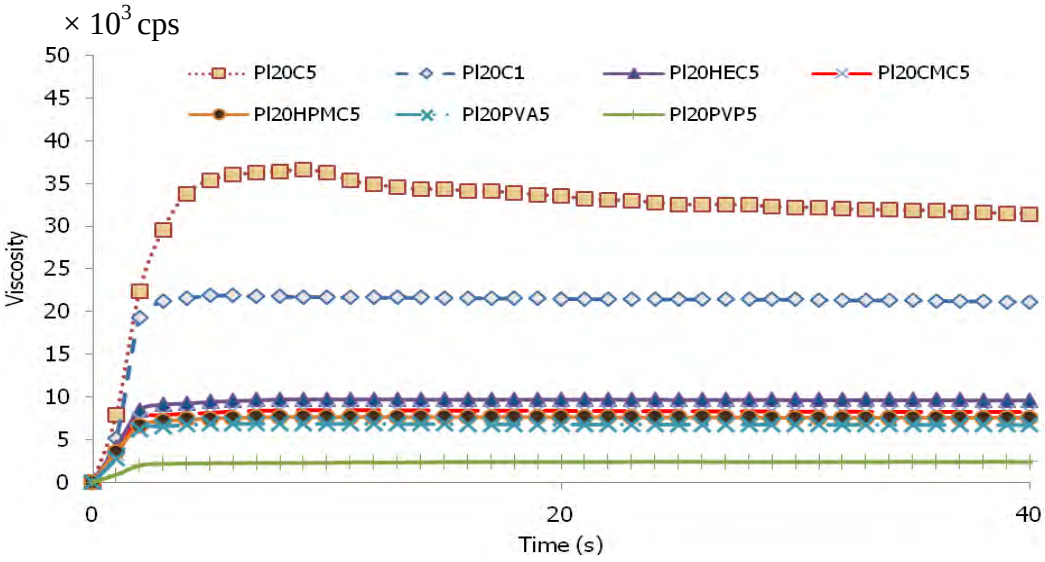

Figure 16 The viscosity profile of hydrophilic-thermoreversible gel with different type of polymers

When systems of poloxamer gel were stored at 37°C environment , the viscosity were increased from the thermoreversible effect. All formulations of poloxamer gel at room temperature were changed to increasing viscosity at 37°C and some system might approach non-newtonian behavior due to the system transformed to cubic phase or hexagonal phase [Manish, 2006] and either the increasing of hydrophilic polymer especially CP and increasing amount of Plo could decreased the gelling temperature [Kramaric et al., 1993]. The viscosity profiles of these systems at 37 °C are shown in Figures 17-24.

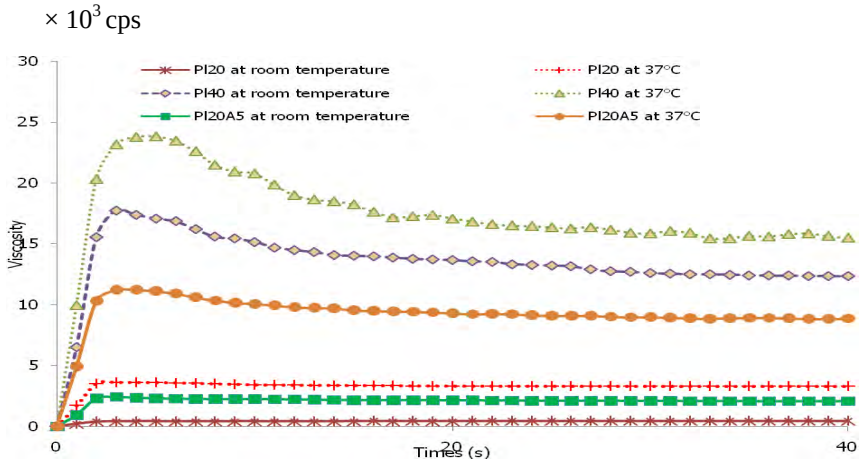

Figure 17 The viscosity profile of poloxamer gel at room temperature and 37°C

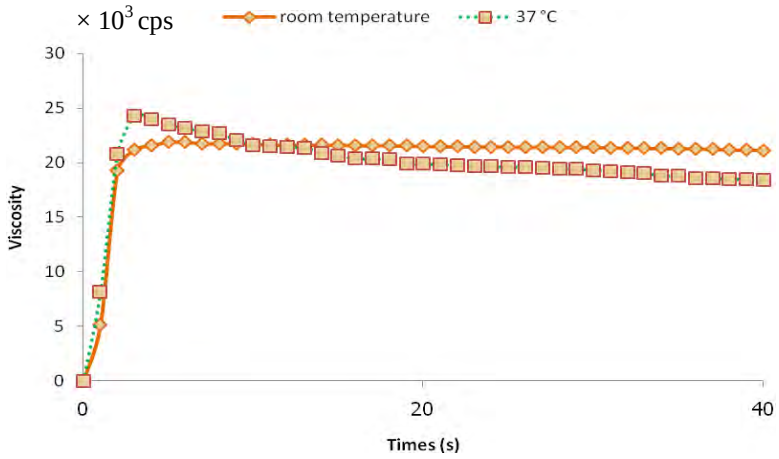

Figure 18 The viscosity profile of $\mathbf{P_{L20}C_1}$ at room temperature and 37°C

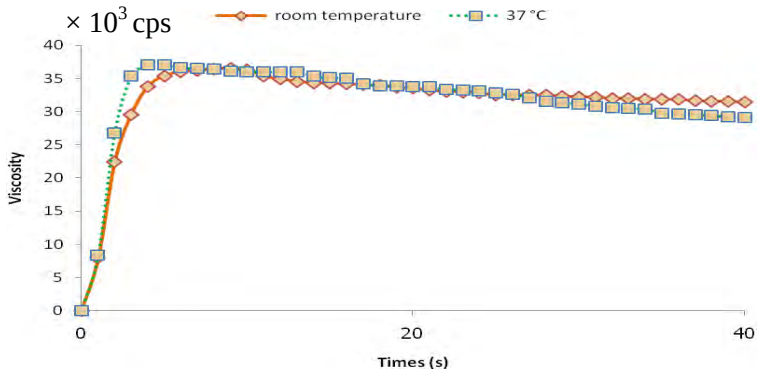

Figure 19 The viscosity profile of $\mathbf{P_{L20}C_5}$ at room temperature and 37°C

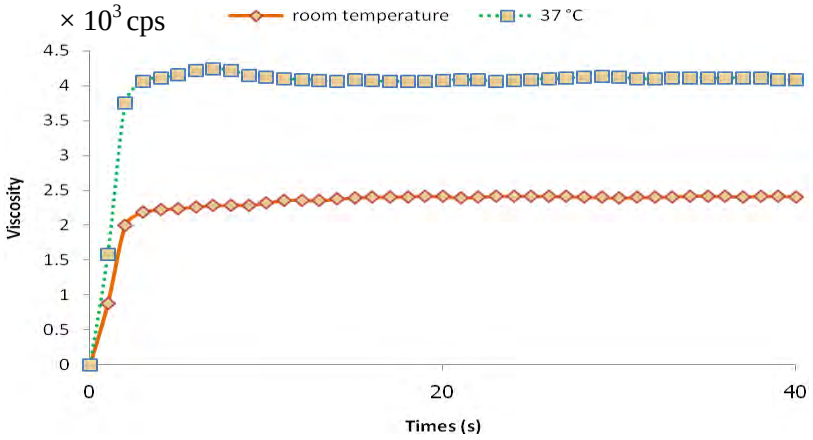

Figure 20 The viscosity profile of $\mathbf{P_{L20}PVP_5}$ at room temperature and 37°C

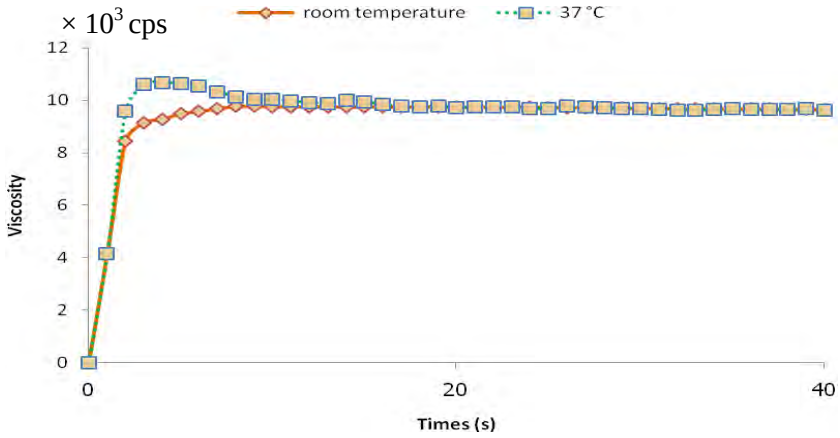

Figure 21 The viscosity profile of $\mathbf{P_{L20}HEC_5}$ at room temperature and 37°C

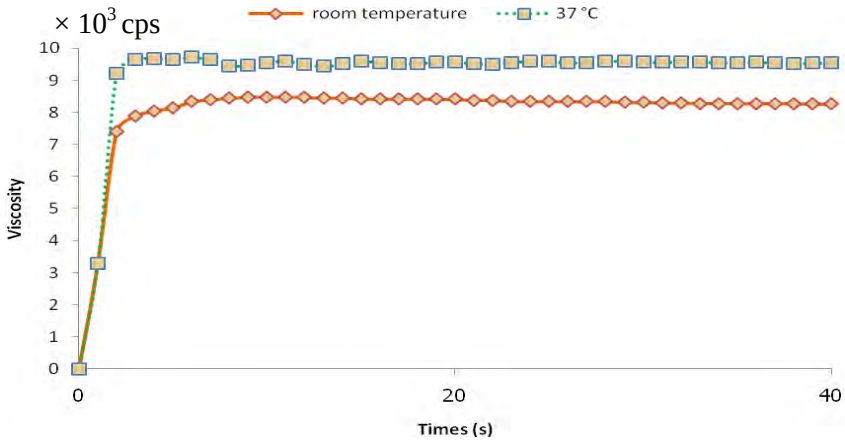

Figure 22 The viscosity profile of $\mathbf{P_{L20}CMC_5}$ at room temperature and 37°C

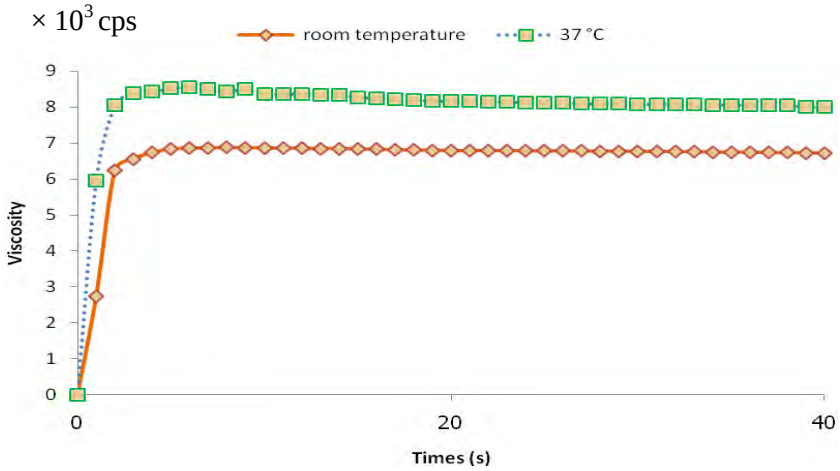

Figure 23 The viscosity profile of $\mathbf{P_{L20}PVA_5}$ at room temperature and 37°C

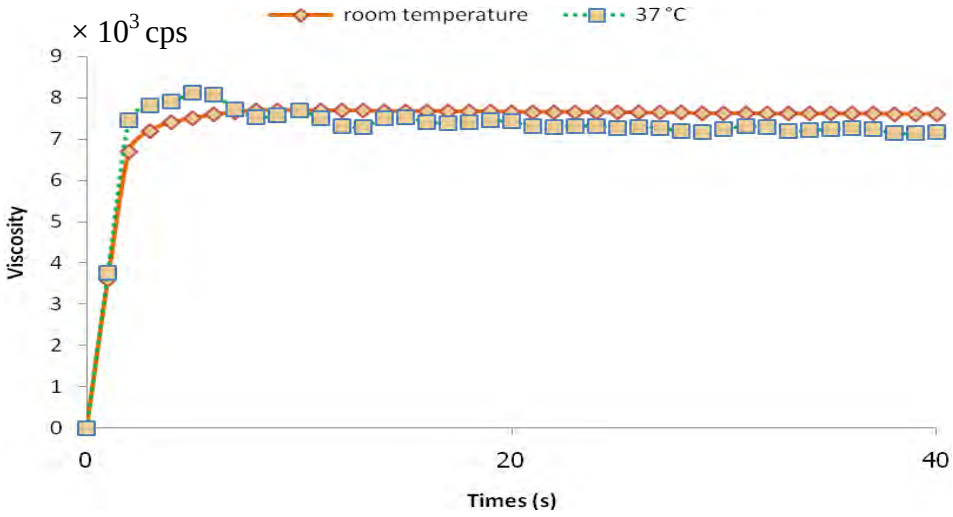

Figure 24 The viscosity profile of $\mathbf{P_{L20}HPMC_5}$ at room temperature and 37°C

Poloxamer 407 (Figure 25), at low concentrations (10^{-4}–10^{-5} %), forms monomolecular micelles, but higher concentrations result in multimolecular aggregates consisting of a hydrophobic central core with their hydrophilic polyoxyethylene chains facing the external medium [Guzmán, 1994]. Micellization from poloxamer407 occurs in dilute solutions of block copolymers in selected solvents above the critical micellar concentration, at a given temperature. At higher concentrations, above a critical gel concentration, the micelles can order into a lattice. These scenarios are illustrated in Figure 26. Poloxamer 407 aqueous solutions of 20

to 30% w/w have the interesting characteristic of reverse thermal gelation [Lenaerts, 1987], i.e., they are liquid or viscous-liquid at refrigerated temperatures (4-5°C), but gel upon warming to room temperature. The gelation is reversible upon cooling.

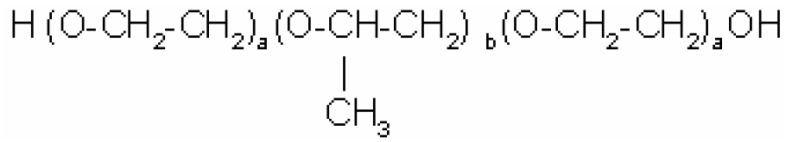

Figure 25 Chemical structure of poloxamer (a, ethylene oxide portion b, propylene oxide portion) [Escobar-Chávez, 2006].

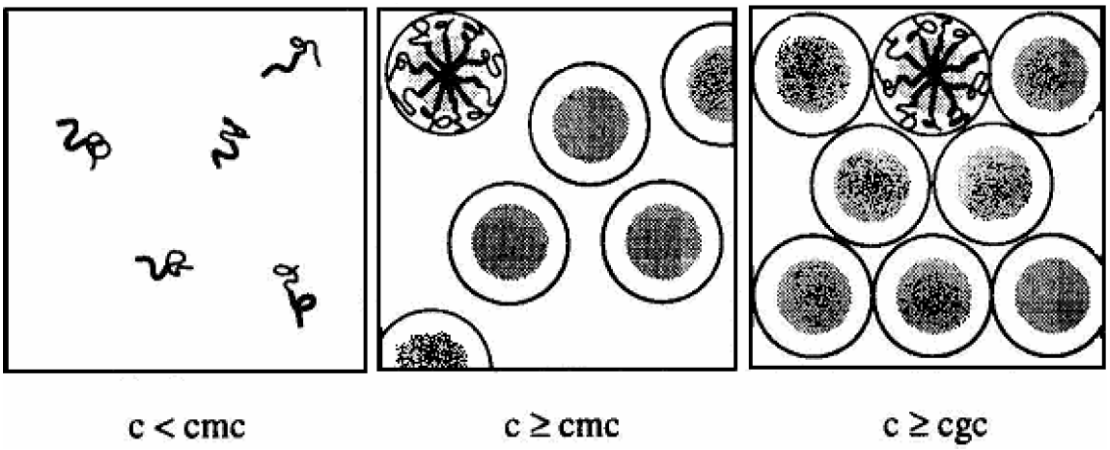

Figure 26 Illustration of the critical micelle concentration (cmc) and critical gel concentration (cgc) in a block copolymer solution [Escobar-Chávez, 2006].

At low temperatures in aqueous solutions, a hydration layer surrounds poloxamer molecules. When the temperature was raised, the hydrophilic chains of the copolymer become desolvated as a result of the breakage of the hydrogen bonds that had been established between the solvent and these chains. This phenomenon favors hydrophobic interactions among the polyoxypropylene domains, and leads to gel formation. Because of the dehydration process, the hydroxyl groups become more accessible and showed the gel as micellar in nature. At higher temperatures, a phase of hexagonal packed cylinders was formed (Figure 27). Gelation of poloxamer is

thought to occur as a result of dehydration of the polymer leading to increased chain friction and entanglement, producing a hydrophobic association.

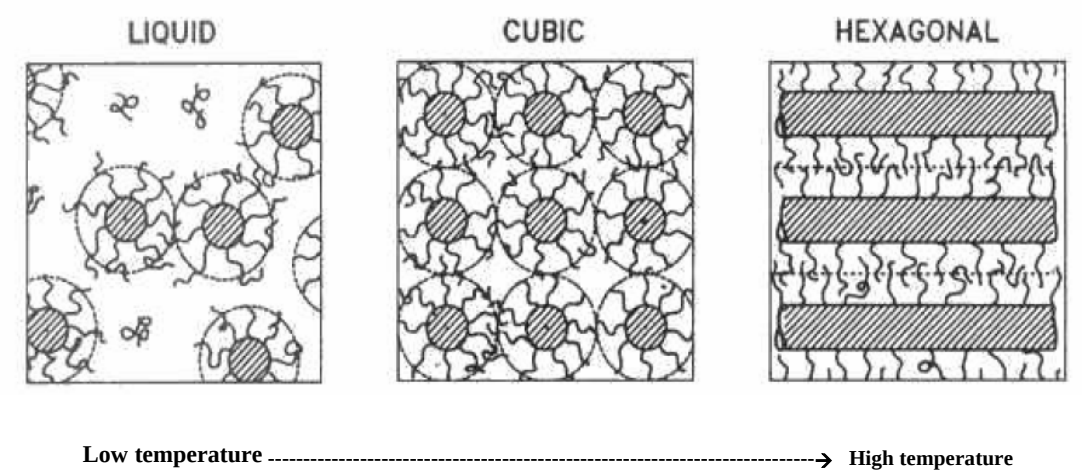

Figure 27 Schematic illustration of micellar phases formed by the poloxamer with increasing temperature. [Escobar-Chávez, 2006]

The viscosity profiles of hydrophobic gels, both polyethylene gel and water insoluble polymers gel based on EC, RS and RL with or without PVP are shown in Figures 28-29, respectively. All formulations of polyethylene gels were sharply increased and then deformation occurred and reached to decreasing of viscosity. $\mathbf{P_{E10}}$ and $\mathbf{P_{E5}}$ exhibited the highest and lowest yield stresses at $13.38 \pm 0.81 \times 10^3$ cps and $2.90 \pm 0.26\ 10^3$ cps, respectively, which implied that higher concentration could increase viscosity profile . Adding 1.5% and 3% of Aerosil would increase the viscosity. Formulation contained 3% Aerosil (yield value = $9.52 \pm 0.84 \times 10^3$ cps) showed higher viscosity than that of 1.5% Aerosil (yield value = $4.50 \pm 0.2 \times 10^3$ cps). This could explain that Aerosil as a thickening agent provided dispersion as a function of volume fraction of solid phase. Aerosil dispersed in the medium affected substantially the structure formation processes in a system and increased the viscosity property [Makarov, Andreeva, and Tretinnik, 2000].

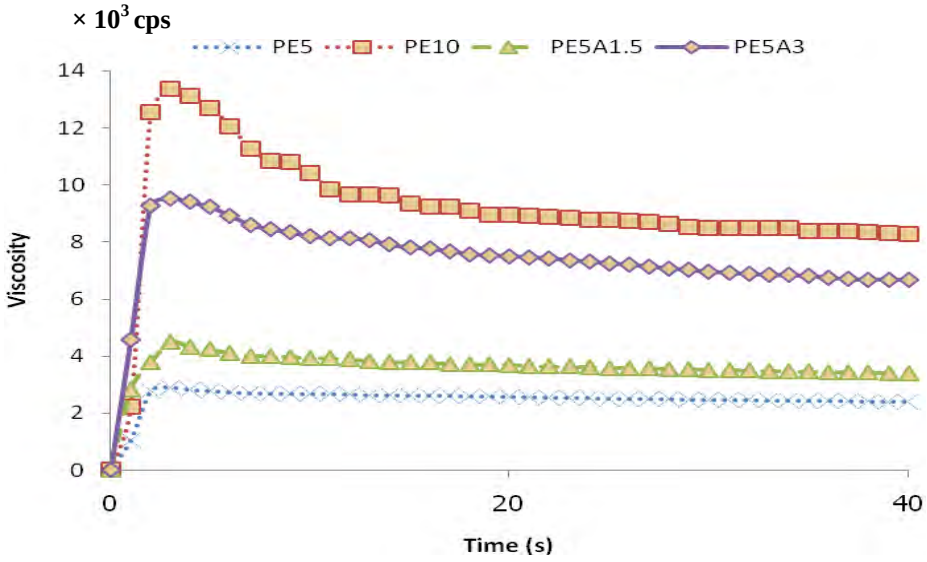

Figure 28 The viscosity profile of polyethylene gel (Semtem 2-1)

The viscosity profile of water insoluble polymer gel based on EC, RS and RL with or without PVP showed similar results as newtonion flow behavior as shown in Figure 29. Increasing the concentration of EC ranged from 12.5% to 17.5% and PVP from 0% to 2% in **ER$_S$-1** formulation would increase the viscosity profile. The viscous gel prepared with RS and RL in different ratios (25:0, 22.5:2.5 and 20:5) exhibited no significant difference (*p-value* = 0.857) in viscosity profile while the formulation incorporated with 2% of PVP, a binder and viscosity agent, could increase viscosity profile to highly viscous gel (*p-value* < 0.5).

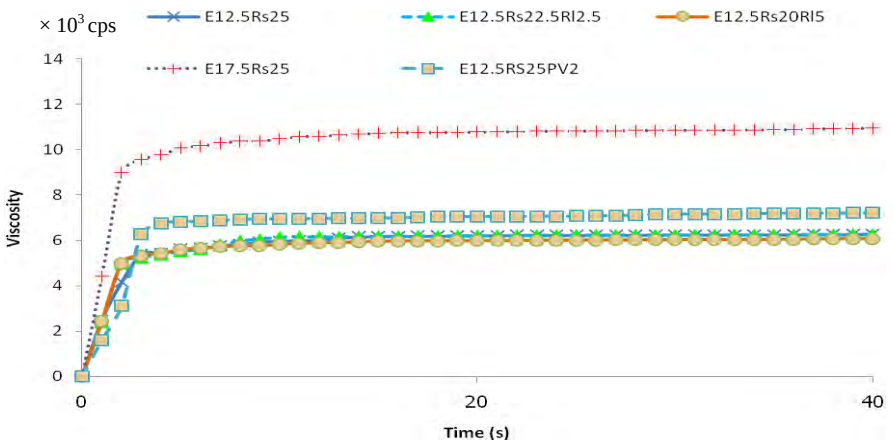

Figure 29 The viscosity profile of hydrophobic gel based on EC, RS and RL with or without PVP (system 2-2)

In this study, the systems of poloxamer gel were prepared in 95% ethanol as a solvent. These systems could not show physical transformation at 37 °C (Figure 30). In fact, system of poloxamer gel could exhibit thermo-reversible gel at the total concentration of more than 20% w/w in water [Maheshwari et al., 2006] or aqueous environment [Escobar-Chávez et al., 2006]. In addition, at higher temperature, the gel underwent dehydration or evaporation and caused the destruction of gel structure. Therefore, poloxamer dissolved with 95% ethanol could not exhibit thermosetting property though room temperature or 37°C.

In addition, Hemelrijck and Goymann [2008] described the influence of isopropyl alcohol (IPA) on the thermogelification of semi-solid and liquid poloxamer 407 systems. The sol/gel transition temperature of 25% (w/w) of poloxamer was increased when increasing the concentration of IPA. The critical temperature (Tc) of system containing 60% IPA was about 42-50°C. Conclusively from this study, polxamer gel as thermoreversible gel had to have at least 15% (w/w) water. Dumortier et al [2006] explained that alcohol could interfere in the poloxamer micellization and alter the dehydration of hydrophobic PO blocks, so reduced the gel strength and bioadhesive force and increased Tc of sol to gel.

Furthermore, Yun-Seok Rhee et al. [2006] reported the effect of alcohol type on the viscosity and gelling point of aqueous poloxamer solution. The results showed that eugenol, citronellol, cinnamyl alcohol, phenethyl alcohol and terpineol added to the aqueous poloxamer solution the gelling point of the aqueous poloxamer 407 solution. It was also found that amount of phenethyl alcohol at 1.0% could decrease the gelling point of the solution to the low 6°C and cinnamyl alcohol decreased the gelling point to approximately 10°C. In the case of citronellol, the gelling point of the poloxamer solution was decreased to 16°C. In general, the gelling point was inversely proportional to the concentration of alcohols. In the view of the structure, the hydrophilic portions of the flavors may bind to the PEO chains of poloxamer 407, and accelerate the entanglement of the polymeric micelle through a hydrophobic interaction. As a result, they appeared to induce the gelation of the poloxamer solution at lower temperatures.

Noticingly, on the other hand, poloxamer gel exhibited clear gel with thermoreversible property, but in this study the appearance was homogeneous dispersion gel (data shows in Table 9). There was undissolved micronized powder of poloxamer suspended in the gel structure thus not transformation into the cubic or hexagonal structure may not occur by increasing the temperature.

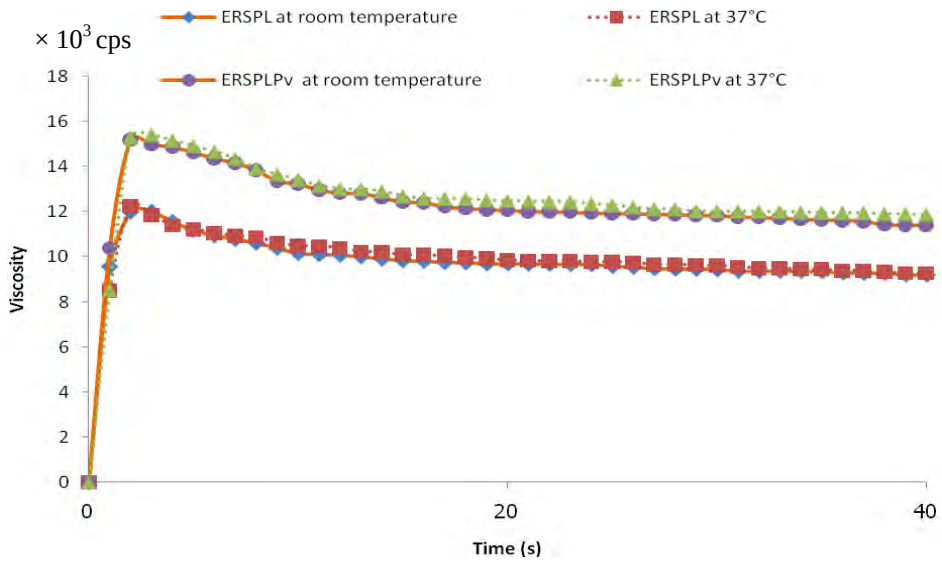

Figure 30 The viscosity profile of hydrophobic gel based on EC, RS and Plo with or without PVP at room temperature and 37°C

All formulations of hydrophobic gel system 3-3 exhibited pseudoplastic flow behavior. Figure 31 shows the formulation composed of 20% WS that deformed and the viscosity was reduced after the system reached to yield stress. When increasing the concentration of WS from 20% to 25%, the concentration of EC from 12.5% to 15 %, the yield stress was changed from 18.52 ± 2.73 to 24.45 ± 1.10 × 10^3 cps and from 23.93 ± 2.01 to 28.88 ± 1.09 × 10^3 cps, respectively. Similar pattern was also exhibited after the system reached the yield stress. Therefore , in this study, it was possible to enhance the stability of this system by increasing the yield stress that could prevent system transformation by adding the component such as hydrohobic, hydrophilic polymers or/and hydrocarbon compound.

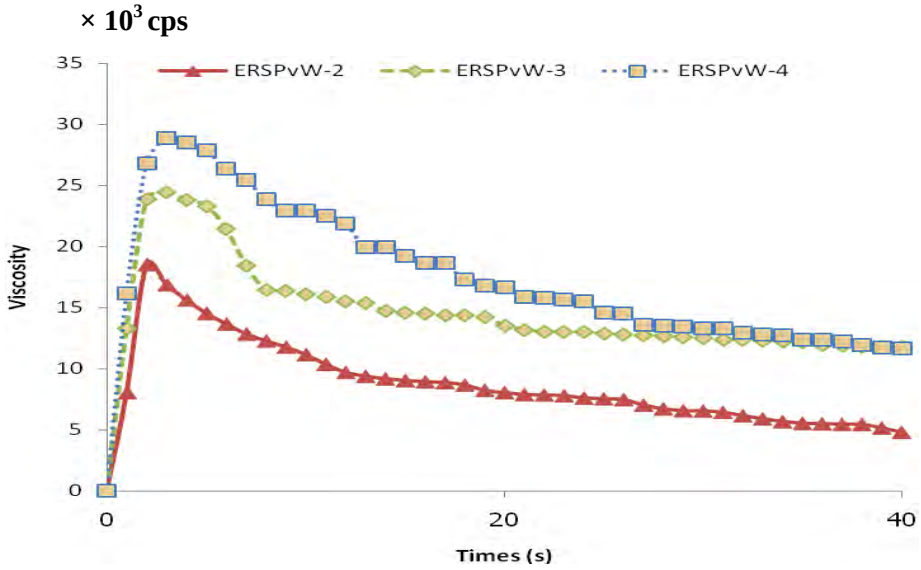

Figure 31 The viscosity profile of hydrophobic gel base (**ER_SP_vW-2**, **ERSPvW-3** and **ER_SP_vW-4**)

The result of the viscosity pattern when different ratios of RS/RL has been incorporated in preparations shown was illustrated Figure 32. Similar viscosity profile was obtained and there were no significant difference in yield stress (*p-value* = 0.276) among these formulations. This might be the closely similar structure RS and RL that shown in Figure 33.

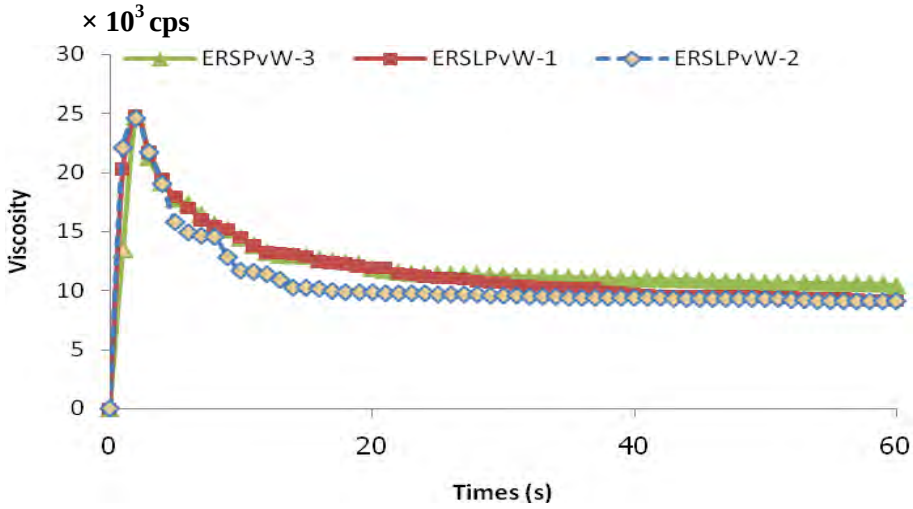

Figure 32 The viscosity profile of hydrophobic gel based on 12.5% EC with different ratio of RS/RL at 25:0, 22.5:2.5, and 20:5 (**ER_SP_vW-3**, **ER_{SL}PvW-1** and **ER_{SL}PvW-2**, respectively)

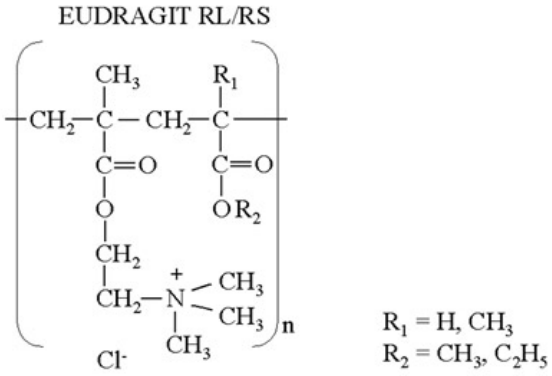

Figure 33 The structure of Eudragit RL/RS [Nguyen et al, 2006]

Figure 34 shows the viscosity profile of **$ER_{SL}P_LP_vW$-1** (20% Plo and 5% WS) and **$ER_{SL}P_LP_vW$-2** (5% Plo and 20% WS). The viscosity profile of **$ER_{SL}P_LP_vW$-1** and **$ER_{SL}P_LP_vW$-2** exhibited closely similar to those of poloxamer gel (system 1-2) and hydrophobic gel (system 3-3) respectively. Consequently, this study was explained that the viscosity profile would be dependent upon the main compositions and could possibly show the similar pattern.

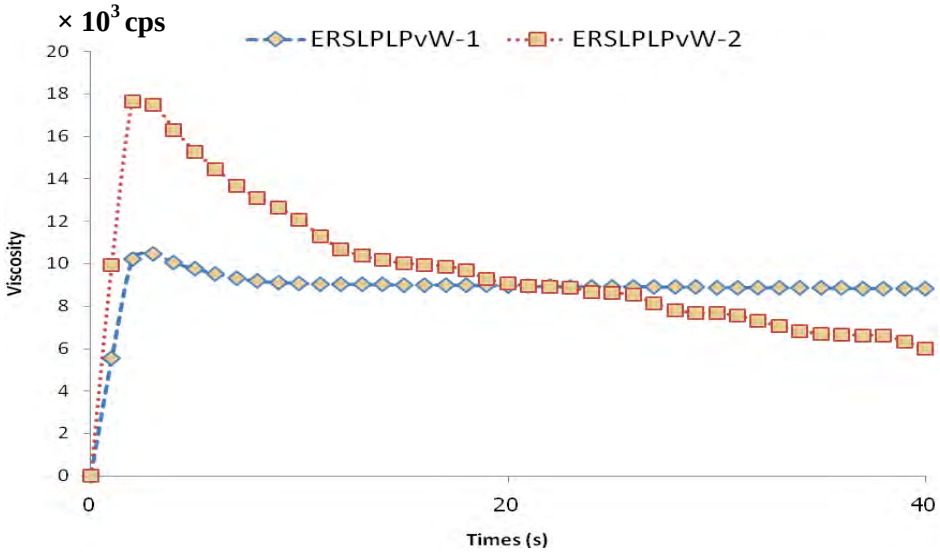

Figure 34 The viscosity profile of **$ER_{SL}P_LP_vW$-1** (20% Plo and 5% WS) and **$ER_{SL}P_LP_vW$-2** (5% Plo and 20% WS)

The viscosity profile of emulsion gel was shown in Figure 35. The viscosity of **EG-1** (1% CP and 6.25% of oil phase) was lower than **EG-2** (1% CP and 12.5% of oil phase) and **EG-3** (5% CP and 6.25% of oil phase) respectively. The increasing concentration of polymers or its component could increase the viscosity profile. These formulations showed 2 rheology pattern as newtonian and pseudoplastic flow behavior. **EG-1** and **EG-2** showed newtonian or free flow behavior similar to **C_1** (1% carbopol) as seen in the viscosity profile while **EG-3** exhibited the pseudoplastic flow from the increasing viscosity due to increasing amount of CP. During testing, the viscosity profile started at yield stress as pseudoplastic flow and then showed a decreased rate of viscosity as newtonian flow.

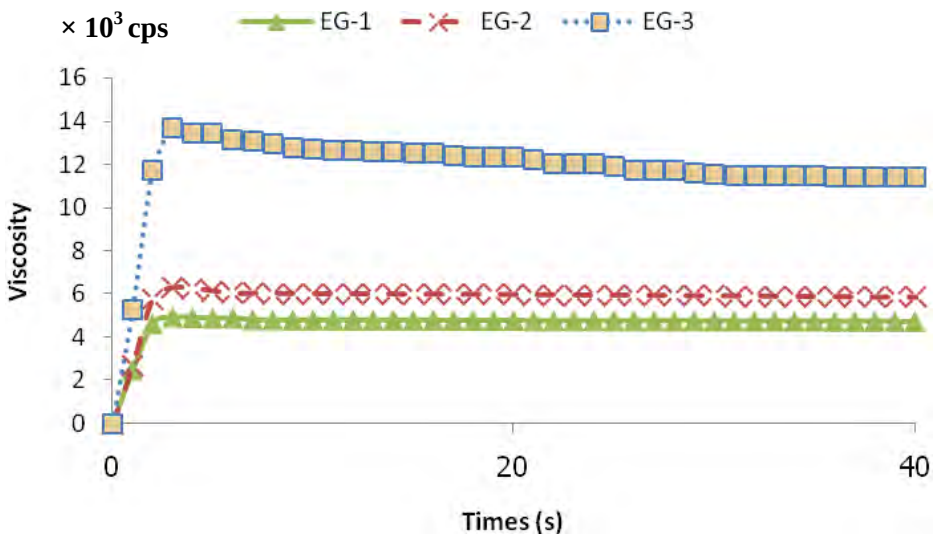

Figure 35 The viscosity profile of **EG-1** (1% CP and 6.25% of oil phase)**, EG-2** (1% CP and 12.5% of oil phase) and **EG-3** (5% CP and 6.25% of oil phase)

Syringeability

Syringeability described the ability of periodontal gel to pass through a hypodermic needle or transfer from the container prior to injection. Syringeability of various formulations was eaxamined to determine the effect of types of periodontal

gel on the force required to expel the product. The syringeability is closely related to the viscosity and flow characteristics [Jones et al., 1997].

Varshosaz et al [2002] reported that the force of syringeability in hydrophilic gel formulations and found that increasing the polymer concentration increase force of syringeability. The drug release profile was decreased according to increasing the polymer concentration. However, the preparation was to hard (high or higher viscosity) and not suitable for the dental application. Higher force, 1,020 N, was shown in the combination of methyl cellulose (MC) and CMC at 10% and 20%, respectively. They recommended that this formulation exhibited zero order kinetic until 48-56 hours, but need higher work of syringeability. Thus, the preparation should be tested by periodontist for further investigation.

In preliminary study, the syringeability test was recommended by the periodontists from the Department of Periodontology, Faculty of Dentistry, Chulalongkorn University. After injecting the periodontal gel that had packed in the 3-ml syringe through a 21-gauge needle, the injection should be applied easily. Consequently, a high syringeability property of periodontal gel base should provide the satisfied system into periodontal pocket. Syringeability in this study was measured in term of force of injection and time. This means the higher of syringeability, the greater performance of flowability of sample which consequence in the ease of application of dosage form. Concerning the design of syringeability test on various formulations, the syringeability test should be initially described the injectability. Injectability refers to the properties of the gel while being injected [Modesto, 2005]. All formulations in this group were tested by the periodontists. The syringeability value was concluded to be at appropriate force of less than 70 N and the highest acceptable force was not more than 100 N.

The syringeability of the prepared hydrophilic gel, hydrophobic gel and hydrophobic-hydrophilic gel is shown in Figures 36-37, 38-39, and 40-42, respectively.

These syringeability property profiles showed the effect of different types and the increasing concentrations of mucoadhesive and non-mucoadhesive polymers. The syringeability of hydrophilic periodontal gel base (system 1-1) through a needle is shown in Figure 36 was increased as a function of force and kept a constant profile. The syringeability profile was ranked: **C_1 > C_5 > C_{11}**, respectively. The decreasing syringeablity was due to the increasing amount of mucoadhesive polymers. In addition, the syringeability profile of formulation with additional polymer was ranked: **$PVP_{10}C$ > $PVA_{10}C$ > $HPMC_{10}C$ > $CMC_{10}C$ > $HEC_{10}C$**, respectively. The influence of these hydrophilic polymers depended on the viscosity grade and molecular weight of polymers [Boylan, Cooper and Chowhan, 1986]. The lowest syringeability of **$HEC_{10}C$** formulation resulted in impossibility or difficulty for periodontal administration. **C_1** (1% CP) exhibited high and appropriate syringeability. Generally, carbopol 0.5-2% is used for preparation of gelling agent and mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents include creams, gels, and ointments for use in ophthalmic, rectal, and topical preparations [Raymond, 2003].

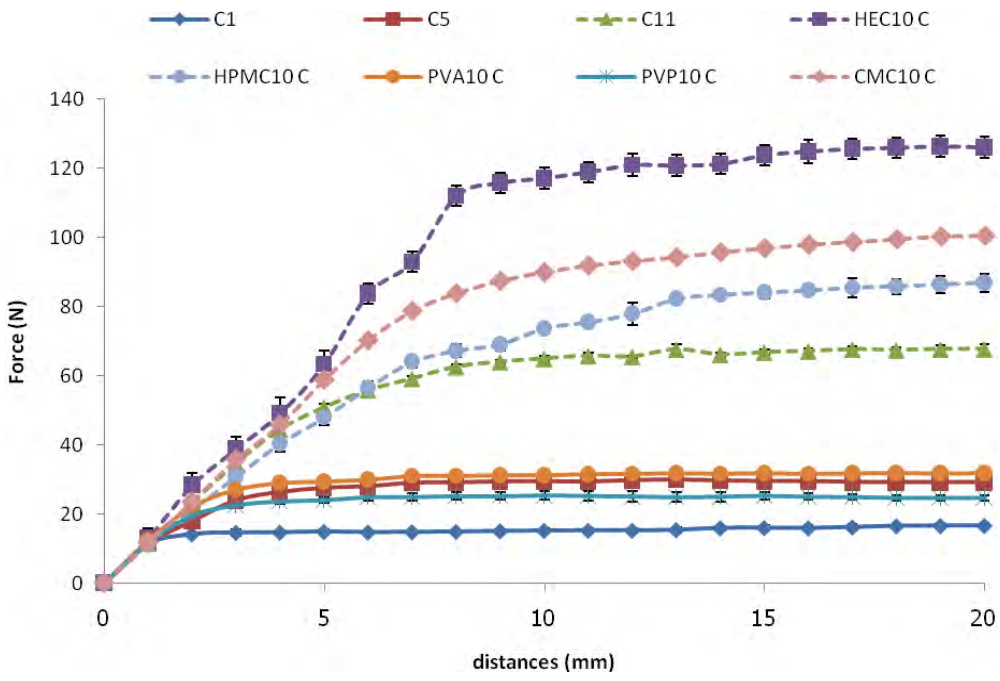

Figure 36 The syringeability profile of hydrophilic gel base (system 1-1)

Figure 37 shows the syringeability property of hydrophilic gel base based on thermoreversible polymer (system 1-2 and 1-3). These formulations exhibited below 100 N of syringeability force, with the exception of $\mathbf{P_{L20}C_5}$ formulation that showed the lowest syringeability nearly 160 N. Interestingly, according to viscosity profile of $\mathbf{P_{L20}C_5}$ formulation reduction of sol viscosity at temperatures under the gelling temperatures (Tc) and a high increase synergy of gel viscosity at room temperature could decreased the syringeability at the same condition.

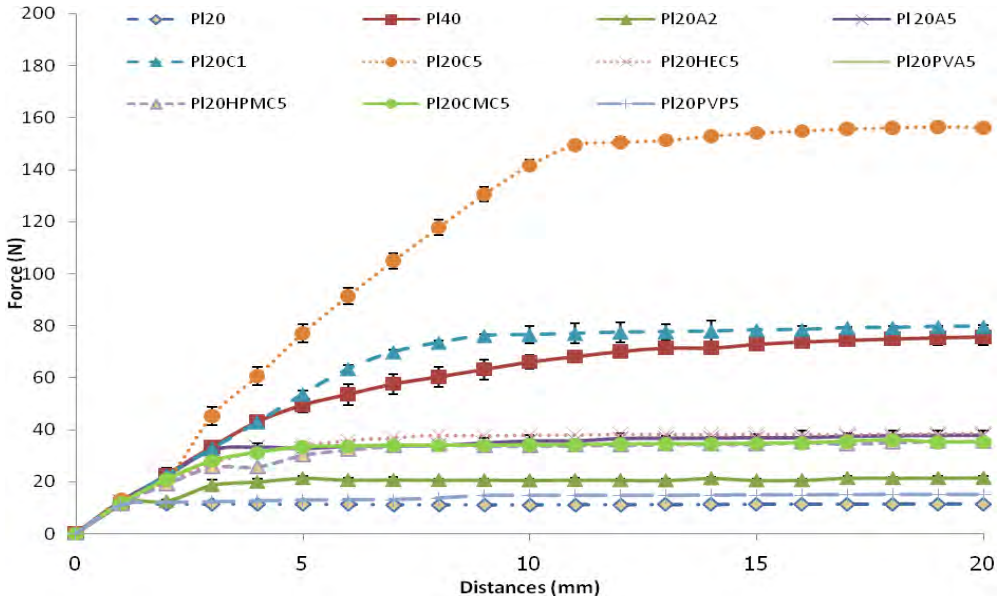

Figure 37 The syringeability profile of hydrophilic gel base (system 1-2 and 1-3)

When the system of Plo was incorporated with 5% of a hydrophilic polymer such as HEC, HPMC, PVP, PVA and CMC in $\mathbf{P_{L20}}$, the syringeability of these formulations was slightly decreased while amounts of CP at 1% and 5% showed a higher decrease. These formulations exhibited that the effect of CP could highly decrease Tc of poloxamer gel and then showed lower syringeability and higher viscosity at room temperature. The result had correlate from previous viscosity data.

When the system of Plo was incorporated with 0.5%, 2% and 5% of Aerosil in $\mathbf{P_{L20}}$. The result indicated that increasing concentration of Aerosil at 2% and 5% that showed statistically significant decreased syringeability than of Aerosil 0.5% (*p-value* < 0.05) previously due to by phase transition [Chen-Chow, 1980]. In addition, when

increasing concentration of Plo from 20% to 40%, the syringiability was also increased the gel viscosity (*p-value* < 0.05) accordingly by decreasing of gelling temperature [Kramaric et al., 1993].

The syringeability of polyethylene gel (a mixture of PE and mineral oil) (system 2-1) is shown in Figure 38. All preparations easily passed though the hypodermic needle with oil as emollient. When increasing the concentration of PE from 5% to 10%, the syringeability was significantly increased (*p-value* < 0.05). This indicated the close volume occurred and given density. When amount of Aerosil at 1.5% and 3% were added in the $\mathbf{P_{E5}}$ formulation, the increasing of syringeability was statistically significant (*p-value* <0.05). In addition, the increasing concentration of Aerosil at 3% showed similar syringeability profile with $\mathbf{P_{E10}}$ formulation (*p-value* =0.967). Therefore, the synergistic effect of the combinations of lower amount of poloxamer gel and higher amount of Aerosil could increase to similar viscosity property at higher amount of poloxamer gel formulation. The syringeability profile of these formulations was in agreement with viscosity results.

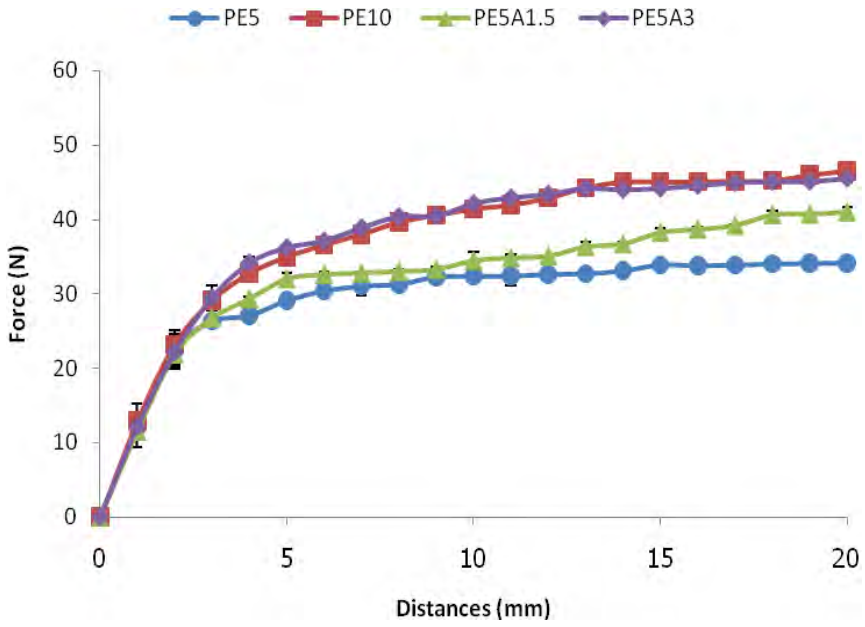

Figure 38 The syringeability profile of polyethylene gel base (system 2-1)

Figure 39 presents the syringeability property of liquid-viscous polymer (system 2-2 and 3-1) based on EC, RS and RL. When increasing the concentration of EC from 12.5% to 17.5%, the syringeability was decreased. These formulations containing 37.5% at total concentration of EC, RS and RL in **ER_S-1**, **ER_S-3** and **ER_S-4** showed no significant difference in syringeability (*p-value* = 0.934), which was in agreement with viscosity profile in Figure 29. In addition, incorporation of 2% PVP in the ER_SP_v formulation slightly increased syringeability property and showed statistically significant higher resistant property (*p-value* = 0.018).

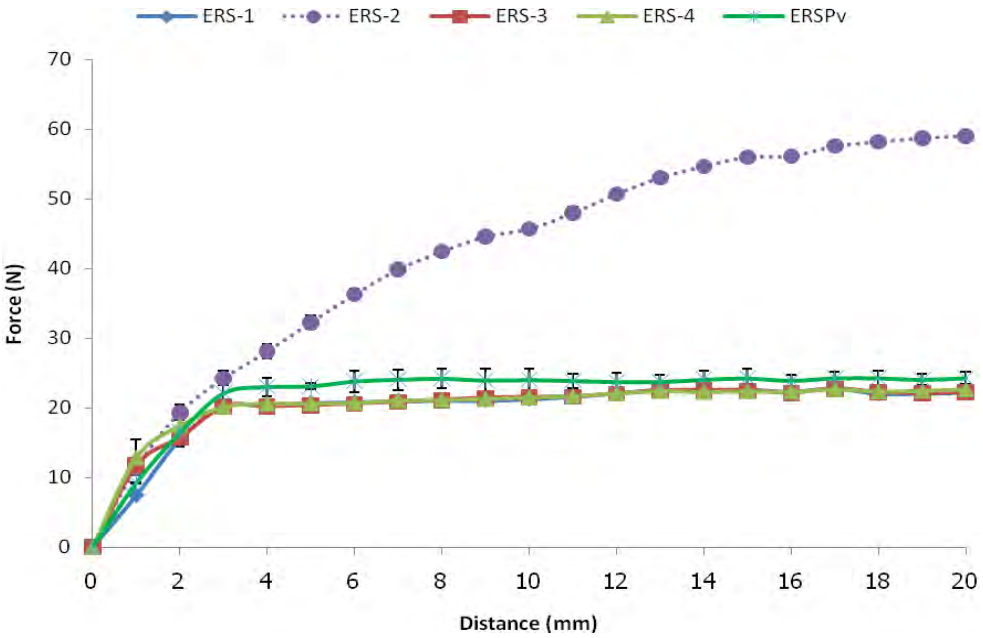

Figure 39 The syringeability profile of EC-R gel base (system 2-2) and EC-R-PVP gel base (system 3-1)

Figure 40 shows the syringeability property of the combination of EC, RS Plo with and without PVP (system 3-2). Both systems passed the hypodermic needle easily. These results would correlate with viscosity profile that according high syringeability to low viscosity. Addition of 0.75% PVP into preparation, the syringeability was slightly changed in a similar pattern and showed insignificant difference (*p-value* = 0.537). This might be required to incorporate a higher concentration of PVP, but concern about viscosity increased had to be accessed.

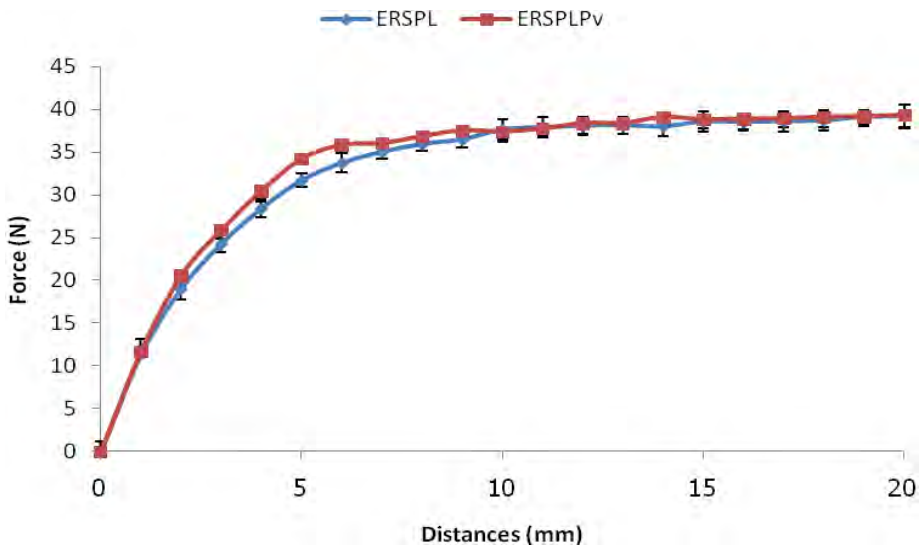

Figure 40 The syringeability profile of EC-R-PVP -Plo gel base (system 3-2)

The syringeability in of the combination of hydrophobic-hydrophilic gel base (system 3-3) based on water insoluble polymer such as EC, RS, RL with WS and PVP property shown in Figure 41. The syringeability profile was decreased when increasing the concentration of EC from 12.5% to 15%. In addition, the formulations containing different ratio of RS/RL exhibited the similar result in the syringeability profile (*p-value* = 0.937) and in agreement with the viscosity profile

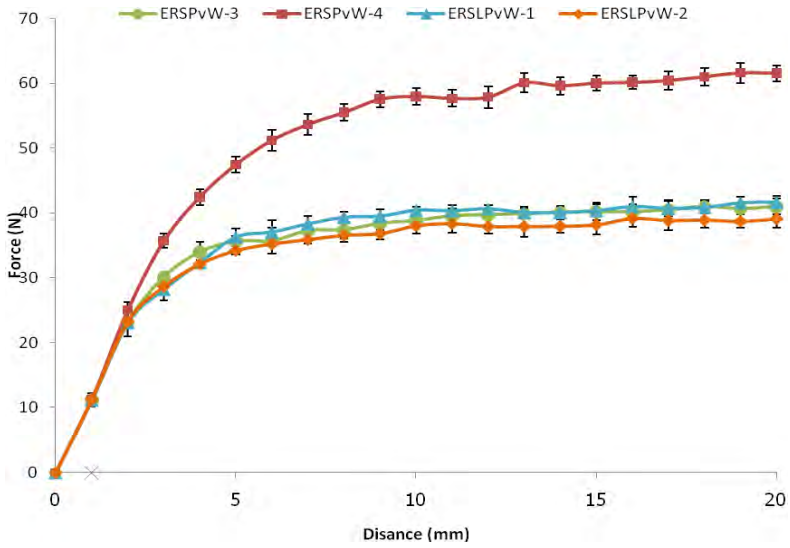

Figure 41 The syringeability profile of EC-R-PVP-WS gel base (system 3-3)

Figure 42 shows the syringeability of hydrophobic-hydrophilic gel base (system 3-4). The two main components, Plo and WS were variable in each formulation while the concentration of EC, RS and PVP were unchanged. The data showed that the formulation containing higher WS (20%) exhibited the higher syringeability than higher Plo (20%). The formulation that contained the increased amount of WS showed a higher volume density than the increase of Plo.

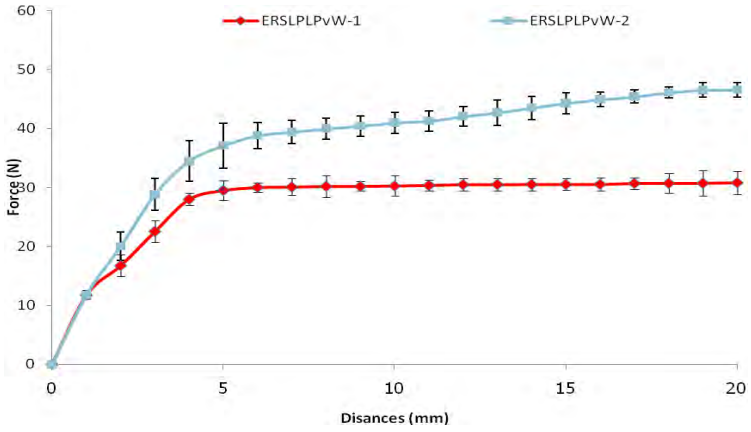

Figure 42 The syringeability profile of EC-R-PVP-Plo-WS gel base(system 3-4)

Figure 43 shows the syringeability of hydrophobic-hydrophilic gel based on CP and oil phase system (system 3-5). The syringeability profile was increased when increasing the concentration of CP and the oil phase ratio, the increasing of viscosity and higher volume density could occur, respectively. Therefore, this system could show both of action above to decreasing the syringeability.

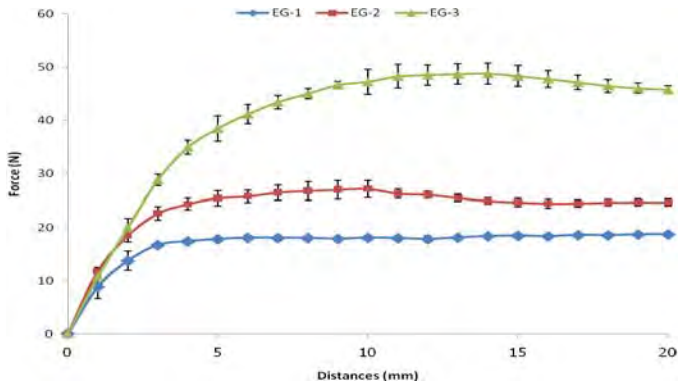

Figure 43 The syringeability profile of Emulsion gel base (system 3-5)

Spreadability

Spreadability shows the capability of periodontal gels to distribute over an area under pressure after applied on a surface. When periodontal pocket is opened and periodontal gel is inserted to the pocket site by 21-guage needle , the gingiva will reattach to the dental root after the device has been removed. Thereafter, the structure of periodontal gels will change to increase the surface area and show adhesive property. The fact, that only small amount of periodontal gel is inserted to the periodontal pocket, the formulated gel would spread over the surface inside the periodontal pocket and cover the infected area. Thereafter, high spreadability could increase drug release and efficiency of drug action.

The spreadability was inversely related to viscosity and syringeability force. Higher spreadability would be obtained from lower viscous gel which required lower syringeability force. Therefore, from this experiment the suitable spreadability should have widest spreadability diameter. However, other properties also needed to be considered. The spreadability of hydrophilic gels is shown in Figure 44. The diameters obtained from hydrophilic gels were the highest and the lowest in $\mathbf{C_1}$ and $\mathbf{HEC_{10}C}$ ranged from 21.93 ± 1.14 to 63.20 ± 2.54 mm, respectively. When increasing the concentration of CP from 1% to 5% and 11%, the diameter was decreased (*p-value* < 0.05). This result was due to the increased viscosity. All formulations showed statistically significant difference in diameters on spreadability profile (*p-value* < 0.05) under the same temperature condition. This explained the displayed behavior of each polymers, except the $\mathbf{C_5}$ ($\eta = 8.38 \pm 0.06 \times 10^3$ cps) had diameters close to $\mathbf{PVA_{10}C}$ ($\eta = 11.66 \pm 0.35 \times 10^3$ cps) and showed insignificant difference (*p-value* = 1.00). This might be resulted from their similar viscosity profiles. In this study, the spreadability property showed close correlation with syringiability and viscosity property. When increasing the syringeability force or increasing the polymer concentration due to increasing the viscosity property, caused a reduction of spreadability. This result was agreed with Varshosaz et al [2002]. They described that viscosity of all formulations were increased with higher hydrophilic polymer concentration and showed non-newtonian with the lower of syringeability and spreading in diameter test.

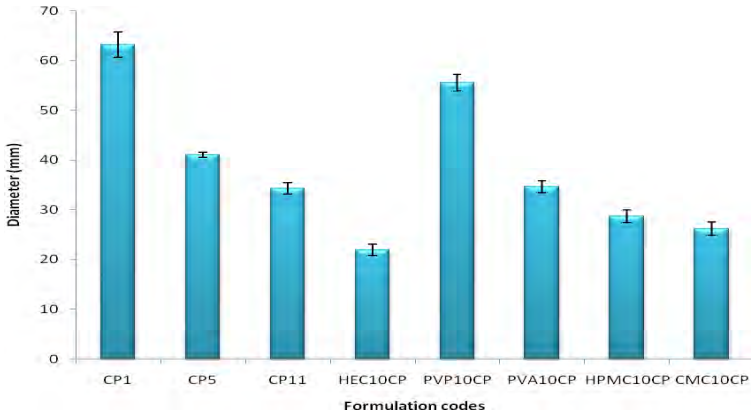

Figure 44 The spreadability of hydrophilic gel (system 1-1) at room temperature

The spreadability of thermoreversible gel and hydrophilic- thermoreversible gels is shown in Figure 45. At room temperature, increasing the concentration of Plo from 20% to 40%, led to an increase in dimeter from 43.93 ± 1.28 to 25.83 ± 0.77 mm. It was found that higher concentration of Plo would increase the resistance of periodontal gel movement. When increasing the concentration of Aerosil from 0.5% to 2% and 5% , and hydrophilic polymers in formulation of 20% Plo, the resistance of all formulated periodontal gels was also increased that showed decreasing in diameters (*p-value* < 0.05). These formulations exhibited viscous gel to highly viscous gel.

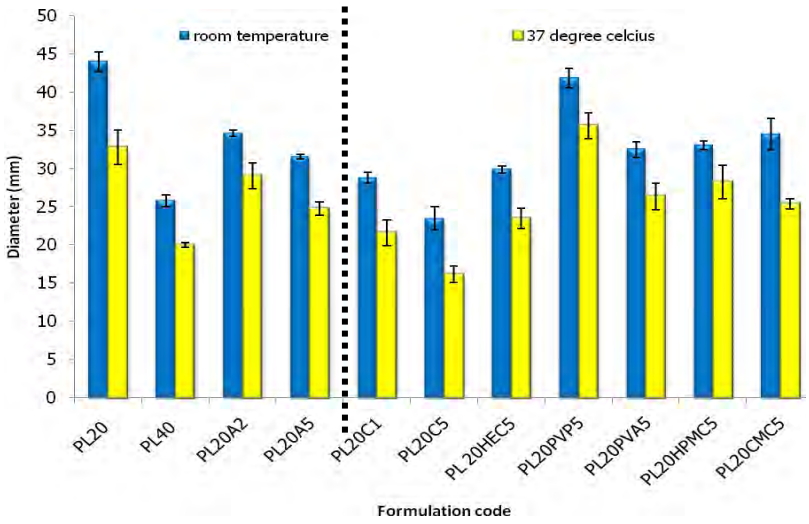

Figure 45 The spreadability of thermoreversible gel base and the hydrophilic-thermoreversible gel base in different temperatures (system 1-2 and 1-3)

When the experiment was tested at 37°C, the spreadability of each formulaion showed higher resistance and decreasing in diameters than when tested at room temperature (*p-value*<0.05). Increasing the concentration of Plo, Aerosil and hydrophilic polymers in poloxamer gel showed decreasing in diameters (*p-value* < 0.05).These formulations showed spreadability results similar to the previous experiment at room temperature, but in narrower diameters. According to Maheshvari et al. [2006], at higher temperature than 25°C, the gel could form high hydrogen bonding and closely packed micelles. Moreover, addition of Aerosil and hydrophilic polymers could exhibit the gel structure more closely packed and reduced the gelling temperature due to increasing the viscosity property. Therefore, the spreadability at 37°C had less spreading diameter than at room temperature.

Figure 46 shows the spreadability of poloxamer periodontal gel based on EC, RS, RL and WS. The results were different from previous data for Figure 43. From room temperature to 37 °C, poloxamer gel should be reversed from liquid to sol-gel, but these formulated gels exhibited slightly decrease in spreadability and showed statistically insignificant difference (*p-value* >0.05). According to previous results were correlated with viscosity profile. From the dispersion gel with evaporation might not establish to thermo-setting property

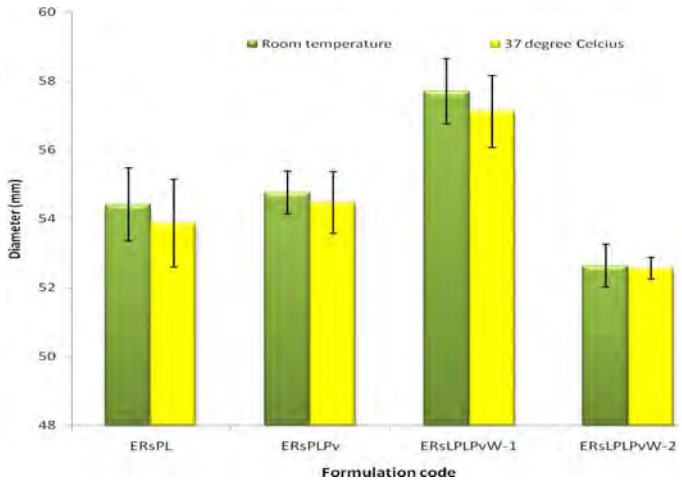

Figure 46 The spreadability of formulated gel containing water insoluble polymer and poloxamer with or without PVP and WS

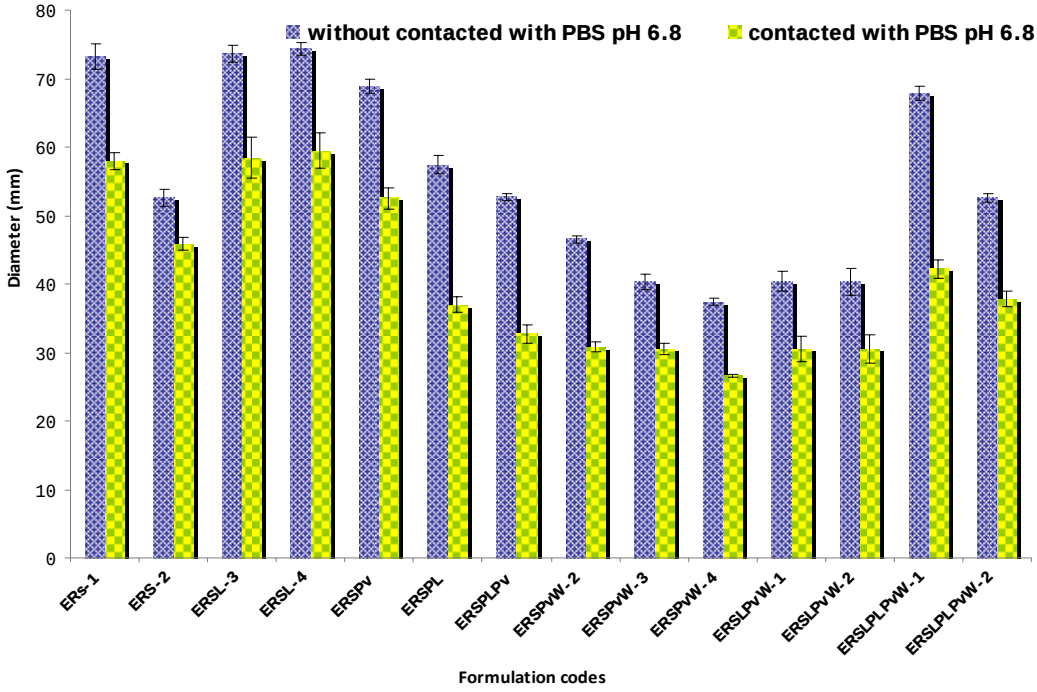

Figure 47 The spreadability of hydrophobic gel base with or without contacted with phosphate buffer solution pH 6.8

The spreadability of hydrophobic gel base prepared from EC, RS and RL polymers (Figure 47) described the effect of water insoluble gel on base movement. In this study, the spreadability property was evaluated after wetted with water. Therefore, since the powder or granule of water insoluble polymers was dissolved in 95% ethanol during preparation, when the preparation contacted the water, the surface immediately returned to solid like state.

From the results, hydrophobic gel based on EC,RS and RL could change to precipitates by aqueous environment. The suitable difference value between with and without contacted PBS should exhibit small difference in diameters because of drug higher release and drug efficiency might upon with spreading in diameter according to increasing the surface area to cover the position of bacteria infection.

The data showed that the mobility of each formulated gel base was reduced (*p-value* < 0.05). At various ratios of RS/RL (25:0, 22.5:2.5, 20:5) with or without

contacting PBS, these gels showed statistically insignificant difference on gel mobility (*p-value* > 0.05). When increasing the concentration of EC from 12.5% to 17 % in system 2-2 and from 12.5% to 15% in system 3-3, the spreadability was statistically reduced (*p-value* < 0.05). EC, RS and RL are water insoluble polymer, freely soluble in 95% ethanol with high and low water permeability for RS and RL, respectively [Rowe et al.,2003]. EC would sediment faster than RS and RL. Consequently, the gels prepared with ethylcellulose immediately transformed to harden structure. Higher the concentration of EC, especially in ER_S-2 (17.5% EC), led to higher resistance due to lower spreading diameter while preparation with RS and RL showed slower conversion and slightly different in spreading diameters. Conclusively, the suitable system for local periodontal pocket contained small amount of EC and water permeable polymers, RS or RL, especially in ER_SP_vW-4, which showed lower spreading diameter and very slight difference value with or without contacting PBS. Which was related to dense gel structure could protect PBS diffusion.

Figures 48-49 shows the spreadability of polyethylene and emulsion gel bases, respectively. The result showed similarly trend as the previous result. To increasing the amount of component or adding another component in the formulations, the spreadability of theses system showed increasing resistance or decreasing of gel movement.

Since high spreadability showed correlation with low viscosity, thus fast swelling, dissolution and erosion in the periodontal pocket [Perioli et al., 2004] while low spreadability due to high viscosity might be difficult to destruction, but these gels were too hard and not suitable to be prepared and used.

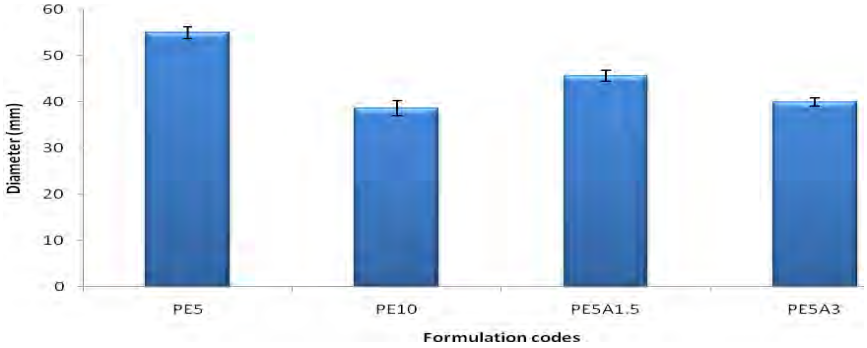

Figure 48 The spreadability of polyethylene gel base (system 2-1)

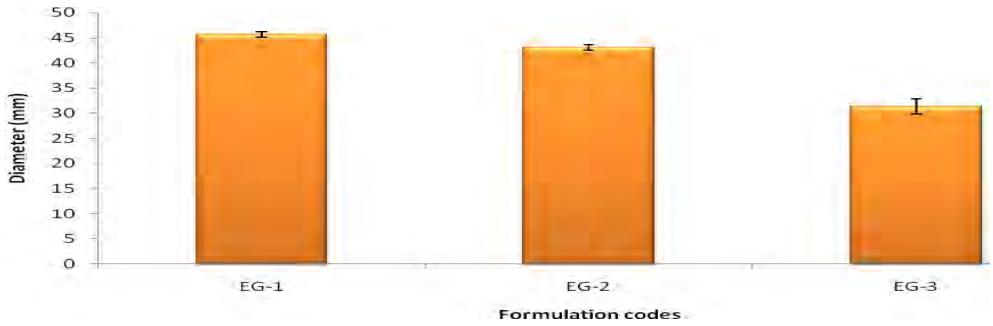

Figure 49 The spreadability of emulsion gel base (system 3-5)

***Ex vivo* mucoadhesive property**

In preliminary, the *ex vivo* mucoadhesive property of all formulated periodontal gel bases were tested by adhesion of gel to the porcine intestinal mucosa. The results showed that the adhesion time of formulations was more than 30 minutes with the rate of 37 °C phosphate buffer solution pH 6.8 flowing at 6.66 ml/min and 20 ml/min. During experiment, the extreme erosion of periodontal gel bases was shown in hydrophilic gel, slight erosion and fragmentation in emulsion gel, and scarce erosion in hydrophobic and hydrophilic-hydrophobic gel. Hydrophilic gel could be swelling due to hydration rate of each polymer, which enhanced the system to weight loss or erosion. The emulsion gels containing CP presented fragmentation due to CP was swollen inside structure, while hydrophobic gel showed water resistance. However, this experiment was not suitable to compare mucoadhesive of gel bases prepared from hydrophilic polymers according to weight loss, swelling, dissolution and erosion [Perioli et al., 2004]. Consequently, the experiment was reevaluated with a different method, using an Instron® universal testing machine.

In the later study, the mucoadhesive strength was determined by measurement of the force of detachment. This parameter is the most frequently studied adhesive property [Jones et al., 1997]. The forces required to detach each formulation from porcine intestinal mucosa are presented in Figures 50-54.

The mucoadhesive property of hydrophilic gel is shown in Figure 50. The adhesive force of only CP containing formulations was significantly increased by

mucoadhesive bonding strength when the concentration of CP increased. The formulations containing 1% CP with the combination of each 10% of PVP, PVA, CMC, HPMC and HEC exhibited significantly higher adhesive force than 1% CP alone. The highest and lowest adhesive forces were shown in **$HPMC_{10}C$** and **C_1** respectively. The results of measurement of adhesion force were in agreement with those of Perioli et al. [2004], who compared the mucoadhesive of buccal tablet using different cellulose and polyacrylic derivative such as hydroxylpropyl cellulose (HPC), hydroxylethylcellulose (HEC), hydroxypropylmethyl cellulose (HPMC), carboxymethyl cellulose (CMC), carbopol (CP) on porcine mucosa and reported that HPMC showed the highest mucoadhesion force and time due to the highest hydration rate and enhanced the adhesion to the mucosa.

Both **C_{11}** and **$HEC_{10}C$** exhibited high viscosity and low syringeability from previous study, **C_{11}** also showed highly adhesive whereas **$HEC_{11}C$** exhibited less adhesion which was closely to PVP adhesive property (*p-vale* =0.112). In fact, HEC, was reported to be the most hygroscopic and showed the fastest hydration [Baumgartner, Kristl and Peppas, 1996] while **$HEC_{11}C$** evidently was a poor adhesive system with the highest syringeability and viscosity. This may be due to the protonation of the OH group and less adhesion force with mucosa [Varshosaz, 2002]. In addition, this gel was too hard and not suitable from a mechanism point of view.

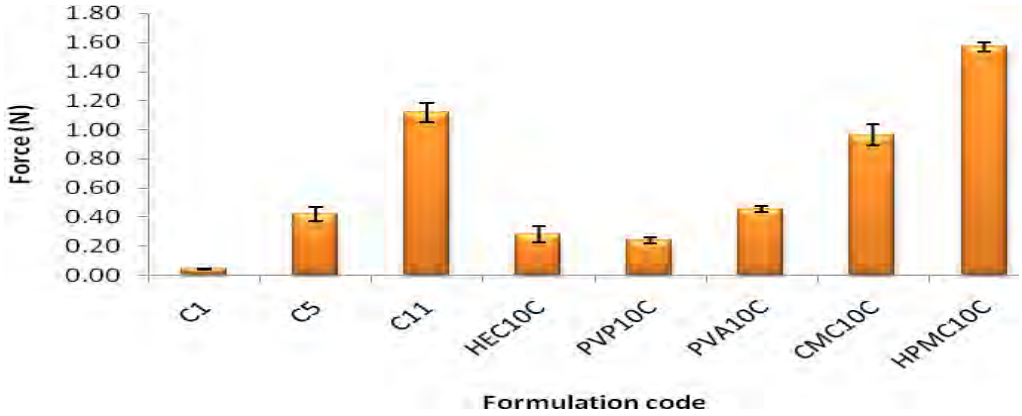

Figure 50 The mucoadhesive force of hydrophilic gel (system 1-1)

El-Kamel et al. [2002] showed an opposite result with Perioli et al. [2004] and explained that CMC showed the higher mucoadhesion than HPMC by initial faster

hydration rate that promotes interpenetration of the polymer chain with the tissue. Polyanions like Carbopol and CMC adhere strongly to the mucus compared to the nonionic polymers like HPMC [Ch'ng et al, 1985; Rao and Buri, 1989]. In addition, CMC could increase surface charge density of the gel. Moreover, the carboxylic group could also form hydrogen bonds with tissue.

Furthermore, Mortazavi and Smart [1994] reported the formation of intermolecular complexes between the glycoprotein and CP molecules. It was summarized that the ionized part of CP had bioadhesion force. Ionization of CP resultd in diminishing the intramolecular hydrogen bonds and generates a stretched cylindrical shape, which was then more able to penetrate a mucin network than the coil form of unionized CP [Hassan and Gallo, 1990]. The adhesion force of HPMC was found to be less than that of CMC and CP. This could be due to formation of thick and viscous-swollen gel. This gel was not continuous and forming localized pockets of polymer [Wan et al, 1993].

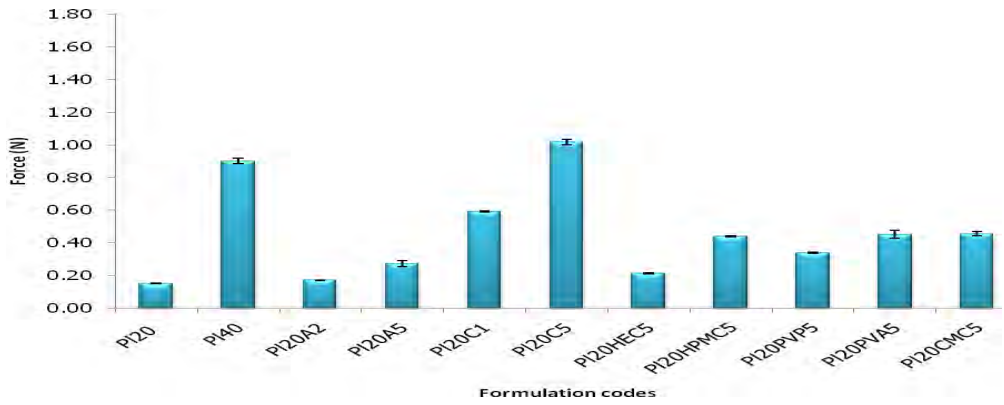

Figure 51 The mucoadhesive force of thermoreversible and hydrophilic-thermo-reversible gel (system 1-2 to 1-3)

The mucoadhesive behavior of poloxamer gel with or without hydrophilic polymers is shown in Figure 51. When increasing the concentration of Plo from 20% to 40 %, the adhesion was also increased from the higher viscous gel that related to gelling temperature decreased. Gelation of thermoreversible gel is affected by a range of factors, such as temperature, polymer concentration, concentration of active ingredient and electrolytes [Killoy, 1998]. Aerosil (Ae) incorporated in poloxamer gel exhibited a

increase adhesive property (*p-value* < 0.05). However, there was no significant difference (*p-value* = 0.051) in adhesion property when percentage of Ae was ranged from 2% to 5%. The mucoadhesive behavior showed in term of detachment force. The results of the detachment force study support the hypothesis that the possible mechanism of the mucoadhesion exhibited by the liquid crystalline poloxamer gel was the dehydration of the mucosa. The amount of water taken up by the liquid crystalline gels governed the mucoadhesive force; the gel higher water uptake capacity showed greater mucoadhesion [Esposito et al., 1996]. In addition, when poloxamer gel incorporated with hydrophilic polymer showed the highest mucoadhesive in $\mathbf{P_{L20}C_5}$ (1.02±0.02 N), which led to synergistically increase the mucoadhesion by the interaction of CP and Plo to mucin when gelling temperature was decreased.

The adhesion of hydrophobic gel base is shown in Figure 52. All formulations showed poor adhesive by itself similar to the results from Jones et al [1997]. The adhesion of hydrophobic gel base was lower when compared with hydrophilic gel. Increasing amount of polyethylene and Ae reduced adhesion of gel base system. In fact, this system containing polyethylene and mineral oil with or without Ae showed poor adhesion. Increasing the concentration of PE from 5% to 10% and Ae increased from 0.5% to 1.5 and 3%, led to significantly decrease the adhesive force (*p*-value < 0.05). These formulations showed strong barrier property when the system was spread on surface of mucosa membrane. Water could not penetrate though destroyed gel structure. When increased amount of PE and Ae that increased system containing higher hydrophobic volume and might decreased the adhesion property. In addition, the adhesion was more decreased from system containing hydrophobic polymer as water insoluble polymer. The attachment force was decreased when the gel would transform semi-solid state to solid like state. The results was correlated to system 2-2

There was statistically insignificant difference in adhesive property when increasing the concentration of polymer and changing the ratio of RS/RL (*p-value* = 0.715), but significant was noted when the formulation was incorporated with 2% PVP (*p-value*< 0.05). Addition of PVP led system changed to viscous gel and could increase

the adhesion while changing the ratio of RS/RL had no effect as previous viscosity results.

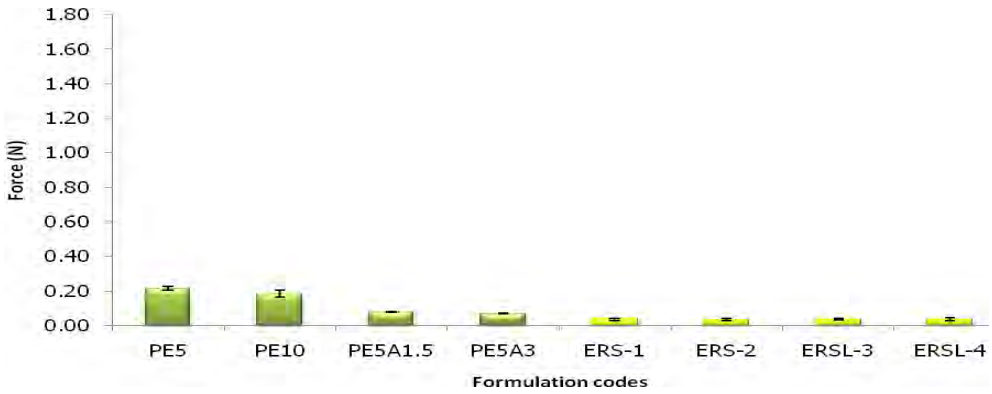

Figure 52 The adhesive force of polyethylene gel and EC-R-WS gel base

Moreover, addition of thermoreversible polymer at 20% Plo with or without 2% PVP to **ER$_S$-1** led to significantly increase the mucoadhesive property (*p-value* < 0.05). The effect of WS on adhesive force is shown in Figure 53. When WS was added in the formulations instead of Plo, the adhesive property was increased more than by Plo. Initially, this might show the adhesion force from viscous polymer while WS could protect themselves from aqueous environment. Subsequently, increasing the concentration of WS from 20% to 25% had no significant effect on adhesive property (*p-value* = 0.867). In contrast, when increasing the amount of EC from 12.5% to 15%, there was a significant reduction adhesive property (*p-value* < 0.05). This result revealed that the amount of EC evidently had more pronounced effect on sedimentation and adhesive property than that of WS.

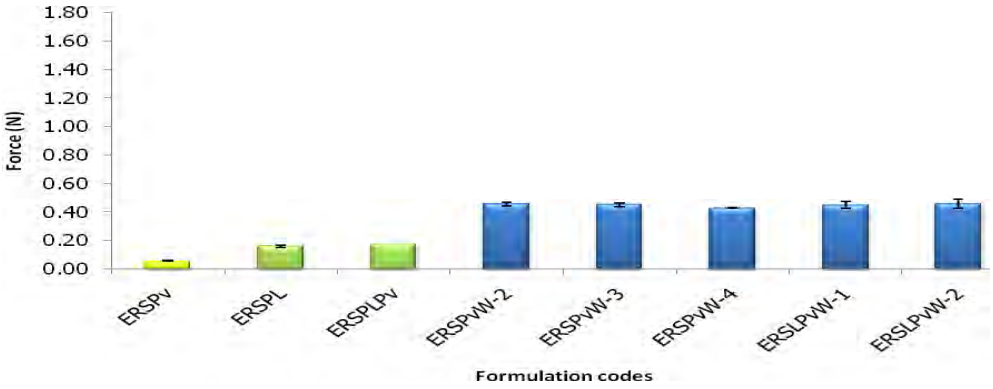

Figure 53 The adhesive force of hydrophobic-hydrophilic gel base (system3-1 to 3-4)

The mucoadhesive property of emulsion gel that contained a water phase, including of 1% and 5% of CP is shown in Figure 54. The mucoadhesive property of these formulations was increased when reduced the amount oil phase and increased amount of mucoadhesive polymers. The fact, oil phase showed poor mucoadhesive while CP could form interaction with mucin [Jones et al., 1996].

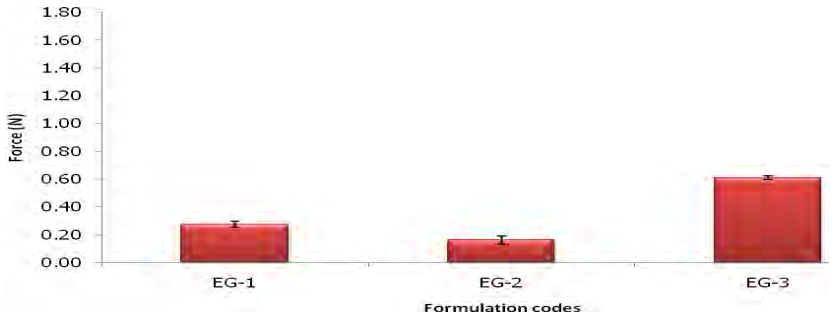

Figure 54 The adhesive emulsion gel base (system 3-5)

Disappearance test

This study was investigated the time of periodontal gel bases disappeared. In all hydrophilic, thermo-reversible and the combination of hydrophilic and thermo-reversible formulations showed the disappearance time between 0.4 ± 0.04 to 7.3 ± 0.25 hours, except that **$HEC_{10}CP$** exhibited the disappearance time of more than 12 hours but less than in 24 hours, It might corelate with highest viscosity The results are showed in Figure 55.

The hydration of hydrophilic polymers could be reached complete dissolution after 24 hours. **Pl_{20}** showed the highest dissolution and completely dissolved after 24.00 ± 2.65 minutes while increasing concentration of Aerosil from 0.5% to 2% and 5% could be decreased degradation system. These results revealed that block copolymer poloxamer 407 gel was reported to be formed by H-bonding in the aqueous system, caused by the attraction of the ether oxygen atom to a proton of water. When the hydrogen bonding is supplemented by adding compounds or excess of water with a hydroxy group, the gelation point decreased [Dumortie et al., 2006]. The gel structure was thought to remain unaltered with water until an excessively high aqueous caused the destruction of the gel structure. As the concentration of the Aerosil increased, the gel became more closely

packed with the arrangement in a lattice pattern. Moreover, Maheshwari et al. [2006] pointed out that when the concentration of Aerosil increased, the poloxamer gel indicated the structure of the gel functioned as a barrierand . Such enhanced resistance may be due to the increase in the size of micelles within the poloxamer gel structure, which led to higher viscosity and lower drug release. Aerosil supplemented hydrogen bonding, which enhanced the dehydration of poloxamer. As a result of these dehydration, micelle entanglements were occurred and could not separated easily from each other, which accounts for the rigidity and slow dissolution of these gels [Esposito et al., 1996].

Disappearing of polyethylene and emulsion gel could be not investigated, but it was noticed that their physical appearance was loosed and flaked after 1 hour for emulsion gel while whole polyethyene gel in PBS showed its unchangeable form. Interestingly, the hydrophobic formulations based on EC, RS,RL and WS with or without Plo and/or PVP transformed from semi-solid to solid like state and appeared intact for more than 24 hours.

In Vivo evaluation of the selected formulation in periodontal pocket and *in vitro* morphology

The retention of selected periodontal gel in the periodontal pocket was the major concern, as it was necessary to ensure that the periodontal gel would remain there for the intended period of drug release.

The concentration of 5% MTZ was added to these selected periodontal gel bases and storage for 24 hours in room temperature for equilibrium. except the hydrophobic gels which were kept and fixed at refrigerated temperature for stabilization.

The disappearance or erosion property of periodontal gels investigated after 5 hours by visual inspection, was a preliminary technique to investigate the selected gels that remained or disappeared. Briefly, this technique used a periodontal probe to seperate the pocket from top to bottom and inspect around periodontal pocket. Moreover, an

available interview from pateints after insertion of seleted gel such as feeling and tasting were additionally used to select the suitable periodontal gel base system.

Initially, hydrophilic gels, **C_1, C_5, $HPMC_{10}C$, P_{L20}, P_{L40}** and **$P_{L20}C_1$** were selected to test on pateints that had the periodontal pocket deeper 3 mm, which met the required criteria. Unpleasant bitter taste was shown after drug release from these formulations. Bleeding is an importance role to clear or remove the periodontal gel after insertion. All pateints showed bleeding immediately. Aproximately 20-80% of periodontal gels were squeezed outside the periodotal pocket by bleeding and GCF flowing pressure. Moreover, Esposito et al [1996] reported that poloxamer gel was complete disappearance after 1 hour after *in vivo* application.

The results in this study suggested that bleeding was an important problem in drug delivery to periodontal pocket. The efficiency management of periodontal gel system based on hydrophilic polymers should initially be self protected from bleeding and immediately adhered with tissue or dental root. These formulations seemed not to be suitable preparations due to bitter taste, fast of product detachment and disappearance. Moreover, the variable environment e.g. bleeding, flooding of GCF, drinking, chewing, compaction and food whih could destroy gels in a mimute to hours [Jones et al., 1996].

Anders and Merkle [1989] described that the initial adhesion was most importance of hydrophilic gel base system that would extent on duration of drug in periodontal pocket with bleeding and higher GCF from imflamation levels. Because hydrophilic polymers, as bioadhesive materials, were affected by water and some may dissolve in oral cavity including periodontal pocket, it was important to establish the duration of the adhesive force provided by the chosen polymer However, adhesion decreased with contact time in human volunteers with bleeding effect may approach to drug delivery system failure.

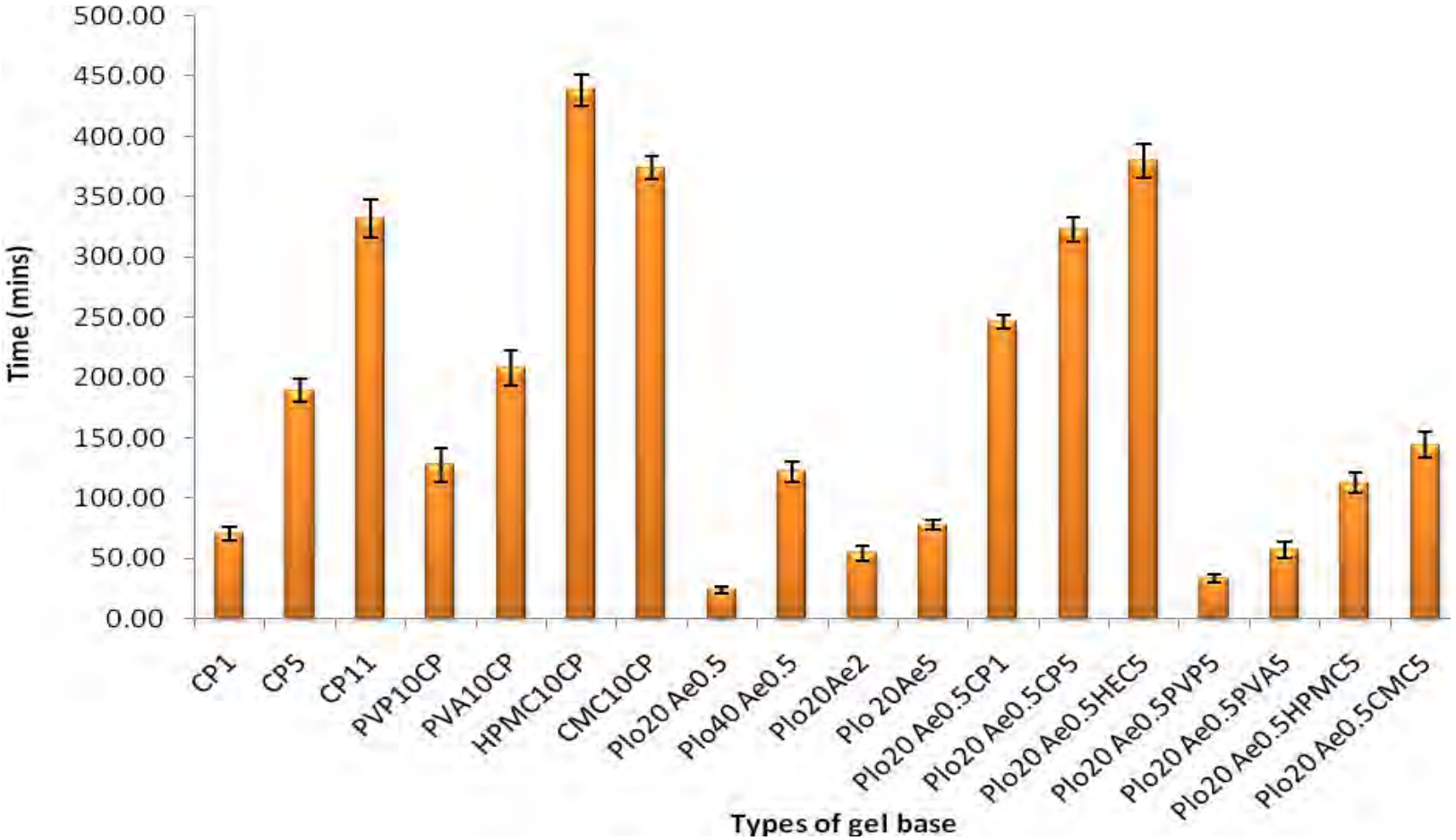

Figure 55 Disappearance time of hydrophilic, thermo-reversible, hydrophilic-thermo-reversible gel

In hydrophobic gels, **P_{E5}**, **P_{E10}**, **$P_{E5}A_3$**, **ER_S-1**, **ER_S-2**, were tested. All formulations showed ease of use. The syringeability values were below 100 N with low viscosity and unchangeable physical structure in water. When the formulated gels was inserted to periodontal pocket, bleeding also washed out and then removed all formulations outside the pocket. In *in vitro* study, polyethylene gel showed no physical transformation and could creat a thin cover to porcine mucosa tissue under pressure from pocket wall whereas *in vivo* study, gels showed easily moved out by bleeding with enhancement from GCF, saliva and abrasive force. [Thau and Charles, 1965]. In additon, polyethylene gel containing mineral oil in approximatly 90-95%, poor retention of oil base formulations within the aqueous environment had been reported [Jones et al., 1996].

All formulations that containing EC, RS and RL, water insoluble polymers, could transform semi-solid to solid like state during gels contacted aqueous environment. These polymer were primarily dissolved in ethanol. This mixtures were incompatible with water and then consequent to solid form as a netwok structure. *In vivo* study, these formulated gels used the aqueous environment from bleeding, GCF, saliva and aqueous additive such as humidity or drinking water that formed the gel structure. A thin sheet intra-periodontal pocket could occurred under pressure of pocket wall of pocket. This system correlated with "**like dissolve like**".

ER_S-1 and **ER_S-2** showed similar results as polyethylene gel. The systems were removed after bleeding, but still adhered to the surface of tooth. *In vitro* morphology of **ER_S-1** and **ER_S-2** is shown in figure 56-57. The physical apperance of this system revealed that the surface of formulated gel immediately transformed to solid like state. The surface of solid like state was smooth with slightly rough and very brittle, hard and easily broken, while liquid-viscous inside structure was still depicted and slowly hardening up.

It was noticed that when incorporation of 5% MTZ in this gel after preparation for 24 hours, sedimentation was observed. Thus freshly prepared gel was obtained before each study.

In addition, transformation of gel structure was irregular, which depend on the site of deposition such as deep of pocket site, aqueous environmentto solid appearance of gel structure was inconsistent form upon the spreadability of gels and the deep of pocket site. Moreover, amount of aqueous environment could faster stimulate transformation of the gel structure.

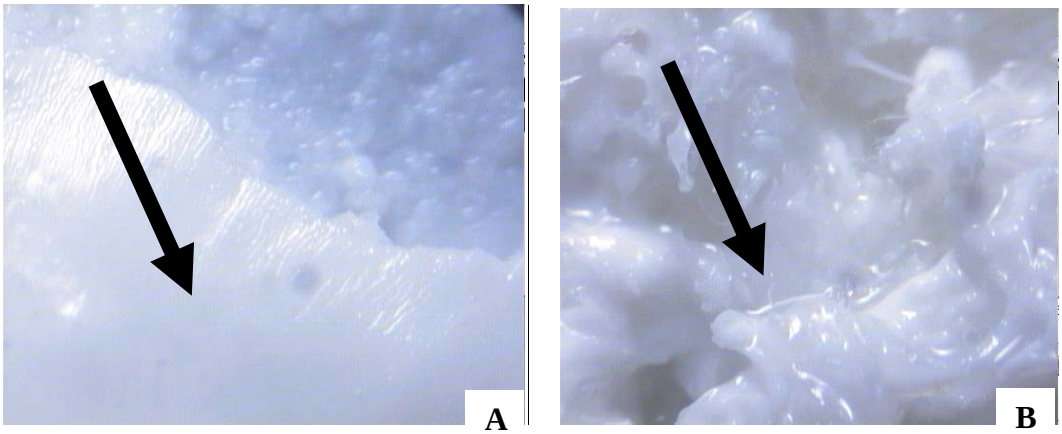

Figure 56 The surface (A) and cross section (B) morphology of **ER_S-1** in vitro test without PVP in aqueous environment by stereo microscope (× 7 times)

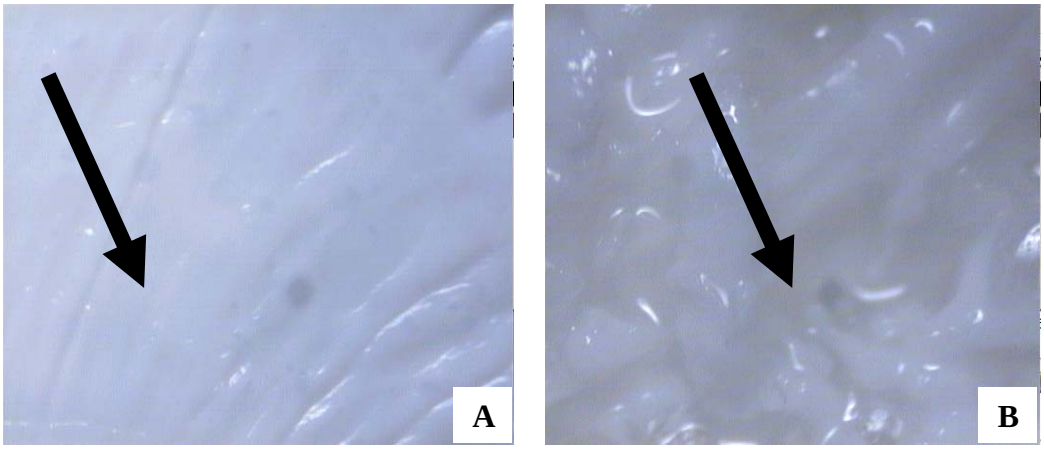

Figure 57 The surface (A) and cross section (B) morphology of **ER_S-2** in vitro test without PVP in aqueous environment by stereo microscope (× 7 times)

For hydrophilic-hydrophobic gels, **ER_SP_v**, **$ER_SP_LP_v$**, **ER_SP_vW-3**, **ER_SP_vW-4** , **$ER_{SL}P_LP_v$W-2**, **EG-1**, **EG-2** and **EG-3** were selected to test in periodontal pocket. Initially, **ER_S-1** was incorporated with 2% PVP (**ER_SP_v**), the viscosity and adhesive

property were increased, but the result was similar to that of **ER_S-1**.The adding of mucoadhesive polymer might intacted the periodontal gels inside the pockets when the bleeding was stopped, but GCF and saliva were still continuously flow. The **ER_S-1** with or without PVP exhibited a solid network as a thin sheet when its contacted with aquoues environment. The *In vitro* mophology of this system is shown in Figure 58.

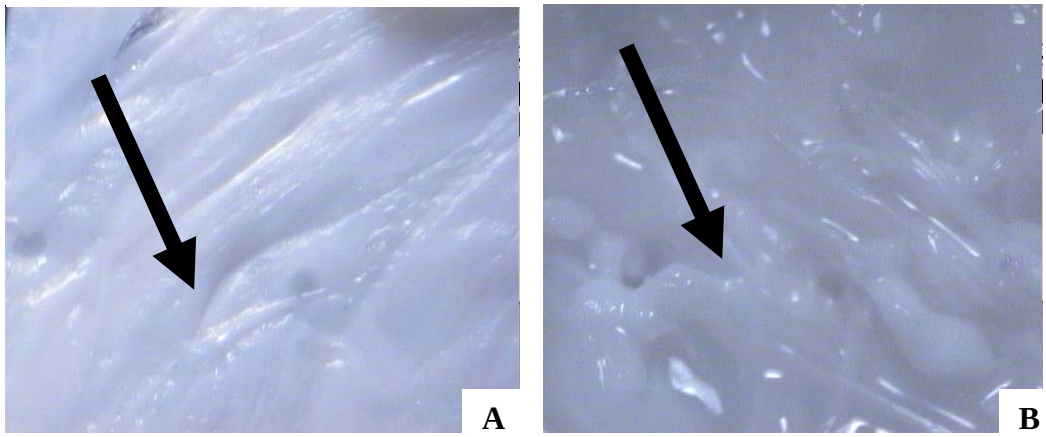

Figure 58 The surface (A) and cross section (B) morphology of **ER_SP_v** in vitro test with aqueous environment by stereo microscope (× 7 times).

$ER_SP_LP_v$ showed a satisfied state inside periodontal pocket. Gel was removed after bleeding and small content of gel could pack and remain in intra-periodontal pocket. This formulation was still seen after 5 hours, but clearly disappeared after 24 hours. The in vitro mophology of this formulation showed in Figure 59.

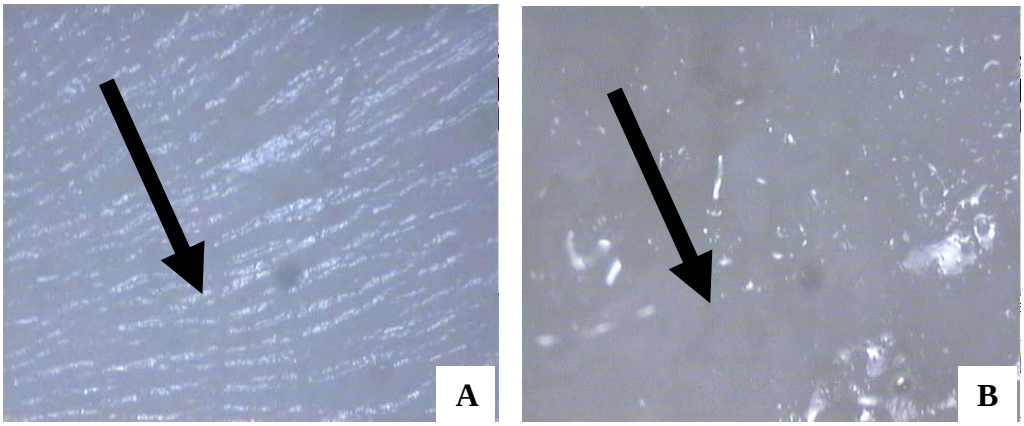

Figure 59 The surface (A) and cross section (B) morphology of **$ER_SP_LP_v$** in vitro test with aqueous environment by stereo microscope (× 7 times).

Interestingly, **ER_SP_vW-3** and **ER_SP_vW-4** were shown to be satified periodontal gel systems and their *in vitro* morphology is showen in Figures 60-61. Although bleeding was occurred but could stop by the effect of these formuations. White soft paraffin (WS) has a function of ointment base, stiffening agent as a component of creams, ointments [Raymond et al., 2003]. The stiffeness property of WS could hold the system to dense network during gel transformation upon contacted with aqueous environment. Bleeding and GCF could help the system to immediately set up as a network. Both formulaltions exhibited high resistance to repulsive pressure from bleeding at periodontal pocket and could be remained inside periodontal pocket for more than 24 hours in all pateints with bleeding at initial period and still remained intact in periodontal pocket deeper than 3 mm

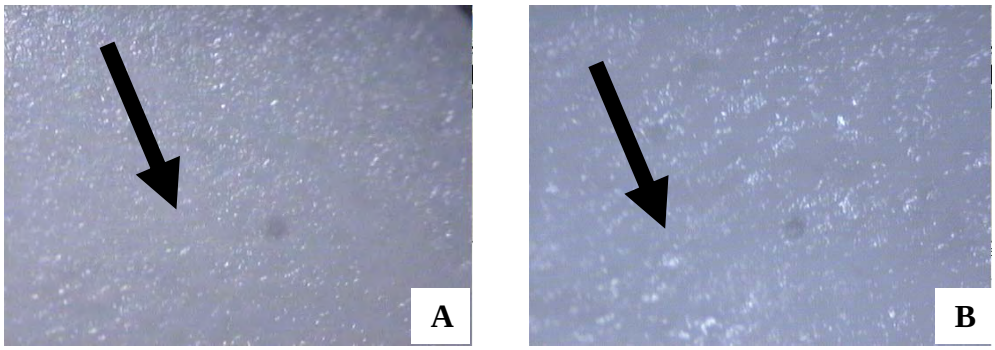

Figure 60 The surface (A) and cross section (B) morphology of **ER_SP_vW-3** *in vitro* test with aqueous environment by stereo microscope (× 7 times).

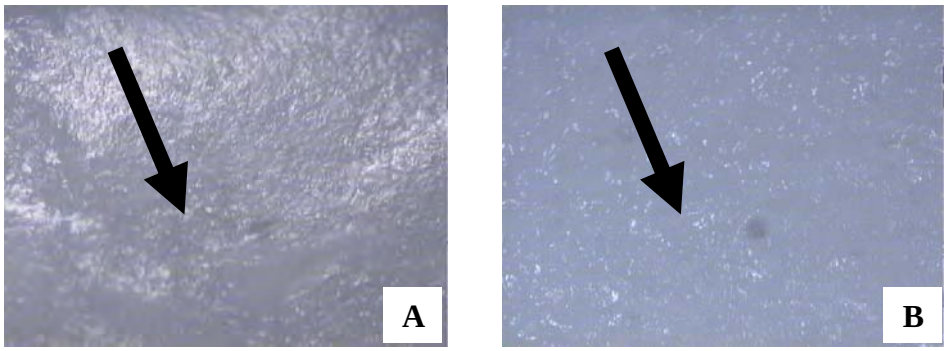

Figure 61 The surface (A) and cross section (B) morphology of **ER_SP_vW-4** *in vitro* test with aqueous environment by stereo microscope (× 7 times).

Both of **$ER_{SL}P_LP_vW$-1** and **$ER_{SL}P_LP_vW$-2** containing amount of poloxamer at 5% and 20% , changed to loose network after gel contacted with water. Poloxamer concentration at below 20% performed as a surfactant or emulsifying agent while white soft paraffin showed as a parafin wax [Raymond, 2003]. When both formulations contacted with water from aqueous environment, surface tension of the system was reduced by emulsifying action from poloxamer. These formulations could be remained inside periodontal pocket at least 5 hours but not more than 24 hours. The in vitro morphology of these formulation is shown in Figures 62-63.

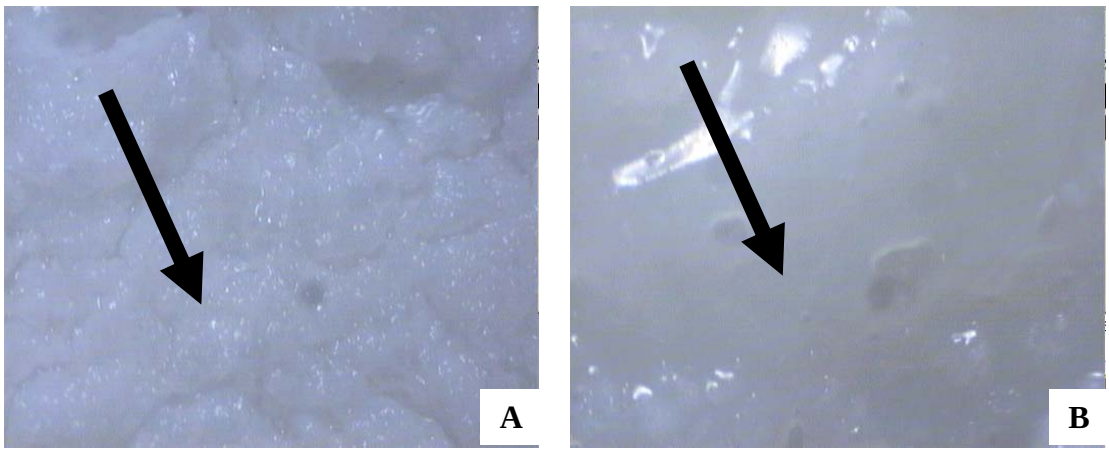

Figure 62 The surface (A) and cross section (B) morphology of **$ER_{SL}P_LP_vW$-1** *in vitro* test with aqueous environment by stereo microscope (× 7 times).

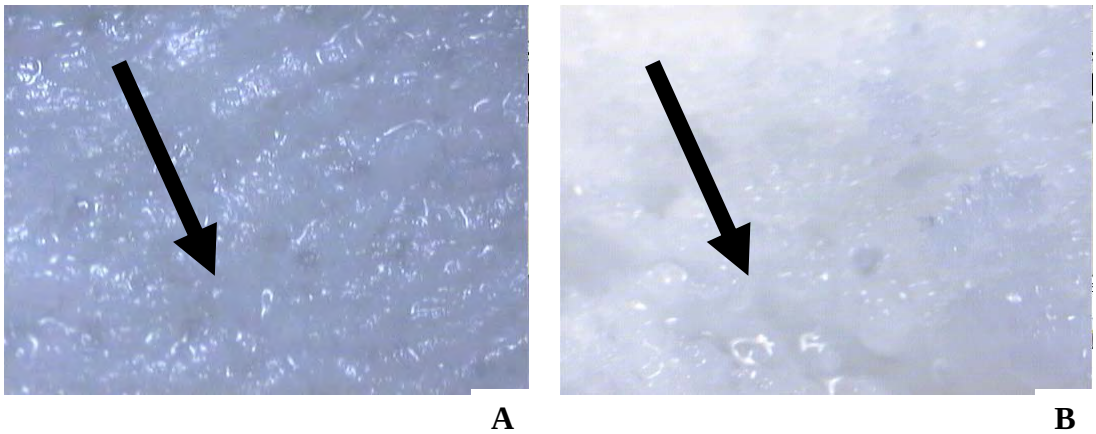

Figure 63 The surface (A) and cross section (B) morphology of **$ER_{SL}P_LP_vW$-2** *in vitro* test with aqueous environment by stereo microscope (× 7 times).

From all emulsion gel preparations, **EG-1**, **EG-2** and **EG-3**, were selected and insert to peridontal pocket. These systems composed of oil phase thus were protected from water surrounding themselves. Gels could adhere to tissue membrane or dental root from amounts of CP and oil phase compositions. However, the systems were then detached as a function of bleeding clearance rate. This system showed poor retention of oil based gel similar to polyethlene gel

In this study, the satisfactory periodontal system was **EC-R-WS-PVP** gel (system 3-3). The formulated gels showed satisfied viscosity and syringeability, good retention within the periodontal pocket and resistance in aqueous environment due to prevention of rapid clearance by the flushing action of bleeding and positive flow of crevicular fluid from the pocket into the oral cavity. In addition, when this periodontal gel was inserted, although the excess gel as residual gel initially exhibited bitter taste but recovery could be obtained by drinking or rinse the mouth with water. Moreover, all pateints could feel like soft solid in periodontal pocket but the irritation which caused the inflammation such as pain, redness and swelling was diappeared. Periodontal gel was further evaluated.

Finally, Two different satified formulations with or without 5% MTZ were further evaluated. One based on a hydrophobic polymers (a mixture of EC, RS and RL in alcohol), liquid-viscous phase could transform to solid phase depending on water content or bleeding. The other based on a lipophilic or hydrocarbon compound, white soft paraffin, as prevention of gel destruction from aqueous enviroment in mouth cavity and might hold the structural system inside periodontal pocket for long-lasting drug release.

1.2 Appropriate periodontal gel base with MTZ incorporation

1.2.1 Incorporation of 5% MTZ in periodontal gel

According to the evaluation of the periodontal gels in *in vivo* periodontal pockets. The appropriate periodontal gel base formulations were system 3-3.There were 5 preparations with different concentrations of EC, RS, RL, WS and PVP which showed stability result after 1 week in previous data (Table 9), and 5% MTZ was incorporated to the gel base. All formulations with or without 5% MTZ were reevaluated at 1 month and 6 months.

Physical stability test

Physical appearance

The stability of all system 3-3 formulations at 1 month and 6 months at refrigerated temperature (4-7°C) showed good appearance as initial, except **ER_SP_vW-2** formulation that was separated into two phases after 1 month. Phase separation might relate to the decreasing of viscosity property (as shown in Figure 31) which might not be enough to hold and fix the system. Therefore high kinetic energy was occurred and obviously revealed phase separation. The term kinetic energy referred to energy attributed to the gel movement and would increase with the high temperature and immiscible liquid in mixing compounds. The result of physical stability was shown in Table 10. In addition, when the concentration of 5% MTZ was incorporated into the periodontal gel base system, the system was yellowish from the color of MTZ. The viscosity property slighly increased from solid dispersion in preparation (as shown in Table 11) .

Evidently, all formualtions showed phase separation at freeze-thawing for 6 cycles [Prince, 1977], Thai-FDA stability testing and room temperature. Freeze-thawing and Thai FDA stability testing were observed under temperature 45-48°C which could melt white soft paraffin into liquid phase and enhanced evaporation of

Table 10 Appearance and stability of system 3-3 gel base with or without 5% MTZ after 6 month storage at room temperature (25-31°C), refrigerated temperature (4-6 °C), after freeze-thawing and After FDA stability testing

Formulation code	**Physical appearance**							
	Gel base				**5% MTZ in gel base**			
	room temperature	**refrigerated temperature**	**after freeze-thawing**	**After FDA stability**	**room temperature**	**refrigerated temperature**	**after freeze-thawing**	**after FDA stability**
ER_SP_vW-3	S	HD	S	S	S	HD	S	S
ER_SP_vW-4	S	HD	S	S	S	HD	S	S
$ER_{SL}P_vW$-1	S	HD	S	S	S	HD	S	S
$ER_{SL}P_vW$-2	S	HD	S	S	S	HD	S	S

where S= phase separation, HD = homogeneous (dispersed gel)

alcohol in preparations. Since the preparations composed of 2 main phases including polar phase from viscous mixture and non-polar phase from white soft paraffin, finally, at room temperature,these gels were be separated. High temperature or stress condition produced high kinetic energy. This might affect or distroy the system. In this study, the incompatibility of polar and non-polar compound could be improved by a fixed the molecular movement at refrigerated temperature .

Viscosity test

All formulations clearly showed plastic flow behavior and showed yield stress (τ_0) after storage at refrigerated temperature for 0, 1 and 6 months. The data are shown in the table 11

Table 11 Yield stress of formulations after storaged at refrigerated temperature

Formulation code	Yield stress ($\times 10^3$ cps)					
	Gel base			5% MTZ gel		
	0 month	1 month	6 month	0 month	1 month	6 month
ER_SP_vW-3	23.92±2.02	23.84±1.89	23.52±1.10	24.03±1.28	24.82±2.84	24.90±1.10
ER_SP_vW-4	26.77±2.47	25.2±2.00	26.51±1.57	27.28±0.73	27.23±1.25	27.75±1.13
$ER_{SL}P_vW$-1	24.69±2.08	23.35±1.00	24.05±0.10	24.84±2.58	24.95±1.77	24.52±1.47
$ER_{SL}P_vW$-2	23.56±1.53	24.50±2.29	24.76±1.52	24.38±1.20	24.61±1.85	24.34±1.62

Viscosity measurement are useful in determining periodontal gel base with and without 5% MTZ. From the results, the viscosity of all tested periodontal gels with and without 5% MTZ showed no significant difference (*p-value >0.05*) in yeild stress between each gel base and 5% MTZ gel after storage at refrigerated temperature. It was possibly from small amounts of drug distributed inside gel structure. The viscosity property was used to predict the physical stability of these system and related to the previous stability result of **ER_SP_vW-2**.

There was statistically insignificant difference (*p-value >0.05*) of 0, 1 and 6 months in viscosity profile. Gel preparations were packed and lower the flowability by refrigerated temperature due to the decrease of kinetic energy by static condition.

Syringeability test

The syringeability of selected periodontal gel base and periodontal gel base containing 5% MTZ at 0, 1 and 6 months were tested and depicted in Figures 64-67. The syringeability was slightly changed when 5% MTZ was incrporated. This might relate to viscosity similar to previous result (Table 11). The syringeability of all formulations at refrigerated temperature showed slightly different at 0 month, 1 and 6 months. The static condition at lower teperature and drug dispersion though gel structure by saturated drug solubility were possibly to be physical stabilization. The syringeability of periodontal gel and periodontal gel containing 5% MTZ were closely related the viscosity profile. For system of inceasing viscosity value, the syringeability force was also increased. Consequently, the syringeability property could also predict the physical stability test as viscosity property.

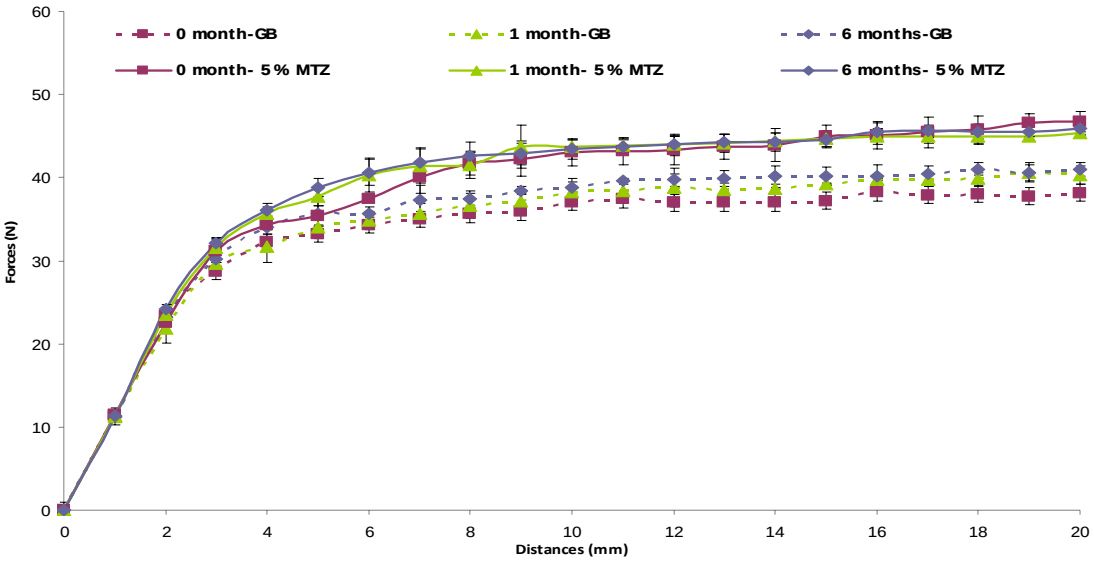

Figure 64 The syringeability of **ER_SP_vW-3** formulation and **ER_SP_vW-3** formulation was incorporated with 5% MTZ at initially, 1 month and 6 months in refrigerated temperature

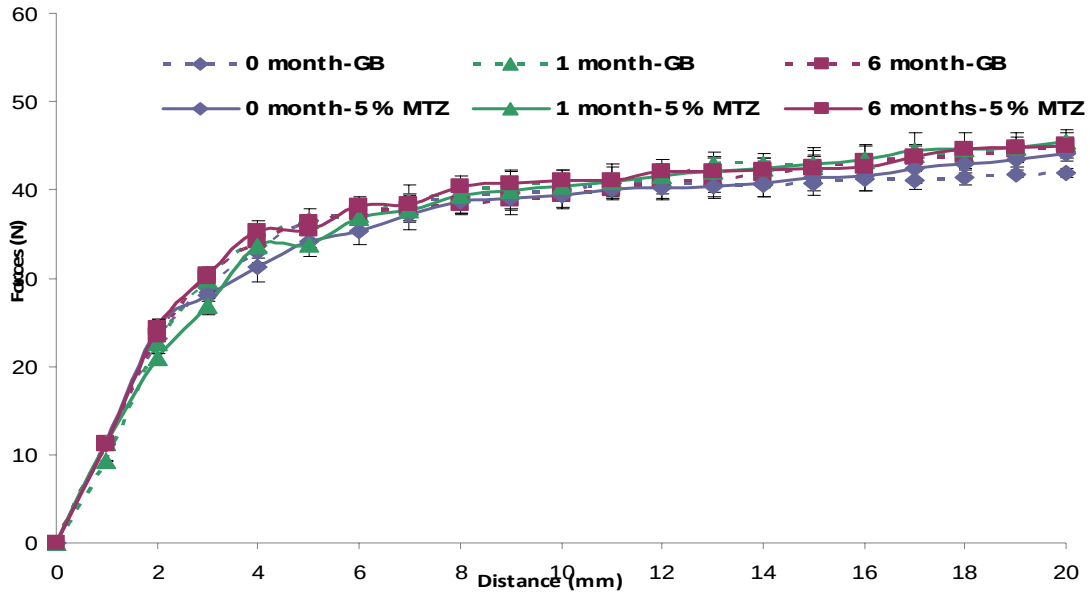

Figure 65 The syringeability of **$ER_{SL}P_vW$-1** formulation and **$ER_{SL}P_vW$-1** formulation was incorporated with 5% MTZ at initially, 1 month and 6 months in refrigerated temperature

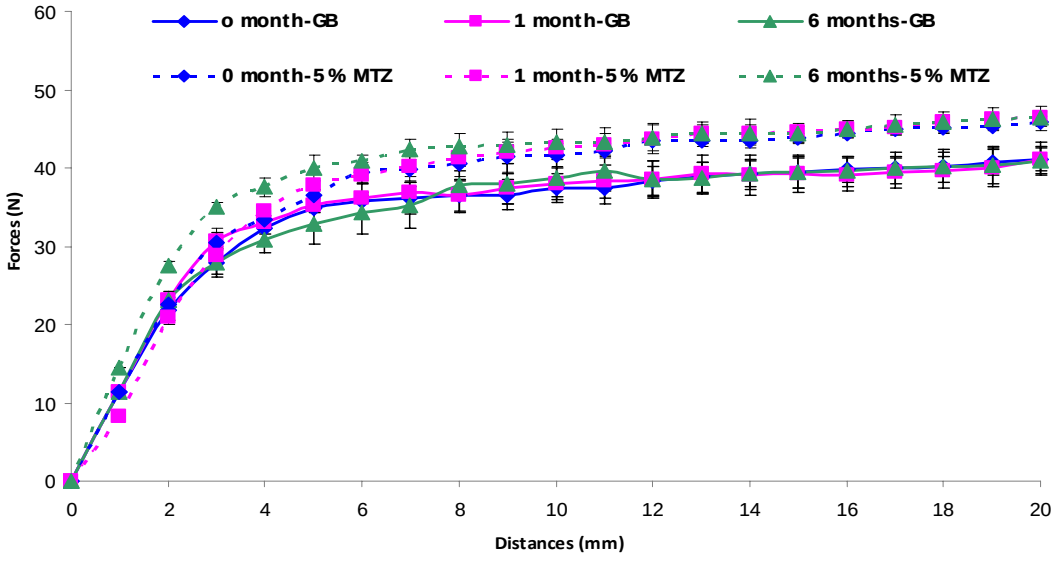

Figure 66 The syringeability of **$ER_{SL}P_vW$-2** formulation and **$ER_{SL}P_vW$-2** formulation was incorporated with 5% MTZ at initially, 1 month and 6 months in refrigerated temperature

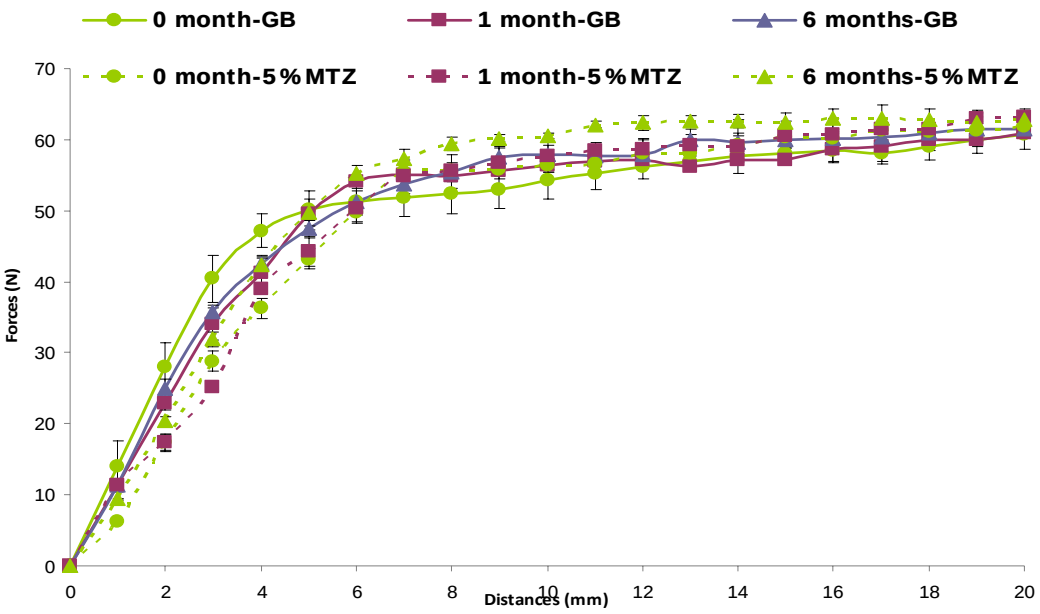

Figure 67 The syringeability of **ER_SP_vW-4** formulation and **ER_SP_vW-4** formulation was incorporated with 5% MTZ at initially, 1 month and 6 months in refrigerated temperature

Effect of Temperature on Syringibility

The periodontal gel at 5% MTZ was higher viscosity and that very difficult for the periodontist use then after formulation had been kept in refrigerated temperature and brought it outside at room temperature (25 - 31°C). This study was to evaluated suitable time after periodontal gel containing 5% MTZ has been removed outside the refrigerated temperature (4-6°C) was about ranges from begining to 60 minutes

The effect of time on syringeability of 5% MTZ periodontal gel is shown in Figure 68-69. Since both formulations of 5% MTZ periodontal gel has been removed. The syringeability of **ER_SP_vW-3** and **ER_SP_vW-4** system ranged from 85.22 ± 1.10 N and 141.72 ± 2.12, respectively.

The suitable time of 5% MTZ periodontal gel of both system were about 30 minutes to 60 minutes. The results showed the closely similar syringeability profile as freshly preparation. Therefore, in this study, the suggestion of these systems should be remove outside refrigerated temperature and keep at room temperature before use at least 30 minutes.

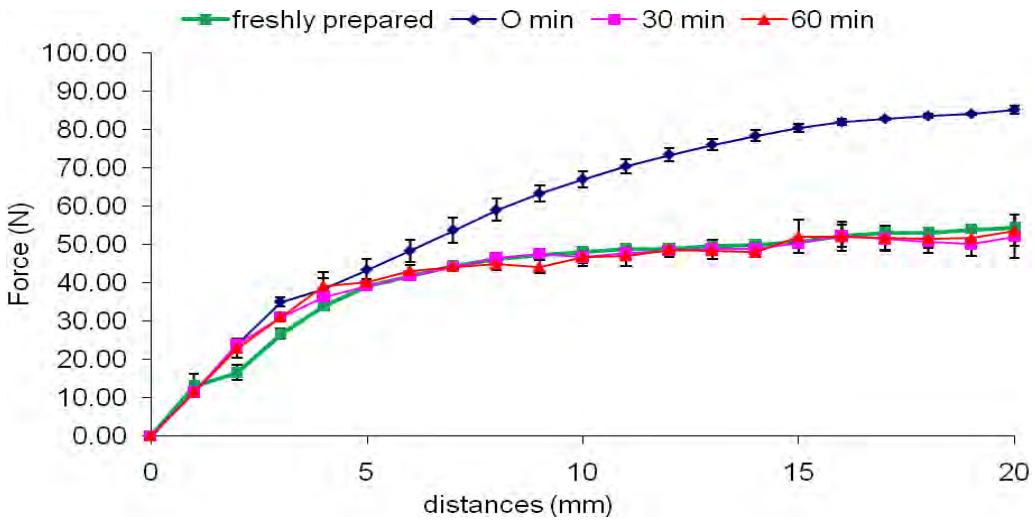

Figure 68 The effect of time on syringeability of 5% MTZ periodontal gel comparing with freshy preapare and then removed from refrigerated temperature.

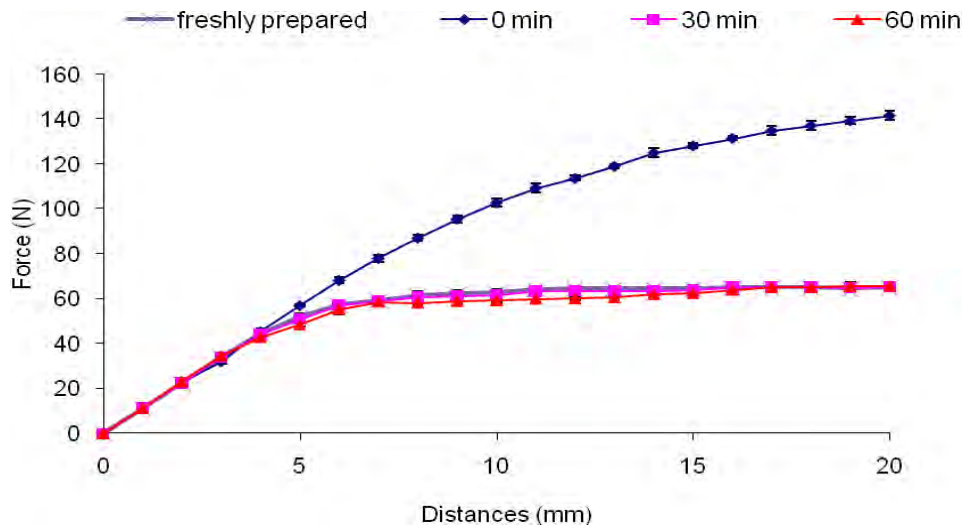

Figure 69 The effect of time on syringeability of 5% MTZ periodontal gel comparing with freshy preapare and then removed from refrigerated temperature.

Spreadability test

There was no significant difference in diameter of all formulations as 0, 1 and 6 months. The diameters of **ER_SP_vW-3**, **ER_SP_vW-4**, **$ER_{SL}P_vW$-1** and **$ER_{SL}P_vW$-2** formulation gel base systems at initial reached to 6 months were 40.97 ± 1.01, 37.52 ± 0.71, 40.61 ± 1.37 and 41.27 ± 1.25 mm, respectively. Adding 5% MTZ, showed diameters of 41.07 ± 1.32, 36.87 ± 0.83, 40.61 ± 1.38 and 40.53 ± 1.55 mm, respectively. When the system of periodontal gel base with or without 5% MTZ were compared at 0, 1 and 6 months, there was no statistically significant. The drug solubility could be used to explain. Ethanol in these formulations ranged from 33.0 to 35.5 % (w/w) could dissolve approximately 495 mg to 533 mg of MTZ (solubility; 15mg/ml) [David et al., 1997]. In fact, these systems containing 5% (w/w) MTZ, could not have completely drug dissolved. There were large amount of drug crystal that spread or suspended in gel structure. The system could be presented an saturated concentration of drug with drug crystal spread in matrix structure.

Most formulations containing 5% MTZ seemed to be decreased in spread diameter, but insignificant difference was noted. It was probably that the physical stability could be stabilized by excessive saturation of drug concentration and static condition at refrigerated temperature (4-6°C). The result was shown in Figure 70

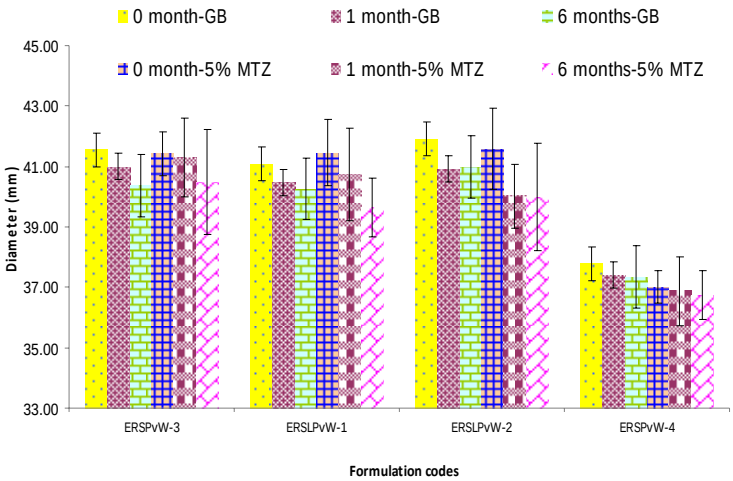

Figure 70 The spreability of selected periodontal gel base with and without 5% MTZ at 0 month, 1 month and 6 months in refrigerated temperature.

Percentage of drug content

The degradation kinetics of MTZ in aqueous solutions of pH 3.1 to 9.9 at 90±0.2°C were studied by Lund et al. [1994]. The reaction order for MTZ in these aquoeus and solvent systems followed pseudo-first order kinetics. MTZ has been also reported to be sensitive to light [Moor et al, 2000]. The degradation of MTZ in different solvent decreased in the order: chloroform > isopropanol > methanol > water.

Reynolds et al. [1996] has pointed out that photodegradation of MTZ gel followed first order reaction. After exposure to accelated light throughout 24 weeks, the color of metrogels changed to yellow whereas that of MTZ gels wrapped in aluminium foil showed no physical change and no degradation occurred.

The percentage of drug content in all formulations at refrigerated temperature ranged from 98.35± 2.45 to 110.22 ± 3.75 % as initially. When after storage for 6 months, the percentage of drug content was ranged from 95.02 ± 3.65 to 108.34 ± 2.71%.

Table 12 Percentage of drug content after 0 month and 6 months at refrigerated temperature

Formulation code	**O month (% amount of drug ± SD)**	**6 months (% amount of drug ± SD)**
ER_SP_vW-3	110.22 ± 3.75	108.34 ± 2.71
ER_SP_vW-4	98.35± 2.45	95.02 ± 3.65
$ER_{SL}P_vW$-1	103.05 ± 1.90	102.43 ± 2.72
$ER_{SL}P_vW$-2	106.10 ± 3.41	105.21 ± 2.41

***In Vitro* Release Studies**

There is no official method to evaluate the release of drug from peridontal gel, but several different methods such as Franz diffusion cell method have been used to characterize the drug release from such gel. Franz diffusion cell method has been mostly uesd in many studies with different formulations of periodontal gel containing drugs of a wide range of aqueous solubility [Chein, 1984]. However, the periodontal pocket, a diseased space or cavity between the inflamed gum and the surface of a tooth is an open system with aqueous environment. Therefore, the suitable method of drug release in periodontal pocket in this study was evaluated by drug dissolution release method. Thus , this experiment using a dissolution method with 2 sieves #40 and size of each screen: 1.5×1.5 cm dimension was carried out the 0.2 g MTZ gel was apply the thickness of the MTZ gel in approximatly 0.40-0.50 mm before study.

The *in vitro* release profile provides insight into the efficiency of the drug delivery system proposed for the controlled release of the drug. Representative of 4 selected periodontal gels that passed stability test of were evaluated in vitro drug release. Before the study, each formulation was assayed of metronidazole content. From the result, metronidazole [MTZ] release from different periodontal gel system were slow, incomplete and could prolonged for more than 24 hours. The result was shown in Figure 71

The percentages of drug release were 93.60 ± 2.76, 90.36 ± 0.52, 93.23 ± 0.78 and 94.51± 3.06 for **ER_SP_vW-3, ER_SP_vW-4, $ER_{SL}P_vW$-1** and **$ER_{SL}P_vW$-2**, respectively, after 24 hours. There were no significant differences (*p-value* > 0.05) of drug release profiles in all formulations, except with **ER_SP_vW-4** and **$ER_{SL}P_vW$-2**. The release of MTZ from periodontal gel from **ER_SP_vW-3** system (12.5% EC) was slightly higher than that for **ER_SP_vW-4** system (15% EC). When decreased the ratio of RE/RL as 25/0, 22.5/2.5 and 20/5, the release profile was also slightly increased. **ER_SP_vW-4** and **$ER_{SL}P_vW$-2** (EC: RS: RL; 12.5 : 20 : 5) were showed significant difference. This mihgt affect from EC and RS/RL to water permeability and drug diffusion. It was possible to increase the concentration of EC and decrease the amount

of RL to improve drug release. Among formulations containing 5% MTZ, the release of all formulations showed similar release profile. The release rate constant was increased when the concentration of EC and RL were decreased and increased, respectively. In this study, all formulations showed better fitted to first order kinetic (table 13, 14), depended on the concentration of drug content similar to report from Manso et al [2005]. Amount of drug incorporation also led to drug release behavior. Conclusively, these periodontal gels were governed by three main processs; drug release by drug solubility, drug diffusion from matrix pore and drug release enhancement by erosion property.

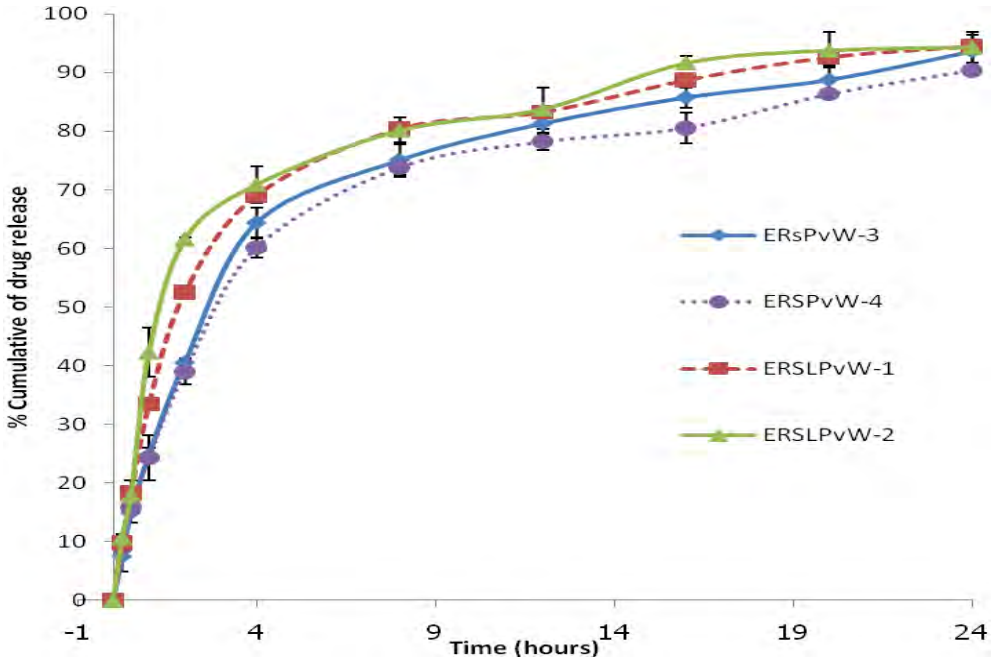

Figure 71 Comparison of the drug release profile of 5% MTZ periodontal gel

Table 13 Comparing the correlation coefficient(r) of drug release data acccording to different kinetics model of 5% MTZ in periodontal gel base in PBS (pH = 6.8) (n=3)

Formulation code	r±SD Zero order ($Q = Q_0 - Kt$)	r±SD First order ($Q = Q_0^{-Kt}$)	r±SD Higuchi ($Q = Kt^{-1/2}$)
ER_SP_vW-3	0.775 ± 0.016	0.950 ± 0.008	0.929 ± 0.007
ER_SP_vW-4	0.784 ± 0.005	0.940 ± 0.006	0.936 ± 0.001
$ER_{SL}P_vW$-1	0.720 ± 0.015	0.943 ± 0.011	0.898 ± 0.010
$ER_{SL}P_vW$-2	0.679 ± 0.024	0.872 ± 0.051	0.865 ± 0.022

Table 14 Comparing the release rate constant (k) of drug release data acccording to first order kinetics model of 5% MTZ in periodontal gel base in PBS (pH = 6.8) (n=3)

Formulation code	Release rate constant (k) First order ($Q = Q_0^{-Kt}$)
ER_SP_vW-3	0.11 ± 0.01
ER_SP_vW-4	0.09 ± 0.00
$ER_{SL}P_vW$-1	0.15 ± 0.08
$ER_{SL}P_vW$-2	0.16 ± 0.08

Anti-microbial activity

Comparison of average minimum inhibition zone againt anaerobic bacteria representive by *P. gingivalis* of each periodontal gel with and without 5% MTZ system (mm) are shown in figure 70. There were no inhibition zone diameter of periodontal gel base. All inhibition zone diameters of these formulations containing 5% MTZ showed no statistically significant difference(*p-value* = 0.479) in diameters. All formulations still had inhibition zone diameters of more than 80 mm. This result indicated that 5% w/w MTZ periodontal gel after storage for 6 months at refrigerated temperature

In comparison of the average minimum inhibitory zone of each 5% MTZ periodontal gel systems (mm), the range of inhitory zone diameters were quite similar of more than 80 mm, which meant that systems had similar antimicrobial activity againt anaerobe bacteria representated by *P. gingivalis*. The diameter results showed statistically insignificance among each formulation although all formulations had quite different amount of polymer content.

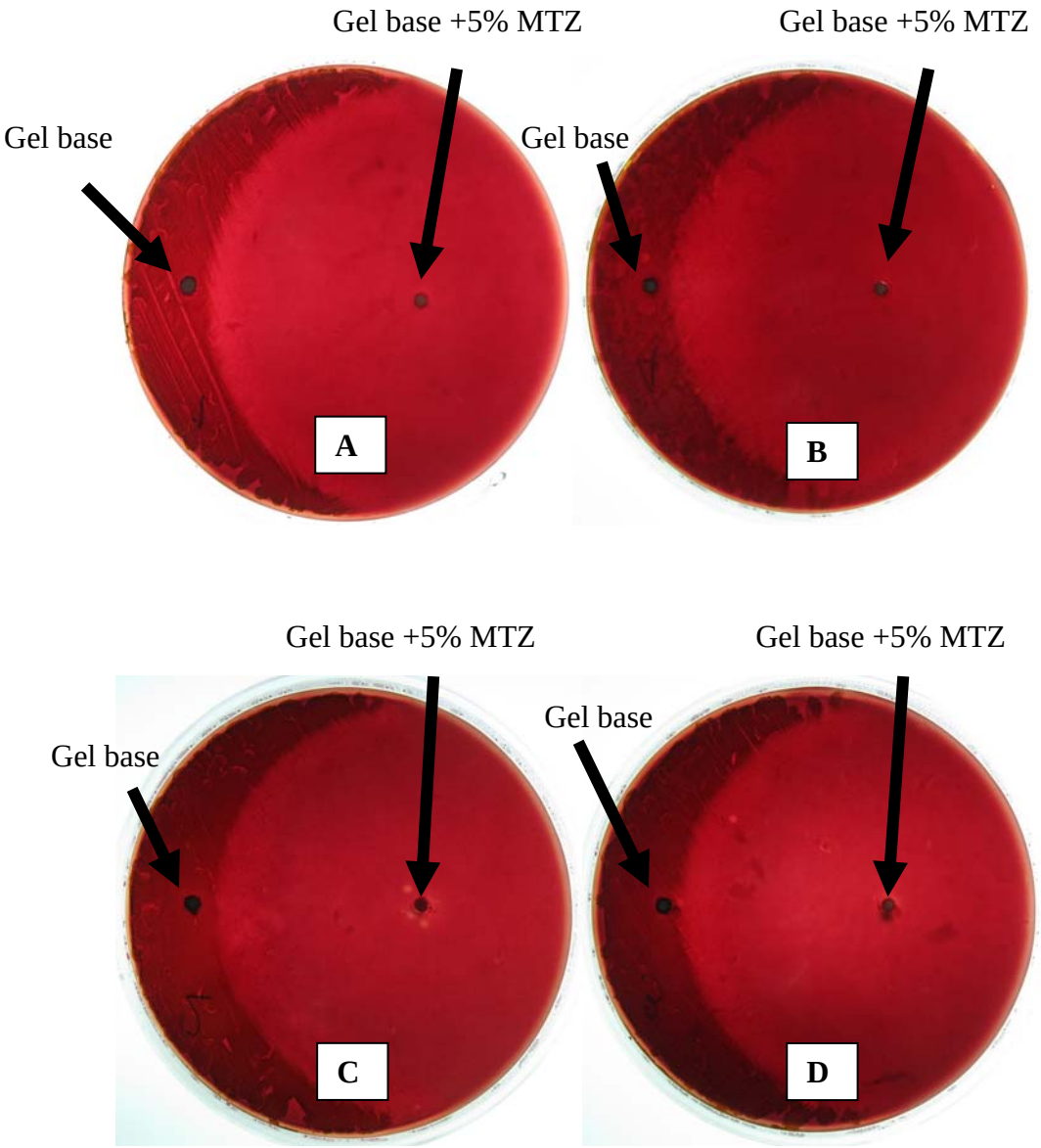

Figure 72 Microbial sensitivity test of **ER_SP_vW-3** (**A**), **ER_SP_vW-4** (**B**), **$ER_{SL}P_vW$-1**(**C**) and **$ER_{SL}P_vW$-2** (**D**) gel base (left) and containing 5% MTZ (right). The duplication of this formulation was performed in the same condition

Effect of amounts of MTZ in selected periodontal gel base

This study was investigated the percentage of drug load at 5% , 10%, 20% and 40% MTZ to selected periodontal gel, **ER_SP_vW-3** and **ER_SP_vW-4**, which had good physicochemical properties and showed higher prolonged release more than 24 hours.

Viscosity property

The effect of the MTZ concentration on viscosity profile is shown in Figure 73. For **ER_SP_vW-3** formulation, when the concentration of MTZ was increased from 5% to 10%, the viscosity was increased to have the yield stress from 24.03 ± 1.28 to 27.28 ± 0.73 $\times 10^3$ cps. The testing experiment could not investigate at 20% and 40% of drug due to limitation of the instrument. The torque was overloaded due to high resistance of drug content.

ER_SP_vW-4 at 5% to 10% of MTZ incorporation showed to increase the yield stress from 27.78 ± 0.66 to 29.85 ± 0.43 $\times 10^3$ cps. The instrument could also not determine the gel of 20% MTZ due to very high viscosity whereas interestingly, 40% of drug had exhibited lower viscosity with yield stress at 25.58 ± 0.56 $\times 10^3$ cps. Which had significantly lower yield stress the 5% MTZ load (*p-value* <0.05). It might be possible that when gel was added with lower concentration of MTZ, which was in crystalline form, the viscosity was increased by the amount of solid packing in the matrix. Oppositely, when increasing the concentration of MTZ up to 40%, viscosity was decreased. It might relate with the excess drug packing in semi-solid matrix system. Since the drug incorporation exceeded the drug solubility, undissolved drug crystal might be spread though the entire network and closely packed in the gel structure. Close packing of drug crystals might be thin coated with WS, an emollient and mainly used in pharmaceutical formulation as a component of ointments. During the gel movement, these moving surfaces of solid might be contacted and the friction was reduce between them, thus improving motion and reducing the viscosity. The syringeability and spreadability were correlated with viscosity result.

In addition, it might be possible the bonding between polymers or gel structure was broken. High amount of drug content might destroy the gel bonding which would decrease the force of bonding, and final gel structure could easily flow due to decreasing of syringeability.

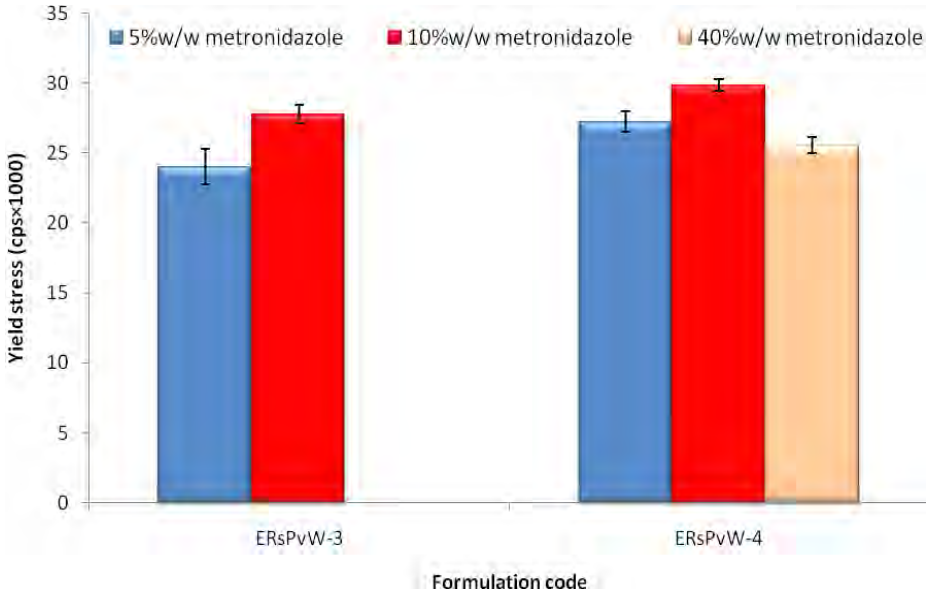

Figure 73 Yield stress of **ER_SP_vW-3** and **ER_SP_vW-4** formulations containing 5%, 10% and 40% MTZ

Syringeability property

The syringeability of both systems seemed to achieve similar to results of viscosity as showsn in Figure 74. The syringeability was lowered in the presence of MTZ increased and also in **ER_SP_vW-4** formulation as EC content increased. **ER_SP_vW-3** with MTZ became higher viscous when the drug was dissolved and the undissolved drug crystals were packed inside the matrix system. The syringeability of both formulations were decreased with the increasing amount of MTZ ranged from 5 %, 10%, 20% and 40% MTZ, respectively, except at 40% MTZ of **ER_SP_vW-4** formulation, the highest amount of 40% MTZ showed the highest syringeability. The observation may be explained by the degree of drug incorporation as in viscosity study.

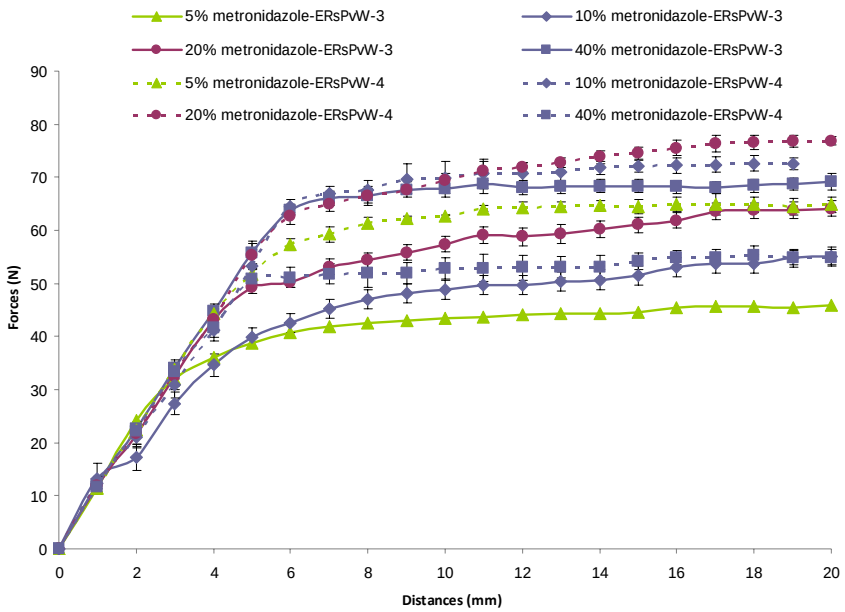

Figure 74 The syringeability of **ER_SP_vW-3 and ER_SP_vW-4** gel base containing MTZ at 5%, 10%, 20% and 40% w/w

Spreadability property

Figure 75 shows the spreadability of both **ER_SP_vW-3** and **ER_SP_vW-4** formulation at 5%, 10%, 20% and 40% MTZ. The spreadability of both systems that contained higher concentration of MTZ were decreased in diameters. This might be the gel formation related to the structure became more closely packed and showed hardened gel. Therefore spreadability could exhibite higher resistance when increasing the concentration of drug content. All formulations was also decreased in diameters by contacting aqueous environment and showed the results similar to the same previous test.

Theoretically, the spreadability of **ER_SP_vW-4** at 40% MTZ should had the highest spreading diameter due to the lowest viscosity and highest syringeability as previous results, but the experiment showed continuously decreased in diameters because of its pseudoplastic behavior that obtained yield stress. According to system deformation, the compression force in this system might not be enough to change the

structure of higher viscosity, thus the spreadability of 40% MTZ formulation is still slightly decreased in diameter by 200 N force.

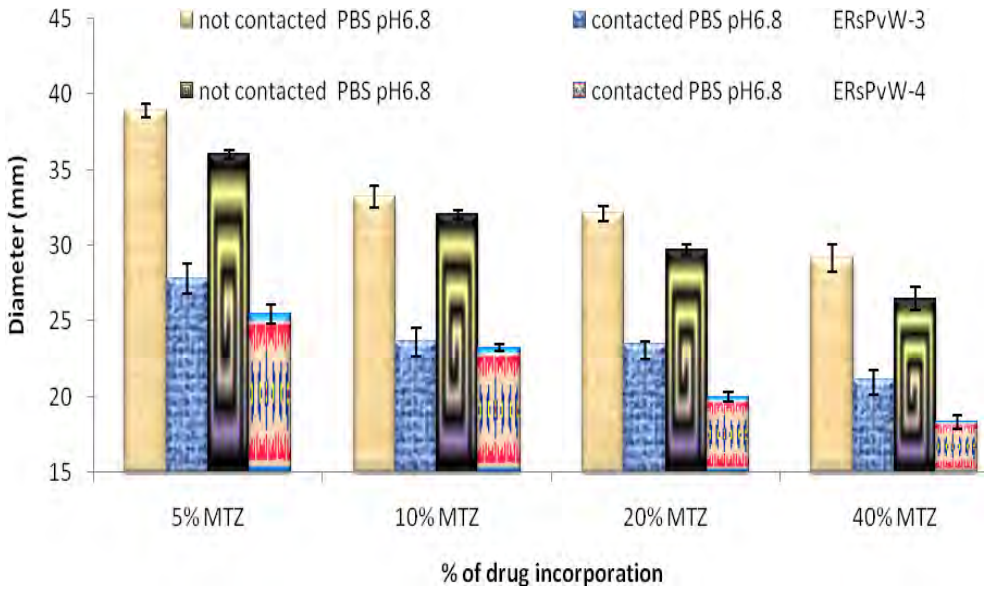

Figure 75 The spreadability of **ER_SP_vW-3** (blue) and ER_SP_vW-4 (red) formulation gel base containing MTZ at 5%, 10%, 20% and 40% w/w

Weight loss study

Percentage of weight loss study was shown in Figure 76. Addition of 5%, 10%, 20% and 40% MTZ to **ER_SP_vW-3** and **ER_SP_vW-4** gel bases showed decreasing in weight loss compared with gel base. It could explain that as the excess of drug concentration was incorporated to gel structure, drug crystal showed more closely packed as homogeneously dispersion though gel structure. The poor water solubility of drug could expel water thoughout gel structure as barrier. Moreover, transformaton of water insouble polymers to solid state also protected aqueous environment the resulted in the decreasing of weight loss. In the result, **ER_SP_vW-3** formulation gel base showed slightly higher (59.00 ± 0.59 %) weight loss than **ER_SP_vW-4** (57.88 ± 0.66 %) but insignificant difference (*p-value* = 0.061) was note. This might be possible to their similar structure during gel contacting water. WS in both formulation could protect these systems from aqueous environment and could

hold a solid phase as binder for the tranformed water insoluble polymers transfromation.

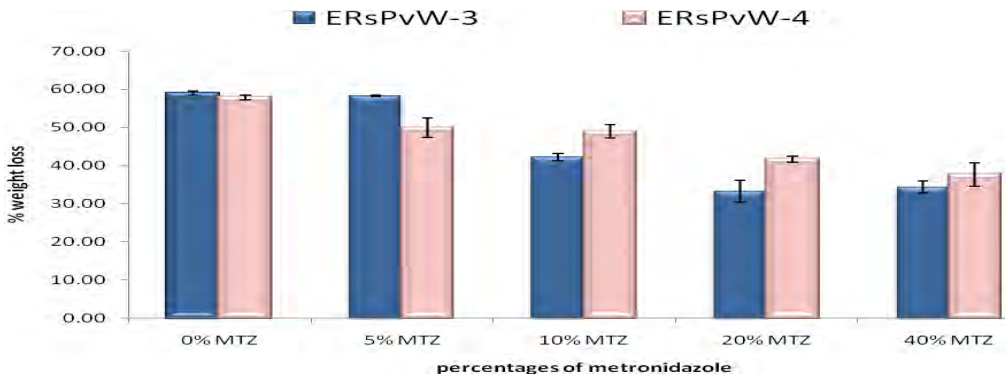

Figure 76 Percentages of weight loss study with the different amount of MTZ at 0%, 5%, 10% 20% and 40% of **ER$_S$P$_v$W-3** and **ER$_S$P$_v$W-4** formulation

***In vitro* release property**

Increasing the MTZ concentration tented to decrease in weight loss. In addition, the concentration of MTZ from in **ER$_S$P$_v$W-3** formulation had an effect to decrease in weight loss higher than in **ER$_S$P$_v$W-4**. This might be possible that increased amount of drug exhibited dense gel structure and would probably be enhance the erosion or brittle of gel structure

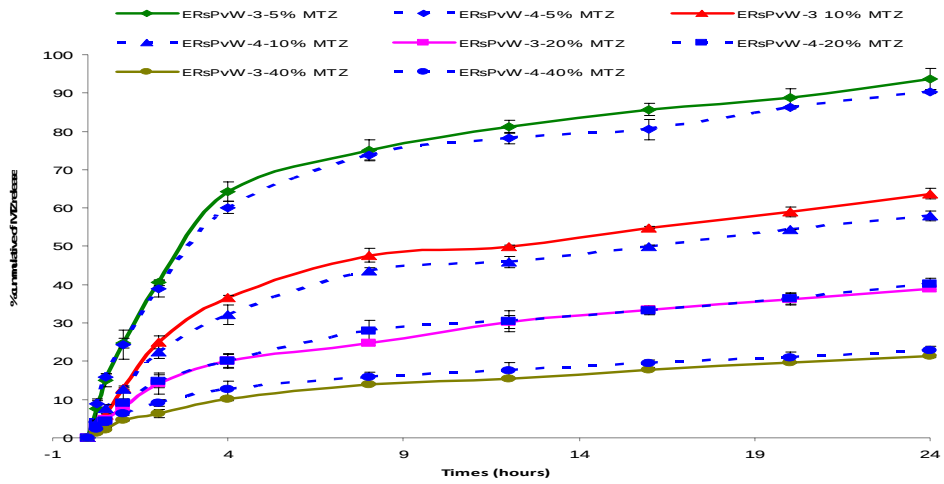

Figure 77 In *vitro* release profile of **ER$_S$P$_v$W-3** and **ER$_S$P$_v$W-4** gel base containing MTZ at 5%, 10%, 20% and 40% w/w.

The release of both **ER_SP_vW-3** and **ER_SP_vW-4** was prolong for more than 24 houres and is shown in Figure 77. The both systems of 10 % to 40% MTZ showed to be better fitted to Higushi equation. The rate of release was decreased with greater concentration of MTZ because of lower solubility of drug in water. MTZ release from **ER_SP_vW-3** system was slightly higher than from **ER_SP_vW-4** system. Increasing the concentration of EC could provided gel structure to dense system of hydrophobic structure as barrier indicating that drug release may be decreased due to the slow diffusion rate of water into gel.

Table 15 The coefficient of determination (R^2) of **ER_SP_vW-3** and **ER_SP_vW-4** formulation with different amount of MTZ in various drug release kinetics calculated from total drug release data

Formulation code	r±SD Zero order ($Q = Q_0 - Kt$)	r±SD First order ($Q = Q_0^{-Kt}$)	r±SD Higuchi ($Q = Kt^{-1/2}$)
ER_SP_vW-3-5% MTZ	0.775 ± 0.016	**0.950 ± 0.008**	0.929 ± 0.007
ER_SP_vW-3-10% MTZ	0.836 ± 0.001	0.911 ± 0.002	**0.960 ± 0.000**
ER_SP_vW-3-20% MTZ	0.889 ± 0.009	0.925 ± 0.006	**0.986 ± 0.004**
ER_SP_vW-3-40% MTZ	0.904 ± 0.005	0.920 ± 0.004	**0.990 ± 0.001**
ER_SP_vW-4-5% MTZ	0.784 ± 0.005	**0.940 ± 0.006**	0.936 ± 0.001
ER_SP_vW-4-10% MTZ	0.846 ± 0.011	0.909 ± 0.007	**0.968 ± 0.004**
ER_SP_vW-4-20% MTZ	0.874 ± 0.018	0.911 ± 0.016	**0.980 ± 0.006**
ER_SP_vW-4-40% MTZ	0.877 ± 0.014	0.883 ± 0.021	**0.976 ± 0.008**

Table16 The release rate constant (k) of **ER_SP_vW-3** and **ER_SP_vW-4** formulation with different amount of MTZ

Formulation code	Release rate constant (k)
ER_SP_vW-3-5% MTZ	0.11 ± 0.01^F
ER_SP_vW-3-10% MTZ	13.66 ± 0.14
ER_SP_vW-3-20% MTZ	8.24 ± 0.08
ER_SP_vW-3-40% MTZ	4.52 ± 0.00
ER_SP_vW-4-5% MTZ	0.16 ± 0.08^F
ER_SP_vW-4-10% MTZ	12.33 ± 0.00
ER_SP_vW-4-20% MTZ	8.32 ± 0.12
ER_SP_vW-4-40% MTZ	4.61 ± 0.12

When, ^{F} = First order kinetic release

The MTZ release profile of **ER_SP_vW-4** system showed significantly lower drug release (p-value < 0.05) than of **ER_SP_vW-3** system, except in 20% and 40%. This might be described by Higuchi model.

Higuchi has described the forgoing dosage forms, the drug is homogeneously dispersed thoughout the matrix of the system and developed an equation for the release of the drug from an oinment base or hydrophobic base,in which the drug entity is distributed uniformly and homogeneously. This equation is based on principles of diffusion as expressed by Fick's first of diffusion equation (2):

$$\frac{dQ}{dt} = \frac{DCs}{l} \quad \text{..... (2)}$$

where *dQ/dt* is the rate of drug released per unit area of eaxposed surface of the system, *D* the diffusion coefficient, *Cs* the saturation concentration or solubility of the drug in the system , and *l* the tickness of the diffusion layer. The drug at the surface of the system, which is in close contact with the medium gels, is released first and sets up a front. As drug passes out of the homogeneous system,the front moves inward, forming the boundary of the drug. In fact, it is assummed that solid drug dissolves from the surface layer of the system first and then, as this layer begins to deplete. The amount of drug depleted per unit area of the system, ***Q***, at time ***t***, is given by Higuchi equation (3):

$$Q = [D(2C-Cs)Cst]^{1/2} \quad \text{.....(3)}$$

where C is the total concentration (amount per unit volume), dissolved or undissolved, of drug in the system.

Consequently, at 10% MTZ in **ER_SP_vW-4** system exhibited lower release profile than **ER_SP_vW-3** system due to higher amount of water insoluble polymer, EC, drug packing of EC. **ER_SP_vW-4** system had a barrier from EC transformation to solid. Increased concentration of MTZ up to 20 and 40%, in both systems showed similar release profile. The result explained that higher amount of

drug content could change to closely pack in gel structure. Drug release property was mainly controlled by drug diffusion at the surface of the system, which was in close contact with the aqueous environment.

Interestingly, at 40% MTZ, the release profile of **ER_SP_vW-3** formulation was slighly higher than that of **ER_SP_vW-4** formulation. It was correlate to the reverse to increase weight loss was shown in Figure 76, thus slightly increased in drug release. In addition, when increasing the concentrations of both **ER_SP_vW-3** and **ER_SP_vW-4** formulation gel bases from 5% to 10%, 20% and 40% MTZ, the release rate constant was significantly decreased (*p-value* < 0.05) and Higuchi release kinetics was readily described. The data is shown in Table 16.

Maheshwari et al., (2006) reported the optimization the concentration of tetracycline in drug delivery system. The amount of tetracycline release was increased with an increase in the concentration of drug in the gel. The rate of release increased with greater concentrations of tetracycline because of higher solubility in water. The release of tetracycline in poloxamer gel decreased as increase concentration of Aerosil, a water insoluble polymer which could change the size of micelles within the gel structure. The following aspects affected the release of drug from the gel system: solubility of the drug in the gel system, diffusion rate of the drug in the gel, and diffusion rate of water into the gel.

In this system, metronidazole (MTZ) , a poor water soluble drug and could exhibit prolong drug release [Rockville, 2006], while gel structure composed of EC, RS and WS as hydrophobic part could controle diffusion rate of the drug in the gel and diffusion rate of water into the gel as a barrier. These reasons indicated that the structure of the gel functioned as a barrier to drug release, Moreover, in this study, MTZ could pack inside gel structure and also enhance system as a homogeneous barrier and released by Higuchi model.

However, in dissolution test, the medium used was 15 ml PBS pH 4.7, approximately the same volume and pH in mouth which drug solution was stable at

this pH. When increasing the amount of drug in gel, 40% MTZ, the release profile was decreased. It might be ralated with sink condition.

Sink condition must be maintained in order to have the correct dissolution. To ensure that sink condition in mantained, the saturation point of the drug in PBS pH 4.7 must be known. The saturation point for any given active drug substance varies from media to media and is greatly influenced by temperature and excipients as well. To calculate sink concentration of the saturated solution reached to 5-10 times of its saturation point would determine the maximum working concentration of the active drug in the proposed media. For metronidazole, the saturated solubility in water is 1:100 or 1 g in 100 ml. The maximum amount of drug added to the dissolution vessel was 80 g. Therefore, the medium of 15 ml might not be enough to maintain sink.

CHAPTER V

CONCLUSIONS

This study elucidated that required periodontal gel base could be prepared by using commercially available and pharmaceutically acceptable excipients. Suitable type of gel base system could remain and release of drug in periodontal pocket more than 24 hours. Importantly, the periodontal gel base system that inserted to periodontal pocket could be resist from GCF, bleeding and the environment variable. The overall results to the development of periodontal gel base to be potential route of periodontal drug delivery were described:

Hydrophilic gel

1. All ingredients used also had effects on viscosity and physical appearance including the color of final product. Increasing the polymer concentration in the gel increased the viscosity, syringeability force and cause a reduction of spreadability.

2. Poloxamer gel could dehydrate at higher temperature (37°C) and transform into cubic or hexagonal phase, thus enhanced the physical property changes such as increasing the viscosity property.

3. As the concentration of Aerosil increased, the poloxamer gel structure became more closely packed and showed viscosity increased.

4. Addition of hydrophilic polymer concentrations especially carbopol to poloxamer gel or increasing the concentration of poloxamer more than 20 % (w/w) could increase viscosity property.

5. The rheology of this system most exhibited pseudoplastic flow from higher viscosity, which depended upon polymer concentration or its component such as Aerosil increased and types of polymer such as molecular weight and grade of polymer used.

6. *Ex vivo* mucodahesive force was increased depended on increasing amounts and type of each hydrophilic polymer.

7. Hydrophilic gel could be destroyed easily by dissolution, swelling and erosion in aqueous environment.

8. *In vivo* study, hydrophilic gel containing 5% MTZ was inserted to intra-periodontal pocket, bleeding and GCF flushed out the gel from the pocket, thus exhibited bitter taste from the drug released.

Hydrophobic gel

1. Increasing the concentration of all ingredients exhibited the viscosity increased.

2. All formulations exhibited poor adhesion while stabilized in aqueous environment.

3. Polyethylene gel contained 5% MTZ could be then removed from the pocket after bleeding and could be easily destroyed, then the bitter taste was reported.

4. Water insoluble gel prepared from EC, RS and RL with 5% MTZ after inserted to the pocket was then easily removed by bleeding and showed strong attachment to the surface of tooth instead.

Hydrophobic-hydrophilic gel

1. All formulations that contained WS were sensitive at room temperature and caused phase separation. These formulations had to be kept at refrigerated temperature (4-6°C) before use.

2. All formulations prepared from 20% (w/w) poloxamer with water insoluble polymers seemed like homogeneous dispersion gel. This system could not exhibit the thermoreversible property at 37°C.

3. From *ex vivo* and *in vitro* tests, all formulations showed poor adhesion in aqueous environment. Gels mainly destroyed by erosion and fragmentation.

Suitable periodontal gel base system (System 3-3)

1. The characterization of rheology including viscosity, syringeabilty and spreadability demonstrated that this system was appropriate as intra-periodontal pocket delivery system for periodontal therapy, Surprisingly, it could transform semi-solid state to solid like state when contacted with excess of bleeding and GCF and provide sustained release of drug.

2. This system could be stabilized by higher viscosity and lower storage temperature (4-6°c). It could not tolerate room temperature, FDA stability testing and freeze-thawing cycles.

3. Eventhough high viscosity, the non-newtonian and shear thinning bahaviors could be obtained. These brought about the good syringeability and injectabilty of this system

4. In *in vivo* study, bleeding and GCF could promote system formation from semi-solid state to solid like state and remain inside at periodontal pocket more than 24 hours

5. Initially, bitter taste was reported and disappeared after the patients rinsed their mouth with water.

6. Most of drug incorporation would disperse into the gel structure and increased the syringeability force, except the higher amount of drug such as 40% MTZ in ER_SP_vW-4, the syringeability force was significantly decreased than gel base preparation (p-value < 0.05).

7. *In vitro* drug release from these formulations was sustained more than 24 hours and their release kinetic was first order only in 10% MTZ system and Higuchi model from 20-40% MTZ.

8. Antimicrobial activity of formulations with increased EC concentration and different ratio of RS/RL showed no statistical difference in inhibition zone diameter (*p-value* > 0.05).

9. According to stability study, most of selected formulations were stable, remained good appearance, good physicochemical property.

This study could be employed as useful basic knowledge for further development of novel periodontal and intra-periodontal drug delivery system. The further investigation should be performed *in vivo* with clinical study toward the patient with periodontal disease under periodontist controlling. Consequently, the potential to prepare in advance industrial scale should be further investigated.

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APPENDICES

APPENDIX A

Material Safety Data of system 3-3

1. Ethylcellulose

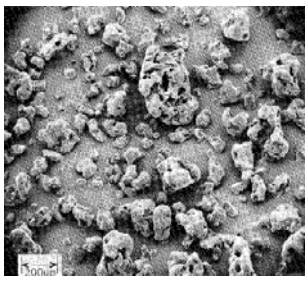

Figure 78 Scanning electron microscope of ethylcellulose

Synonyms

Aquacoat ECD; Aqualon; E462; Ethocel; Surelease.

Structural Formula

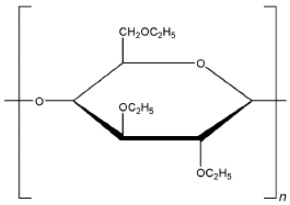

Figure 79 Structural Formula of ethylcellulose

Functional Category

Coating agent; flavoring fixative; tablet binder; tablet filler; viscosity-increasing agent.

Description

Ethylcellulose is a tasteless, free-flowing, white to light tan-colored powder.

Stability and Storage Conditions

Ethylcellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters. Ethylcellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives that absorb light in the 230–340 nm range. Ethylcellulose should be stored at a temperature not exceeding 32°C (90°F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

Incompatibilities

Incompatible with paraffin wax and microcrystalline wax.

Safety

Ethylcellulose is widely used in oral and topical pharmaceutical formulations. It is also used in food products. Ethylcellulose is not metabolized following oral consumption and is therefore a noncalorific substance. Because ethylcellulose is not metabolized it is not recommended for parenteral products; parenteral use may be harmful to the kidneys. Ethylcellulose is generally regarded as a nontoxic, nonallergenic, and nonirritating material. As ethylcellulose is not considered to be a health hazard, the WHO has not specified an acceptable daily intake. LD_{50}(rabbit, skin): >5 g/kg and LD_{50}(rat, oral): >5 g/kg

2. Eudragit® RL-100 and Eudragit® RS-100

Eudragit® polymers, are the favorite choice for solid oral formulations. They have become indispensable for the manufacture of enteric coatings on solid dosage forms, for sustained release formulations as well as immediate release coatings. New product

developments for controlled release enable the use of most sophisticated coating processes and innovative drug delivery techniques to the benefit of our customers.

Chemical structure

Eudragit® RL-100 and Eudragit® RS-100 are copolymers of ethyl acrylate, methyl methacrylate and a low content of a methacrylic acid ester with quaternary ammonium groups (trimethylammonioethyl methacrylate chloride). The ammonium groups are present as salts and make the polymers permeable. The average molecular weight is approx. 150,000

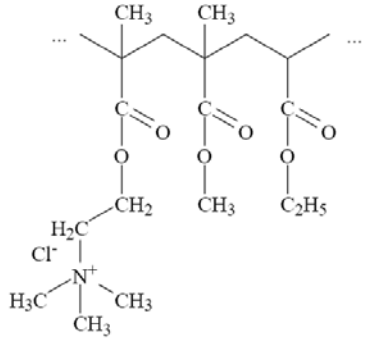

Figure 80 The ammonium groups of Eudragit® RL-100 and Eudragit® RS-100

Description

Colourless to light yellow liquids of low viscosity, clear to slightly cloudy. The odour is characteristic of the solvents.

Solubility

Eudragit® RL 12,5 and Eudragit® RS 12,5 are miscible with methanol, ethanol and isopropyl alcohol (containing approx. 3 % water), as well as with acetone, ethyl acetate and methylene chloride in a ratio of 1:1. The polymer is precipitated from Eudragit® RL 12.5 and Eudragit® RS 12.5 when mixed with petroleum ether in a ratio of 1:1. When mixed with 1 N sodium hydroxide or water, the solution becomes cloudy or precipitates.

Storage

Protect from warm temperatures (USP, General Notices). Keep in tightly closed containers.

Safety

LD_{50}: Oral rat LD_{50} > 5000mg/kg.

3. Polyvinylpyrrolidone (PVP)

Figure 81 Scanning electron microscope of PVP

Synonyms

E1201; Kollidon; Plasdone; poly[1-(2-oxo-1-pyrrolidinyl)ethylene]; polyvidone; polyvinylpyrrolidone; PVP; 1-vinyl-2- pyrrolidinone polymer.

Structural Formula

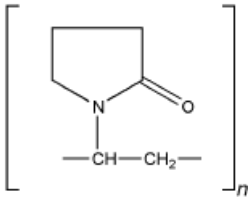

Figure 82 Structural Formula of PVP

Description

PVP occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder. PVPs with K-values equal to or lower than 30 are manufactured by

spray-drying and occur as spheres. PVP K-90 and higher K-value PVPs are manufactured by drum drying and occur as plates.

Melting point

Its physical appearance softens at 150°C.

Solubility

Freely soluble in acids, chloroform, ethanol, ketones, methanol, and water; practically insoluble in ether, hydrocarbons, and mineral oil. In water, the concentration of a solution is limited only by the viscosity of the resulting solution, which is a function of the K-value.

Stability and Storage Conditions

PVP darkens to some extent on heating at 150°C, with a reduction in aqueous solubility. It is stable to a short cycle of heat exposure around 110–130°C; steam sterilization of an aqueous solution does not alter its properties. Aqueous solutions are susceptible to mold growth and consequently require the addition of suitable reservatives. PVP may be stored under ordinary conditions without undergoing decomposition or degradation. However, since the powder is hygroscopic, it should be stored in an airtight container in a cool, dry place.

Incompatibilities

PVP is compatible in solution with a wide range of inorganic salts, natural and synthetic resins, and other chemicals. It forms molecular adducts in solution with such as sulfathiazole, sodium salicylate, salicylic acid, phenobarbital, tannin.The efficacy of some preservatives, e.g., thimerosal, may be adversely affected by the formation of complexes with PVP.

Safety

PVP has been used in pharmaceutical formulations for many years, being first used in the 1940s as a plasma expander, although it has now been superseded for this purpose by dextran. PVP is widely used as an excipient, particularly in oral tablets and solutions. When consumed orally, PVP may be regarded as essentially nontoxic since it is not absorbed from the gastrointestinal tract or mucous membranes. PVP additionally has no irritant effect on the skin and causes no sensitization.

Reports of adverse reactions to PVP primarily concern the formation of subcutaneous granulomas at the injection site of intramuscular injections formulated with PVP. Evidence also exists that PVP may accumulate in the organs of the body following intramuscular injection. A temporary acceptable daily intake for PVP has been set by the WHO at up to 25 mg/kg body-weight. LD_{50}(mouse, IP): 12 g/kg(12)

4. White soft paraffin

Synonyms

Mineral hydrocarbons; hard wax; paraffinum durum; paraffin wax.

Structure formula

Paraffin is a purified mixture of solid saturated hydrocarbons having the general formula CnH2n+2, and is obtained from petroleum or shale oil

Description

Paraffin is an odorless and tasteless, translucent, colorless, or white solid. It feels slightly greasy to the touch and may show a brittle fracture. Microscopically, it is a mixture of bundles of microcrystals. Paraffin burns with a luminous, sooty flame. When melted, paraffin is essentially without fluorescence in daylight; a slight odor may be apparent.

Melting point

melting point ranges from 38-60 °C.

Solubility

Soluble in chloroform, ether, volatile oils, and most warm fixed oils; slightly soluble in ethanol; practically insoluble in acetone, ethanol (95%), and water. Paraffin can be mixed with most waxes if melted and cooled.

Stability and Storage Conditions

Paraffin is stable, although repeated melting and congealing may alter its physical properties. Paraffin should be stored at a temperature not exceeding 40ฐC in a well-closed container.

Incompatibilities

—

Safety

Paraffin is generally regarded as an essentially nontoxic and nonirritant material when used in topical ointments and as a coating agent for tablets and capsules. However, granulomatous reactions (paraffinomas) may occur following injection of paraffin into tissue for cosmetic purposes or to relieve pain. Long-term inhalation of aerosolized paraffin may lead to interstitial pulmonary disease. Ingestion of a substantial amount of white soft paraffin has led to intestinal obstruction in one instance.

APPENDIX B

Table 17 Calibration data of metronidazole/tinidazole in phosphate buffer solution pH 4.7 at 275 nm

Concentration of metronidazole (µg/ml)	Area (metronidazole/tinidazole)
0	0
21.2	0.28
42.4	0.56
63.6	0.84
84.8	1.13
106.0	1.40

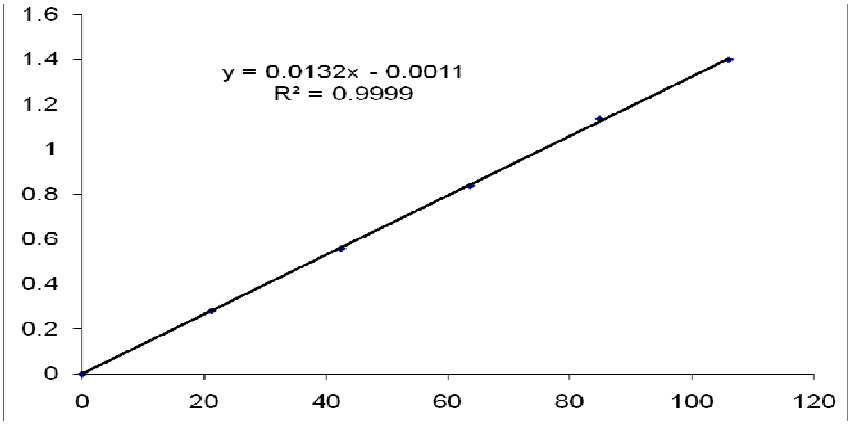

Figure 83 Calibration curve of metronidazole/tinidazole in phosphate buffer solution pH 4.7 at 275 nm

Table 18 Data of precision of metronidazole/tinidazole

Number	Area at 275 nm		
	1st day	2nd day	3rd day
1	3553544/4261844	3554758/4239409	3598320/4273889
2	3566271/4270787	3568131/4242849	3579973/4219440
3	3565662/4279302	3567762/4258378	3567352/4217465
4	3587313/4279124	3565373/4256866	3560247/4201016
5	3557668/4202710	3556696/4245833	3566772/4258378
6	3732386/4243745	3567433/4258188	3534837/4198392
Average	0.837673	0.838389	0.843874
SD	0.008024	0.001309	0.004117
%CV	0.96	0.16	0.49

Table 19 The percentage of recovery of metronidazole/tinidazole with gel bases

Concentration (µg/ml)	% Recovery of metronidazole/tinidazole			Mean	SD
	1	2	3		
Assay 30	99.44	98.95	100.00	97.81	0.43
60	101.98	101.40	98.33	100.57	1.96
90	97.69	97.51	98.34	99.46	0.53
Assay (Spiking) 30	96.02	97.80	97.05	96.95	0.89
60	100.23	99.01	98.23	99.17	1.01
90	102.20	98.71	99.02	99.98	1.93

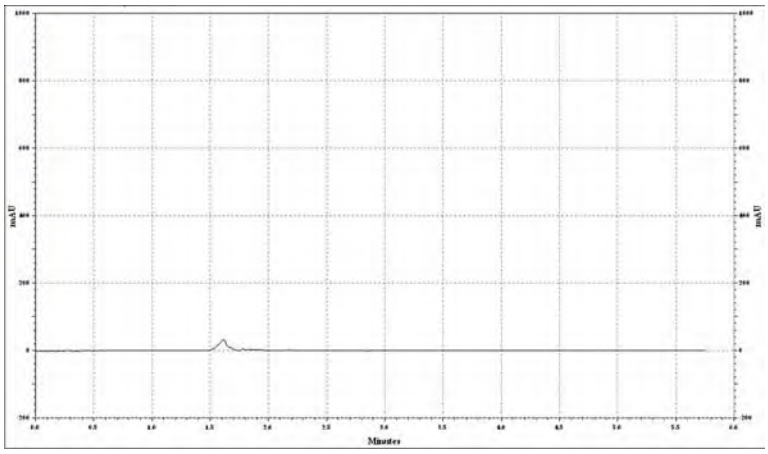

Figure 84 HPLC chromatogram of placebo solution in phosphate buffer solution pH 4.7

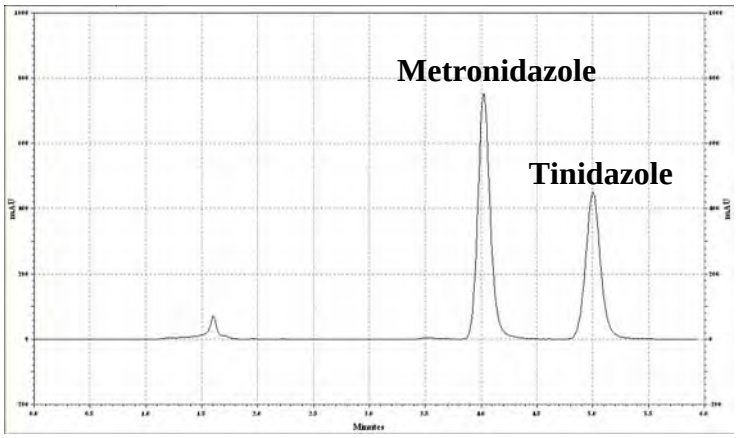

Figure 85 HPLC chromatogram of metronidazole/tinidazole in phosphate buffer solution pH 4.7

APPENDIX C

The diffusion of drug from selected periodontal gels

Table c1 The release of metronidazole from **ER_SP_vW-3** formulation

Time (hours)	% release			
	5% MTZ	**10% MTZ**	**20% MTZ**	**40% MTZ**
0.25	7.51 ± 2.60	3.87 ± 0.57	2.72 ± 0.10	**1.19 ± 0.13**
0.5	15.01 ± 1.77	5.82 ± 0.20	4.80 ± 0.87	**2.13 ± 0.07**
1	24.81 ± 1.28	12.86 ± 0.20	8.04 ± 2.18	**4.55 ± 0.08**
2	40.53 ± 0.75	25.00 ± 1.69	14.20 ± 2.78	**6.27 ± 1.07**
4	64.34 ± 2.53	36.59 ± 0.57	20.00 ± 1.70	**10.17 ± 0.42**
8	75.04 ± 2.77	47.63 ± 1.83	24.82 ± 0.63	**13.96 ± 0.13**
12	81.28 ± 1.54	49.97 ± 0.31	30.27 ± 1.66	**15.51 ± 0.36**
16	85.71 ± 1.67	54.84 ± 0.35	33.45 ± 1.01	**17.80 ± 0.03**
20	88.72 ± 2.48	58.98 ± 1.21	36.24 ± 1.65	**19.63 ± 0.17**
24	**93.60 ± 2.76**	**63.71 ± 1.35**	**38.92 ± 0.60**	**21.31 ± 0.36**

Table c2 The release of 5% metronidazole from **ER_SP_vW-4** formulation

Time (hours)	% release			
	5% MTZ	**10% MTZ**	**20% MTZ**	**40% MTZ**
0.25	8.95 ± 0.81	3.93 ± 0.70	2.90 ± 1.18	**2.24 ± 0.76**
0.5	15.90 ± 0.72	7.52 ± 1.21	4.54 ± 1.40	**4.03 ± 1.23**
1	24.33 ± 3.85	12.65 ±0.91	9.15 ± 2.62	**6.27 ± 1.88**
2	38.96 ± 2.09	22.51 ± 1.81	14.84 ± 1.69	**9.12 ± 0.78**
4	60.11 ± 1.65	32.15 ± 2.45	20.13 ± 1.84	**12.64 ± 2.18**
8	73.77 ± 1.30	43.55 ± 0.92	27.89 ± 2.86	**15.79 ± 1.35**
12	78.15 ± 1.39	45.80 ± 1.47	30.38 ± 2.79	**17.58 ± 2.18**
16	80.51 ± 2.62	49.80 ± 0.42	33.16 ± 0.95	**19.38 ± 0.89**
20	86.29 ± 0.44	54.41 ± 0.02	36.33 ± 1.37	**21.03 ± 1.40**
24	**90.32 ± 0.52**	**57.99 ± 1.25**	**40.14 ± 1.42**	**22.85 ± 1.05**

APPENDIX D

Spreading diameter of formulated gels

Table d1 Spreading diameter of hydrophilic gel bases

Formulation code	Spreading diameter (mm) n = 6							
	1	2	3	4	5	6	mean	SD
C_1	64.73	60.22	63.83	59.79	65.67	64.94	63.20	2.54
C_5	40.54	40.78	41.58	40.54	41.12	41.56	41.02	0.48
C_{11}	34.65	33.66	33.76	35.6	32.77	35.35	34.30	1.09
$HEC_{10}C_1$	21.27	20.19	22.29	22.58	21.78	23.49	21.93	1.14
$HPMC_{10}C_1$	53.95	57.29	55.24	53.34	57.42	55.86	55.52	1.68
$PVA_{10}C_1$	34.55	33.54	33	35	35.8	36	34.65	1.20
$PVP_{10}C_1$	27.89	27.43	30.95	28.24	29	28.51	28.67	1.24
$CMC_{10}C_1$	27.89	26.48	23.73	26.26	26.32	26.55	26.21	1.35
P_{L20}	45.93	42.46	43.22	44.28	43.01	44.7	43.93	1.28
P_{L40}	26.88	24.99	25.23	25.44	26.64	25.78	25.83	0.77
$P_{L20}A_2$	34.76	35.36	34.16	34.63	34.35	34.37	34.61	0.43
$P_{L20}A_5$	31.9	31.7	31.5	31	31.7	31.5	31.55	0.31
$P_{L20}C_1$	29.51	29.05	28.19	29.64	28.64	27.77	28.80	0.74
$P_{L20}C_5$	24.92	23.32	21.37	23.9	22.07	25.16	23.46	1.52
$P_{L\ 20}HEC_5$	29.42	29.44	29.95	30.73	29.64	30.01	29.87	0.49
$P_{L20}HPMC_5$	39.44	41.51	42.1	42.24	42.62	42.93	41.81	1.26
$P_{L20}PVP_5$	32.06	31.75	31.5	34	33.53	32.2	32.51	1.02
$P_{L20}PVA_5$	32.87	32.08	33.08	33.37	33.81	32.92	33.02	0.58
$P_{L20}CMC_5$	32.51	35.89	33.59	36.21	32.05	36.85	34.52	2.06

Table d2 Spreading diameter of hydrophobic gel bases

Formulation code	Spreading diameter (mm) n = 6							
	1	2	3	4	5	6	mean	SD
P_{E5}	54.32	55.85	55.01	53.69	53.97	57	54.97	1.26
P_{E10}	37.39	40.01	38.04	37.64	37.2	41.16	38.57	1.62
$P_{E5}A_{1.5}$	44.59	45.88	44	45.49	46.87	46.82	45.61	1.16
$P_{E5}A_3$	38.92	39.65	41.45	40.57	39.52	39.94	40.01	0.89
ER_S-1	75.93	73.4	71.48	72.25	74.81	71.43	73.22	1.85
ER_S-2	50.73	52.34	51.89	54.33	52.29	53.92	52.58	1.33
ER_{SL}-3	75.3	72.8	73.67	73.98	74.29	71.69	73.62	1.25
ER_{SL}-4	74.02	75.74	73.36	75.38	74.12	73.87	74.42	0.93

Table d3 Spreading diameter of hydrophilic-hydrophobic gel bases

Formulation code	Spreading diameter (mm) n = 6							
	1	2	3	4	5	6	mean	SD
ER_SP_v	69.51	68.58	68.29	67.29	69.19	70.5	68.89	1.10
ER_SP_L	57.56	56.99	59.63	57.77	55.54	56.82	57.39	1.35
$ER_SP_LP_v$	52.65	52.68	52.29	53.59	52.47	52.54	52.70	0.46
ER_SP_vW-2	45.73	46.86	46.84	47.13	46.25	46.66	46.58	0.51
ER_SP_vW-3	40.61	41	38	40.75	40.83	40.96	40.36	1.16
ER_SP_vW-4	37.01	37.5	37.85	37.92	37.29	36.85	37.40	0.44
$ER_{SL}P_vW$-1	38.5	41.25	42.51	41.22	39.52	39.89	40.48	1.45
$ER_{SL}P_vW$-2	38.12	37.95	42.12	41.56	41.85	40.28	40.31	1.87
$ER_{SL}P_LP_vW$-1	67.77	66.7	69.4	68.33	67.74	66.76	67.78	1.01
$ER_{SL}P_LP_vW$-2	53.17	52.6	51.62	52.6	53.39	52.43	52.64	0.62
EG-1	45.27	45.56	45.66	46.76	45.23	45.7	45.70	0.56
EG-2	42.56	42.66	42.72	43.23	43.7	43.52	43.07	0.48
EG-3	32.87	31.41	29.89	33.46	30.54	29.85	31.34	1.54

Table d4 Spreading diameter of hydrophilic-hydrophobic gel bases prepared from water insoluble polymer with contacted PBS pH 6.8

Formulation code	Spreading diameter (mm) n = 6							
	1	2	3	4	5	6	mean	SD
ERs-1	57.44	59.78	58.31	56.04	57.32	58.35	57.87	1.26
ER_S-2	45.24	46.73	47.12	46.09	44.46	45.58	45.87	0.98
ER_{SL}-3	55.14	58.66	59.21	54.68	60.52	62.25	58.41	2.98
ER_{SL}-4	57.58	56.92	61.55	63.58	58.95	58.12	59.45	2.58
ER_SP_v	52.6	54.1	54.5	51.25	50.8	51.75	52.50	1.52
ER_SP_L	35.37	36.58	36.4	37.59	38.4	37.5	36.97	1.07
$ER_SP_LP_v$	33.42	32.3	31.95	30.65	33.86	34	32.70	1.30
ER_SP_vW-2	31.05	31.55	29.39	30.56	31	31.29	30.81	0.77
ER_SP_vW-3	29.5	31.5	30.53	29.48	30.76	30.97	30.46	0.81
ER_SP_vW-4	26.4	26.3	26.59	27	26.88	26.45	26.60	0.28
$ER_{SL}P_vW$-1	28.75	31.52	30.89	27.85	31.95	32.25	30.54	1.81
$ER_{SL}P_vW$-2	26.95	32.85	31.58	31.21	30.65	29.95	30.53	2.01
$ER_{SL}P_LP_vW$-1	41.45	43	39.8	42.55	42.85	43.45	42.18	1.35
$ER_{SL}P_LP_vW$-2	36.8	37.61	36.05	38.79	38.66	38.55	37.74	1.13

Table d5 Spreading diameter of hydrophilic gel base based on poloxamer at 37°C

Formulation code	Spreading diameter (mm) n= 6							
	1	2	3	4	5	6	mean	SD
P_{L20}	32.03	31.56	30.78	33.98	36.76	31.45	32.76	2.24
P_{L40}	20.36	20.35	20.17	19.85	19.54	19.85	20.02	0.33
$P_{L20}A_2$	31.2	30.25	29.84	27.35	26.98	28.63	29.04	1.68
$P_{L20}A_5$	25.45	24.36	25.81	25.13	24.2	23.58	24.76	0.85
$P_{L20}C_1$	19.85	19	22.25	23	22.85	22.61	21.59	1.72
$P_{L20}C_5$	15.21	15.47	16.98	17.5	16.93	15	16.18	1.08
$P_{L\ 20}HEC_5$	22.1	22.69	22.85	25.36	24.85	22.95	23.47	1.31
$P_{L20}PVP_5$	33.52	35.84	37.21	37.23	33.54	36.22	35.59	1.69
$P_{L20}PVA_5$	25.12	24.36	27.89	27.68	28.23	24.95	26.37	1.74
$P_{L20}HPMC_5$	24.75	27.85	29.68	31.03	28.99	27.15	28.24	2.19
$P_{L20}CMC_5$	25.66	24.95	25.43	25.25	24.5	26.5	25.38	0.68

APPENDIX E

Adhesive property of formulated gels

Table e1 Adhesive forces of hydrophilic gel bases

Formulation code	Adhesive forces (N) n = 6							
	1	2	3	4	5	6	mean	SD
C_1	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.00
C_5	0.40	0.41	0.48	0.47	0.37	0.37	0.42	0.05
C_{11}	1.19	1.05	1.10	1.05	1.15	1.19	1.12	0.07
$HEC_{10}C_1$	0.39	0.25	0.28	0.25	0.24	0.29	0.28	0.06
$PVP_{10}C_1$	0.25	0.24	0.23	0.21	0.26	0.27	0.24	0.02
$PVA_{10}C_1$	0.47	0.47	0.42	0.48	0.45	0.44	0.46	0.02
$CMC_{10}C_1$	0.91	1.10	0.95	0.93	0.99	0.90	0.96	0.07
$HPMC_{10}C_1$	1.55	1.57	1.53	1.60	1.56	1.61	1.57	0.03
P_{L20}	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.00
P_{L40}	0.91	0.93	0.89	0.90	0.91	0.88	0.90	0.02
$P_{L20}A_2$	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.00
$P_{L20}A_5$	0.29	0.28	0.24	0.26	0.29	0.28	0.27	0.02
$P_{L20}C_1$	0.60	0.60	0.59	0.59	0.60	0.59	0.59	0.00
$P_{L20}C_5$	1.02	1.04	1.00	1.01	1.04	1.01	1.02	0.02
$P_{L\ 20}HEC_5$	0.19	0.21	0.23	0.21	0.23	0.21	0.21	0.00
$P_{L20}HPMC_5$	0.42	0.43	0.45	0.44	0.44	0.48	0.44	0.00
$P_{L20}PVP_5$	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.00
$P_{L20}PVA_5$	0.46	0.43	0.49	0.47	0.44	0.44	0.45	0.02
$P_{L20}CMC_5$	0.46	0.46	0.44	0.44	0.47	0.48	0.46	0.01

Table e2 Adhesive of hydrophobic gel bases

Formulation code	Adhesive force (N) n = 6							
	1	2	3	4	5	6	Mean	SD
P_{E5}	0.22	0.20	0.23	0.21	0.22	0.21	0.21	0.01
P_{E10}	0.19	0.22	0.18	0.17	0.16	0.18	0.18	0.02
$P_{E5}A_{1.5}$	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.00
$P_{E5}A_3$	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.00
ER_S-1	0.04	0.03	0.04	0.03	0.04	0.04	0.04	0.01
ER_{SL}-2	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.01
ER_S-3	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.01
ER_{SL}-4	0.03	0.05	0.04	0.04	0.04	0.03	0.04	0.01

Table e3 Adhesive of hydrophilic-hydrophobic gel bases

Formulation code	Adhesive force (N) n = 6							
	1	**2**	**3**	**4**	**5**	**6**	**Mean**	**SD**
ER_SP_v	0.16	0.15	0.17	0.16	0.17	0.15	0.16	0.01
ER_SP_L	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.00
$ER_SP_LP_v$	0.17	0.17	0.18	0.17	0.18	0.17	0.17	0.00
ER_SP_vW-2	0.45	0.45	0.45	0.48	0.44	0.47	0.46	0.01
ER_SP_vW-3	0.47	0.43	0.45	0.44	0.48	0.45	0.45	0.01
ER_SP_vW-4	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.00
$ER_{SL}P_v$W-1	0.43	0.46	0.47	0.49	0.42	0.44	0.45	0.02
$ER_{SL}P_v$W-2	0.41	0.49	0.46	0.50	0.44	0.45	0.46	0.03
$ER_{SL}P_LP_v$W-1	0.37	0.35	0.36	0.36	0.37	0.37	0.36	0.01
$ER_{SL}P_LP_v$W-2	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.00
EG-1	0.31	0.25	0.26	0.28	0.30	0.26	0.28	0.02
EG-2	0.17	0.11	0.19	0.15	0.18	0.18	0.16	0.03
EG-3	0.62	0.62	0.62	0.60	0.59	0.62	0.61	0.01

APPENDIX F

Statistic Evaluation

Table f1 The result of one-way ANOVA of viscosity test of each $\mathbf{P_{L20}}$ containing various hydrophilic polymers

ANOVA

Viscosity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	119.128	5	23.826	47.390	.000
Within Groups	6.033	12	.503		
Total	125.161	17			

Multiple Comparisons

Dependent Variable: Viscosity

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HPMC	PVA	1.28630	.57894	.465	-.9952	3.5678
	CMC	-.21681	.57894	1.000	-2.4983	2.0647
	HEC	-1.90304	.57894	.127	-4.1845	.3784
	Carbopol	-2.70108*	.57894	.017	-4.9825	-.4196
	PVP	5.25184*	.57894	.000	2.9704	7.5333
PVA	HPMC	-1.28630	.57894	.465	-3.5678	.9952
	CMC	-1.50310	.57894	.310	-3.7846	.7784
	HEC	-3.18934*	.57894	.005	-5.4708	-.9079
	Carbopol	-3.98737*	.57894	.001	-6.2688	-1.7059
	PVP	3.96554*	.57894	.001	1.6841	6.2470
CMC	HPMC	.21681	.57894	1.000	-2.0647	2.4983
	PVA	1.50310	.57894	.310	-.7784	3.7846
	HEC	-1.68624	.57894	.210	-3.9677	.5952
	Carbopol	-2.48427*	.57894	.030	-4.7657	-.2028
	PVP	5.46865*	.57894	.000	3.1872	7.7501
HEC	HPMC	1.90304	.57894	.127	-.3784	4.1845
	PVA	3.18934*	.57894	.005	.9079	5.4708
	CMC	1.68624	.57894	.210	-.5952	3.9677
	Carbopol	-.79803	.57894	.853	-3.0795	1.4834
	PVP	7.15488*	.57894	.000	4.8734	9.4363
Carbopol	HPMC	2.70108*	.57894	.017	.4196	4.9825
	PVA	3.98737*	.57894	.001	1.7059	6.2688
	CMC	2.48427*	.57894	.030	.2028	4.7657
	HEC	.79803	.57894	.853	-1.4834	3.0795
	PVP	7.95292*	.57894	.000	5.6715	10.2344
PVP	HPMC	-5.25184*	.57894	.000	-7.5333	-2.9704
	PVA	-3.96554*	.57894	.001	-6.2470	-1.6841
	CMC	-5.46865*	.57894	.000	-7.7501	-3.1872
	HEC	-7.15488*	.57894	.000	-9.4363	-4.8734
	Carbopol	-7.95292*	.57894	.000	-10.2344	-5.6715

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

Table f2 The result of one-way ANOVA of viscosity test with different ratio of RS/RL

ANOVA

Viscosity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.449	2	2.724	1.609	.276
Within Groups	10.160	6	1.693		
Total	15.609	8			

Multiple Comparisons

Dependent Variable: Viscosity

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RS/RL 25:0	RS/RL 22.5:2.	1.46550	1.06251	.438	-1.9422	4.8732
	RS/RL 20:5	1.78800	1.06251	.314	-1.6197	5.1957
RS/RL 22.5:2.	RS/RL 25:0	-1.46550	1.06251	.438	-4.8732	1.9422
	RS/RL 20:5	.32250	1.06251	.955	-3.0852	3.7302
RS/RL 20:5	RS/RL 25:0	-1.78800	1.06251	.314	-5.1957	1.6197
	RS/RL 22.5:2.	-.32250	1.06251	.955	-3.7302	3.0852

Table f3 The result of One-Way ANOVA of syringeability test of 0%, 2% and 5% of Aerosil® in hydrophilic gel base system 1-2

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Grou	944.246	2	472.123	670.465	.000
Within Groups	4.225	6	.704		
Total	948.472	8			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ae 0.5%	Ae 2%	-9.68708*	.68516	.000	-11.8846	-7.4896
	Ae 5%	-24.88708*	.68516	.000	-27.0846	-22.6896
Ae 2%	Ae 0.5%	9.68708*	.68516	.000	7.4896	11.8846
	Ae 5%	-15.20000*	.68516	.000	-17.3975	-13.0025
Ae 5%	Ae 0.5%	24.88708*	.68516	.000	22.6896	27.0846
	Ae 2%	15.20000*	.68516	.000	13.0025	17.3975

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

Table f4 The result of independent T test of syringeability test of P_{L20} and $P_{L20}PVP_5$ in hydrophilic gel base system 1-2

Independent Samples Test

		Levene's Test for Equality of Variance		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	12.980	.023	-5.999	4	.004	-3.22697	.53790	-4.72043	-1.73351
	Equal variances not assumed			-5.999	2.024	.026	-3.22697	.53790	-5.51521	-.93873

Table f5 The result of paired-sample T test of syringeability test of 5% and 10% of PE in hydrophobic gel base system 2-1

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1 Formulation -	-6.37116	4.95028	2.02094	-11.56616	-1.17616	-17.997	5	.000

Table f6 The result of independent T test of syringeability test of $P_{E5}A_3$ and P_{E10} of hydrophobic gel base system 2-1

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.002	.967	-.534	4	.622	-.07890	.14785	-.48941	.33161
	Equal variances not assumed			-.534	3.970	.622	-.07890	.14785	-.49063	.33284

Table f7 The result of One-Way ANOVA of syringeability test of 0%, 1.5% and 3% of Aerosil® in hydrophobic gel base system 2-1

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Group	152.391	2	76.195	2931.244	.000
Within Groups	.156	6	.026		
Total	152.547	8			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ae 0%	Ae 1.5%	-3.60313*	.13164	.000	-4.0253	-3.1809
	Ae 3%	-9.95378*	.13164	.000	-10.3760	-9.5316
Ae 1.5%	Ae 0%	3.60313*	.13164	.000	3.1809	4.0253
	Ae 3%	-6.35064*	.13164	.000	-6.7729	-5.9284
Ae 3%	Ae 0%	9.95378*	.13164	.000	9.5316	10.3760
	Ae 1.5%	6.35064*	.13164	.000	5.9284	6.7729

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

Table f8 The result of One-Way ANOVA of syringeability test of 25:0%, 2.5:2.5% and 20:5% of Eudragit® RS/Eudragit® RL in hydrophobic gel base system 2-2

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	2	.000	.069	.934
Within Groups	.041	6	.007		
Total	.042	8			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RS 25% RL 0%	RS 22.5% RL 2.5%	-.00300	.06763	.999	-.2199	.2139
	Ae 3%	-.02316	.06763	.944	-.2401	.1937
RS 22.5% RL 2.5%	RS 25% RL 0%	.00300	.06763	.999	-.2139	.2199
	Ae 3%	-.02016	.06763	.957	-.2371	.1967
Ae 3%	RS 25% RL 0%	.02316	.06763	.944	-.1937	.2401
	RS 22.5% RL 2.5%	.02016	.06763	.957	-.1967	.2371

Table f9 The result of independent T test of syrigeability test of ER_SP_L and $ER_SP_LP_v$ in hydrophobic- hydrophilic gel base system 3-2

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Force	Equal variances assumed	.455	.537	-5.074	4	.007	-3.98731	.78578	-6.16899	-1.80564
	Equal variances not assumed			-5.074	3.777	.008	-3.98731	.78578	-6.22080	-1.75382

Table f10 The result of One-Way ANOVA of syringeability test of Eudragit® RS/Eudragit® RL, (25:0, 22.5:2.5, 20:5) in system 3-3

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.421	2	.210	.066	.937
Within Groups	19.208	6	3.201		
Total	19.629	8			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RS 25% RL 0%	RS 22.5% RL 2.5%	.52128	1.46090	.939	-4.1642	5.2068
	RS 20% RL 5%	.17926	1.46090	.993	-4.5062	4.8647
RS 22.5% RL 2.5%	RS 25% RL 0%	-.52128	1.46090	.939	-5.2068	4.1642
	RS 20% RL 5%	-.34202	1.46090	.973	-5.0275	4.3434
RS 20% RL 5%	RS 25% RL 0%	-.17926	1.46090	.993	-4.8647	4.5062
	RS 22.5% RL 2.5%	.34202	1.46090	.973	-4.3434	5.0275

Table f11 The result of One-Way ANOVA of spreadability test of hydrophilic gel base system 1-1

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8758.763	7	1251.252	594.684	.000
Within Groups	84.163	40	2.104		
Total	8842.926	47			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CP	CP5	22.1767*	.8375	.000	18.854	25.500
	CP11	28.8983*	.8375	.000	25.575	32.221
	HEC10CP	41.2633*	.8375	.000	37.940	44.586
	PVP10CP	7.6800*	.8375	.000	4.357	11.003
	PVA10CP	28.5483*	.8375	.000	25.225	31.871
	HPMC10CP	34.5267*	.8375	.000	31.204	37.850
	CMC10CP	36.9917*	.8375	.000	33.669	40.315
CP5	CP	-22.1767*	.8375	.000	-25.500	-18.854
	CP11	6.7217*	.8375	.000	3.399	10.045
	HEC10CP	19.0867*	.8375	.000	15.764	22.410
	PVP10CP	-14.4967*	.8375	.000	-17.820	-11.174
	PVA10CP	6.3717*	.8375	.000	3.049	9.695
	HPMC10CP	12.3500*	.8375	.000	9.027	15.673
	CMC10CP	14.8150*	.8375	.000	11.492	18.138
CP11	CP	-28.8983*	.8375	.000	-32.221	-25.575
	CP5	-6.7217*	.8375	.000	-10.045	-3.399
	HEC10CP	12.3650*	.8375	.000	9.042	15.688
	PVP10CP	-21.2183*	.8375	.000	-24.541	-17.895
	PVA10CP	-.3500	.8375	1.000	-3.673	2.973
	HPMC10CP	5.6283*	.8375	.000	2.305	8.951
	CMC10CP	8.0933*	.8375	.000	4.770	11.416
HEC10CP	CP	-41.2633*	.8375	.000	-44.586	-37.940
	CP5	-19.0867*	.8375	.000	-22.410	-15.764
	CP11	-12.3650*	.8375	.000	-15.688	-9.042
	PVP10CP	-33.5833*	.8375	.000	-36.906	-30.260
	PVA10CP	-12.7150*	.8375	.000	-16.038	-9.392
	HPMC10CP	-6.7367*	.8375	.000	-10.060	-3.414
	CMC10CP	-4.2717*	.8375	.003	-7.595	-.949
PVP10CP	CP	-7.6800*	.8375	.000	-11.003	-4.357
	CP5	14.4967*	.8375	.000	11.174	17.820
	CP11	21.2183*	.8375	.000	17.895	24.541
	HEC10CP	33.5833*	.8375	.000	30.260	36.906
	PVA10CP	20.8683*	.8375	.000	17.545	24.191
	HPMC10CP	26.8467*	.8375	.000	23.524	30.170
	CMC10CP	29.3117*	.8375	.000	25.989	32.635
PVA10CP	CP	-28.5483*	.8375	.000	-31.871	-25.225
	CP5	-6.3717*	.8375	.000	-9.695	-3.049
	CP11	.3500	.8375	1.000	-2.973	3.673
	HEC10CP	12.7150*	.8375	.000	9.392	16.038
	PVP10CP	-20.8683*	.8375	.000	-24.191	-17.545
	HPMC10CP	5.9783*	.8375	.000	2.655	9.301
	CMC10CP	8.4433*	.8375	.000	5.120	11.766
HPMC10CP	CP	-34.5267*	.8375	.000	-37.850	-31.204
	CP5	-12.3500*	.8375	.000	-15.673	-9.027
	CP11	-5.6283*	.8375	.000	-8.951	-2.305
	HEC10CP	6.7367*	.8375	.000	3.414	10.060
	PVP10CP	-26.8467*	.8375	.000	-30.170	-23.524
	PVA10CP	-5.9783*	.8375	.000	-9.301	-2.655
	CMC10CP	2.4650	.8375	.306	-.858	5.788
CMC10CP	CP	-36.9917*	.8375	.000	-40.315	-33.669
	CP5	-14.8150*	.8375	.000	-18.138	-11.492
	CP11	-8.0933*	.8375	.000	-11.416	-4.770
	HEC10CP	4.2717*	.8375	.003	.949	7.595
	PVP10CP	-29.3117*	.8375	.000	-32.635	-25.989
	PVA10CP	-8.4433*	.8375	.000	-11.766	-5.120
	HPMC10CP	-2.4650	.8375	.306	-5.788	.858

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

Table f12 The result of One-Way ANOVA of spreadability test thermo-reversible gel base system 1-2 at room temperature

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Grou	031.047	3	343.682	545.813	.000
Within Groups	12.593	20	.630		
Total	043.641	23			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulatic	(J) Formulatic	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Plo20%	Plo40%	18.1067*	.4581	.000	16.710	19.503
	Plo20%Ae2%	9.3283*	.4581	.000	7.932	10.725
	Plo20%Ae5%	12.3833*	.4581	.000	10.987	13.780
Plo40%	Plo20%	-18.1067*	.4581	.000	-19.503	-16.710
	Plo20%Ae2%	-8.7783*	.4581	.000	-10.175	-7.382
	Plo20%Ae5%	-5.7233*	.4581	.000	-7.120	-4.327
Plo20%Ae2%	Plo20%	-9.3283*	.4581	.000	-10.725	-7.932
	Plo40%	8.7783*	.4581	.000	7.382	10.175
	Plo20%Ae5%	3.0550*	.4581	.000	1.658	4.452
Plo20%Ae5%	Plo20%	-12.3833*	.4581	.000	-13.780	-10.987
	Plo40%	5.7233*	.4581	.000	4.327	7.120
	Plo20%Ae2%	-3.0550*	.4581	.000	-4.452	-1.658

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

Table f13 The result of One-Way ANOVA of spreadability test thermo-reversible gel base system 1-2 at 37 °C

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Grou	543.600	3	181.200	83.788	.000
Within Groups	43.252	20	2.163		
Total	586.852	23			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PI20	PI40	12.7400*	.8490	.000	10.151	15.329
	PI20A2	3.7183*	.8490	.003	1.130	6.307
	PI20a5	8.0050*	.8490	.000	5.416	10.594
PI40	PI20	-12.7400*	.8490	.000	-15.329	-10.151
	PI20A2	-9.0217*	.8490	.000	-11.610	-6.433
	PI20a5	-4.7350*	.8490	.000	-7.324	-2.146
PI20A2	PI20	-3.7183*	.8490	.003	-6.307	-1.130
	PI40	9.0217*	.8490	.000	6.433	11.610
	PI20a5	4.2867*	.8490	.001	1.698	6.875
PI20a5	PI20	-8.0050*	.8490	.000	-10.594	-5.416
	PI40	4.7350*	.8490	.000	2.146	7.324
	PI20A2	-4.2867*	.8490	.001	-6.875	-1.698

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

Table f14 The result of One-Way ANOVA of spreadability test of hydrophilic-thermo-reversible gel base system 1-3 at room temperature

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Group	1765.180	6	294.197	162.687	.000
Within Groups	63.293	35	1.808		
Total	1828.472	41			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CP	CP5	5.3433*	.7764	.000	2.415	8.272
	HEC10CP	-3.0033*	.7764	.041	-5.932	-.075
	PVP10CP	-17.6733*	.7764	.000	-20.602	-14.745
	PVA10CP	-3.7067*	.7764	.005	-6.635	-.778
	HPMC10CP	-4.2217*	.7764	.001	-7.150	-1.293
	CMC10CP	-5.7167*	.7764	.000	-8.645	-2.788
CP5	CP	-5.3433*	.7764	.000	-8.272	-2.415
	HEC10CP	-8.3467*	.7764	.000	-11.275	-5.418
	PVP10CP	-23.0167*	.7764	.000	-25.945	-20.088
	PVA10CP	-9.0500*	.7764	.000	-11.979	-6.121
	HPMC10CP	-9.5650*	.7764	.000	-12.494	-6.636
	CMC10CP	-11.0600*	.7764	.000	-13.989	-8.131
HEC10CP	CP	3.0033*	.7764	.041	.075	5.932
	CP5	8.3467*	.7764	.000	5.418	11.275
	PVP10CP	-14.6700*	.7764	.000	-17.599	-11.741
	PVA10CP	-.7033	.7764	.990	-3.632	2.225
	HPMC10CP	-1.2183	.7764	.867	-4.147	1.710
	CMC10CP	-2.7133	.7764	.087	-5.642	.215
PVP10CP	CP	17.6733*	.7764	.000	14.745	20.602
	CP5	23.0167*	.7764	.000	20.088	25.945
	HEC10CP	14.6700*	.7764	.000	11.741	17.599
	PVA10CP	13.9667*	.7764	.000	11.038	16.895
	HPMC10CP	13.4517*	.7764	.000	10.523	16.380
	CMC10CP	11.9567*	.7764	.000	9.028	14.885
PVA10CP	CP	3.7067*	.7764	.005	.778	6.635
	CP5	9.0500*	.7764	.000	6.121	11.979
	HEC10CP	.7033	.7764	.990	-2.225	3.632
	PVP10CP	-13.9667*	.7764	.000	-16.895	-11.038
	HPMC10CP	-.5150	.7764	.998	-3.444	2.414
	CMC10CP	-2.0100	.7764	.373	-4.939	.919
HPMC10CP	CP	4.2217*	.7764	.001	1.293	7.150
	CP5	9.5650*	.7764	.000	6.636	12.494
	HEC10CP	1.2183	.7764	.867	-1.710	4.147
	PVP10CP	-13.4517*	.7764	.000	-16.380	-10.523
	PVA10CP	.5150	.7764	.998	-2.414	3.444
	CMC10CP	-1.4950	.7764	.714	-4.424	1.434
CMC10CP	CP	5.7167*	.7764	.000	2.788	8.645
	CP5	11.0600*	.7764	.000	8.131	13.989
	HEC10CP	2.7133	.7764	.087	-.215	5.642
	PVP10CP	-11.9567*	.7764	.000	-14.885	-9.028
	PVA10CP	2.0100	.7764	.373	-.919	4.939
	HPMC10CP	1.4950	.7764	.714	-1.434	4.424

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

Table f15 The result of One-Way ANOVA of spreadability test of hydrophilic - thermoreversible gel base system 1-3 at 37 **°C**

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1299.220	6	216.537	78.064	.000
Within Groups	97.084	35	2.774		
Total	1396.304	41			

Multiple Comparisons

Dependent Variable: Spread
Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CP	CP5	5.4117*	.9616	.001	1.784	9.039
	HEC10CP	-1.8733	.9616	.703	-5.501	1.754
	PVP10CP	-14.0000*	.9616	.000	-17.627	-10.373
	PVA10CP	-4.7783*	.9616	.003	-8.406	-1.151
	HPMC10CP	-6.6483*	.9616	.000	-10.276	-3.021
	CMC10CP	-4.4550*	.9616	.007	-8.082	-.828
CP5	CP	-5.4117*	.9616	.001	-9.039	-1.784
	HEC10CP	-7.2850*	.9616	.000	-10.912	-3.658
	PVP10CP	-19.4117*	.9616	.000	-23.039	-15.784
	PVA10CP	-10.1900*	.9616	.000	-13.817	-6.563
	HPMC10CP	-12.0600*	.9616	.000	-15.687	-8.433
	CMC10CP	-9.8667*	.9616	.000	-13.494	-6.239
HEC10CP	CP	1.8733	.9616	.703	-1.754	5.501
	CP5	7.2850*	.9616	.000	3.658	10.912
	PVP10CP	-12.1267*	.9616	.000	-15.754	-8.499
	PVA10CP	-2.9050	.9616	.200	-6.532	.722
	HPMC10CP	-4.7750*	.9616	.003	-8.402	-1.148
	CMC10CP	-2.5817	.9616	.329	-6.209	1.046
PVP10CP	CP	14.0000*	.9616	.000	10.373	17.627
	CP5	19.4117*	.9616	.000	15.784	23.039
	HEC10CP	12.1267*	.9616	.000	8.499	15.754
	PVA10CP	9.2217*	.9616	.000	5.594	12.849
	HPMC10CP	7.3517*	.9616	.000	3.724	10.979
	CMC10CP	9.5450*	.9616	.000	5.918	13.172
PVA10CP	CP	4.7783*	.9616	.003	1.151	8.406
	CP5	10.1900*	.9616	.000	6.563	13.817
	HEC10CP	2.9050	.9616	.200	-.722	6.532
	PVP10CP	-9.2217*	.9616	.000	-12.849	-5.594
	HPMC10CP	-1.8700	.9616	.705	-5.497	1.757
	CMC10CP	.3233	.9616	1.000	-3.304	3.951
HPMC10CP	CP	6.6483*	.9616	.000	3.021	10.276
	CP5	12.0600*	.9616	.000	8.433	15.687
	HEC10CP	4.7750*	.9616	.003	1.148	8.402
	PVP10CP	-7.3517*	.9616	.000	-10.979	-3.724
	PVA10CP	1.8700	.9616	.705	-1.757	5.497
	CMC10CP	2.1933	.9616	.529	-1.434	5.821
CMC10CP	CP	4.4550*	.9616	.007	.828	8.082
	CP5	9.8667*	.9616	.000	6.239	13.494
	HEC10CP	2.5817	.9616	.329	-1.046	6.209
	PVP10CP	-9.5450*	.9616	.000	-13.172	-5.918
	PVA10CP	-.3233	.9616	1.000	-3.951	3.304
	HPMC10CP	-2.1933	.9616	.529	-5.821	1.434

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

Table f16 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.958	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-36.8467	6.5913	1.9027	-41.0346	-32.6588	-19.365	11	.000

Table f17 The result of paired-sample T test of spreadability test of $\mathbf{P_{L40}}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.983	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-21.4233	3.5993	1.0390	-23.7102	-19.1364	-20.619	11	.000

Table f18 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}A_2}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.928	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-30.3233	3.6204	1.0451	-32.6236	-28.0230	-29.014	11	.000

Table f19 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}A_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.986	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-26.6525	4.1158	1.1881	-29.2676	-24.0374	-22.432	11	.000

Table f20 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}C_1}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.948	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-23.6967	4.4677	1.2897	-26.5353	-20.8580	-18.373	11	.000

Table f21 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}C_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.950	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-18.3192	4.4999	1.2990	-21.1783	-15.4600	-14.102	11	.000

Table f22 The result of paired-sample T test of spreadability test of $\mathbf{P_{L\ 20}HEC_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.962	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-25.1658	3.9775	1.1482	-27.6930	-22.6386	-21.917	11	.000

Table f23 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}PVP_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.968	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-39.5333	6.3787	1.8414	-43.5862	-35.4805	-21.469	11	.000

Table f24 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}PVA_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.921	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-27.9392	3.9656	1.1448	-30.4588	-25.4195	-24.406	11	.000

Table f25 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}HPMC_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.889	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-29.2733	3.1157	.8994	-31.2530	-27.2937	-32.547	11	.000

Table f26 The result of paired-sample T test of spreadability test of $\mathbf{P_{L20}CMC_5}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.960	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-28.2408	5.6978	1.6448	-31.8610	-24.6207	-17.170	11	.000

Table f27 The result of One-Way ANOVA of spreadability test of hydrophobic-hydrophilic gel base system 3-3

ANOVA

Force

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.421	2	.210	.066	.937
Within Groups	19.208	6	3.201		
Total	19.629	8			

Multiple Comparisons

Dependent Variable: Force

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
RS25% RL0%	RS22.5% RL2.5%	.52128	1.46090	.939	-4.1642	5.2068
	RS20%RL5%	.17926	1.46090	.993	-4.5062	4.8647
RS22.5% RL2.5%	RS25% RL0%	-.52128	1.46090	.939	-5.2068	4.1642
	RS20%RL5%	-.34202	1.46090	.973	-5.0275	4.3434
RS20%RL5%	RS25% RL0%	-.17926	1.46090	.993	-4.8647	4.5062
	RS22.5% RL2.5%	.34202	1.46090	.973	-4.3434	5.0275

Table f28 The result of paired-sample T test of spreadability test of $\mathbf{ER_{SL}P_LP_vW\text{-}1}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.308	.331

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-55.9100	1.2588	.3634	-56.7098	-55.1102	-153.863	11	.000

Table f29 The result of paired-sample T test of spreadability test of $\mathbf{ER_{SL}P_LP_vW\text{-}2}$ at room temperature and 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.083	.798

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-51.0975	.7329	.2116	-51.5631	-50.6319	-241.527	11	.000

Table f30 The result of paired-sample T test of spreadability test of $\mathbf{ER_SP_L}$ and $\mathbf{ER_SP_LP_v}$ at room temperature

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.931	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-53.54417	3.11862	.90027	-55.52564	-51.56269	-59.476	11	.000

Table f31 The result of paired-sample T test of spreadability test of $\mathbf{ER_SP_L}$ And $\mathbf{ER_SP_LP_v}$ at 37 °C

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.891	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	-33.33500	2.98109	.86057	-35.22909	-31.44091	-38.736	11	.000

Table f32 The result of one-way ANOVA of spreadability test of $\mathbf{ER_S}$**-1**, $\mathbf{ER_S}$**-3** and $\mathbf{ER_S}$**-4** without contacted PBS pH 6.8

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.459	2	2.229	1.145	.345
Within Groups	29.218	15	1.948		
Total	33.677	17			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
EC12.5 RS25	EC12.5 RS22.5 RL2.5	-.40500	.80578	.882	-2.5917	1.7817
	EC12.5 RS20 RL5	-1.19833	.80578	.356	-3.3851	.9884
EC12.5 RS22.5 RL2.5	EC12.5 RS25	.40500	.80578	.882	-1.7817	2.5917
	EC12.5 RS20 RL5	-.79333	.80578	.625	-2.9801	1.3934
EC12.5 RS20 RL5	EC12.5 RS25	1.19833	.80578	.356	-.9884	3.3851
	EC12.5 RS22.5 RL2.5	.79333	.80578	.625	-1.3934	2.9801

Table f33 The result of one-way ANOVA of spreadability test of $\mathbf{ER_S}$**-1**, $\mathbf{ER_S}$**-3** and $\mathbf{ER_S}$**-4** with contacted PBS pH 6.8

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.711	2	3.855	.674	.524
Within Groups	85.798	15	5.720		
Total	93.509	17			

Multiple Comparisons

Dependent Variable: Spread
Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
EC12.5 RS25	EC12.5 RS22.5 RL2.5	-.53667	1.38080	.928	-4.2839	3.2105
	EC12.5 RS20 RL5	-1.57667	1.38080	.535	-5.3239	2.1705
EC12.5 RS22.5 RL2.5	EC12.5 RS25	.53667	1.38080	.928	-3.2105	4.2839
	EC12.5 RS20 RL5	-1.04000	1.38080	.757	-4.7872	2.7072
EC12.5 RS20 RL5	EC12.5 RS25	1.57667	1.38080	.535	-2.1705	5.3239
	EC12.5 RS22.5 RL2.5	1.04000	1.38080	.757	-2.7072	4.7872

Table f34 The result of One-Way ANOVA of spreadability of **ER_SP_vW-3** base containing 5, 10, 20 and 40 % MTZ without contacted PBS pH 6.8

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	651.720	7	93.103	286.478	.000
Within Groups	13.000	40	.325		
Total	664.720	47			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	5.71000*	.32914	.000	4.4041	7.0159
	3-20% MTZ	6.77333*	.32914	.000	5.4674	8.0793
	3-40% MTZ	9.76833*	.32914	.000	8.4624	11.0743
	4-5% MTZ	2.88333*	.32914	.000	1.5774	4.1893
	4-10% MTZ	6.85000*	.32914	.000	5.5441	8.1559
	4-20% MTZ	9.17333*	.32914	.000	7.8674	10.4793
	4-40% MTZ	12.41167*	.32914	.000	11.1057	13.7176
3-10% MTZ	3-5% MTZ	-5.71000*	.32914	.000	-7.0159	-4.4041
	3-20% MTZ	1.06333	.32914	.198	-.2426	2.3693
	3-40% MTZ	4.05833*	.32914	.000	2.7524	5.3643
	4-5% MTZ	-2.82667*	.32914	.000	-4.1326	-1.5207
	4-10% MTZ	1.14000	.32914	.133	-.1659	2.4459
	4-20% MTZ	3.46333*	.32914	.000	2.1574	4.7693
	4-40% MTZ	6.70167*	.32914	.000	5.3957	8.0076
3-20% MTZ	3-5% MTZ	-6.77333*	.32914	.000	-8.0793	-5.4674
	3-10% MTZ	-1.06333	.32914	.198	-2.3693	.2426
	3-40% MTZ	2.99500*	.32914	.000	1.6891	4.3009
	4-5% MTZ	-3.89000*	.32914	.000	-5.1959	-2.5841
	4-10% MTZ	.07667	.32914	1.000	-1.2293	1.3826
	4-20% MTZ	2.40000*	.32914	.000	1.0941	3.7059
	4-40% MTZ	5.63833*	.32914	.000	4.3324	6.9443
3-40% MTZ	3-5% MTZ	-9.76833*	.32914	.000	-11.0743	-8.4624
	3-10% MTZ	-4.05833*	.32914	.000	-5.3643	-2.7524
	3-20% MTZ	-2.99500*	.32914	.000	-4.3009	-1.6891
	4-5% MTZ	-6.88500*	.32914	.000	-8.1909	-5.5791
	4-10% MTZ	-2.91833*	.32914	.000	-4.2243	-1.6124
	4-20% MTZ	-.59500	.32914	.853	-1.9009	.7109
	4-40% MTZ	2.64333*	.32914	.000	1.3374	3.9493
4-5% MTZ	3-5% MTZ	-2.88333*	.32914	.000	-4.1893	-1.5774
	3-10% MTZ	2.82667*	.32914	.000	1.5207	4.1326
	3-20% MTZ	3.89000*	.32914	.000	2.5841	5.1959
	3-40% MTZ	6.88500*	.32914	.000	5.5791	8.1909
	4-10% MTZ	3.96667*	.32914	.000	2.6607	5.2726
	4-20% MTZ	6.29000*	.32914	.000	4.9841	7.5959
	4-40% MTZ	9.52833*	.32914	.000	8.2224	10.8343
4-10% MTZ	3-5% MTZ	-6.85000*	.32914	.000	-8.1559	-5.5441
	3-10% MTZ	-1.14000	.32914	.133	-2.4459	.1659
	3-20% MTZ	-.07667	.32914	1.000	-1.3826	1.2293
	3-40% MTZ	2.91833*	.32914	.000	1.6124	4.2243
	4-5% MTZ	-3.96667*	.32914	.000	-5.2726	-2.6607
	4-20% MTZ	2.32333*	.32914	.000	1.0174	3.6293
	4-40% MTZ	5.56167*	.32914	.000	4.2557	6.8676
4-20% MTZ	3-5% MTZ	-9.17333*	.32914	.000	-10.4793	-7.8674
	3-10% MTZ	-3.46333*	.32914	.000	-4.7693	-2.1574
	3-20% MTZ	-2.40000*	.32914	.000	-3.7059	-1.0941
	3-40% MTZ	.59500	.32914	.853	-.7109	1.9009
	4-5% MTZ	-6.29000*	.32914	.000	-7.5959	-4.9841
	4-10% MTZ	-2.32333*	.32914	.000	-3.6293	-1.0174
	4-40% MTZ	3.23833*	.32914	.000	1.9324	4.5443
4-40% MTZ	3-5% MTZ	-12.41167*	.32914	.000	-13.7176	-11.1057
	3-10% MTZ	-6.70167*	.32914	.000	-8.0076	-5.3957
	3-20% MTZ	-5.63833*	.32914	.000	-6.9443	-4.3324
	3-40% MTZ	-2.64333*	.32914	.000	-3.9493	-1.3374
	4-5% MTZ	-9.52833*	.32914	.000	-10.8343	-8.2224
	4-10% MTZ	-5.56167*	.32914	.000	-6.8676	-4.2557
	4-20% MTZ	-3.23833*	.32914	.000	-4.5443	-1.9324

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

Table f35 The result of One-Way ANOVA of spreadability of **ER_SP_vW-3** base containing 5, 10, 20 and 40 % MTZ with contacted PBS pH 6.8

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	408.002	7	58.286	227.440	.000
Within Groups	10.251	40	.256		
Total	418.253	47			

Multiple Comparisons

Dependent Variable: spread

Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	4.19167*	.35376	.000	2.7880	5.5953
	3-20% MTZ	4.36333*	.35376	.000	2.9597	5.7670
	3-40% MTZ	6.68500*	.35376	.000	5.2814	8.0886
	4-5% MTZ	2.38500*	.35376	.000	.9814	3.7886
	4-10% MTZ	4.60333*	.35376	.000	3.1997	6.0070
	4-20% MTZ	7.84667*	.35376	.000	6.4430	9.2503
	4-40% MTZ	9.53167*	.35376	.000	8.1280	10.9353
3-10% MTZ	3-5% MTZ	-4.19167*	.35376	.000	-5.5953	-2.7880
	3-20% MTZ	.17167	.35376	1.000	-1.2320	1.5753
	3-40% MTZ	2.49333*	.35376	.000	1.0897	3.8970
	4-5% MTZ	-1.80667*	.35376	.003	-3.2103	-.4030
	4-10% MTZ	.41167	.35376	.985	-.9920	1.8153
	4-20% MTZ	3.65500*	.35376	.000	2.2514	5.0586
	4-40% MTZ	5.34000*	.35376	.000	3.9364	6.7436
3-20% MTZ	3-5% MTZ	-4.36333*	.35376	.000	-5.7670	-2.9597
	3-10% MTZ	-.17167	.35376	1.000	-1.5753	1.2320
	3-40% MTZ	2.32167*	.35376	.000	.9180	3.7253
	4-5% MTZ	-1.97833*	.35376	.001	-3.3820	-.5747
	4-10% MTZ	.24000	.35376	.999	-1.1636	1.6436
	4-20% MTZ	3.48333*	.35376	.000	2.0797	4.8870
	4-40% MTZ	5.16833*	.35376	.000	3.7647	6.5720
3-40% MTZ	3-5% MTZ	-6.68500*	.35376	.000	-8.0886	-5.2814
	3-10% MTZ	-2.49333*	.35376	.000	-3.8970	-1.0897
	3-20% MTZ	-2.32167*	.35376	.000	-3.7253	-.9180
	4-5% MTZ	-4.30000*	.35376	.000	-5.7036	-2.8964
	4-10% MTZ	-2.08167*	.35376	.000	-3.4853	-.6780
	4-20% MTZ	1.16167	.35376	.182	-.2420	2.5653
	4-40% MTZ	2.84667*	.35376	.000	1.4430	4.2503
4-5% MTZ	3-5% MTZ	-2.38500*	.35376	.000	-3.7886	-.9814
	3-10% MTZ	1.80667*	.35376	.003	.4030	3.2103
	3-20% MTZ	1.97833*	.35376	.001	.5747	3.3820
	3-40% MTZ	4.30000*	.35376	.000	2.8964	5.7036
	4-10% MTZ	2.21833*	.35376	.000	.8147	3.6220
	4-20% MTZ	5.46167*	.35376	.000	4.0580	6.8653
	4-40% MTZ	7.14667*	.35376	.000	5.7430	8.5503
4-10% MTZ	3-5% MTZ	-4.60333*	.35376	.000	-6.0070	-3.1997
	3-10% MTZ	-.41167	.35376	.985	-1.8153	.9920
	3-20% MTZ	-.24000	.35376	.999	-1.6436	1.1636
	3-40% MTZ	2.08167*	.35376	.000	.6780	3.4853
	4-5% MTZ	-2.21833*	.35376	.000	-3.6220	-.8147
	4-20% MTZ	3.24333*	.35376	.000	1.8397	4.6470
	4-40% MTZ	4.92833*	.35376	.000	3.5247	6.3320
4-20% MTZ	3-5% MTZ	-7.84667*	.35376	.000	-9.2503	-6.4430
	3-10% MTZ	-3.65500*	.35376	.000	-5.0586	-2.2514
	3-20% MTZ	-3.48333*	.35376	.000	-4.8870	-2.0797
	3-40% MTZ	-1.16167	.35376	.182	-2.5653	.2420
	4-5% MTZ	-5.46167*	.35376	.000	-6.8653	-4.0580
	4-10% MTZ	-3.24333*	.35376	.000	-4.6470	-1.8397
	4-40% MTZ	1.68500*	.35376	.008	.2814	3.0886
4-40% MTZ	3-5% MTZ	-9.53167*	.35376	.000	-10.9353	-8.1280
	3-10% MTZ	-5.34000*	.35376	.000	-6.7436	-3.9364
	3-20% MTZ	-5.16833*	.35376	.000	-6.5720	-3.7647
	3-40% MTZ	-2.84667*	.35376	.000	-4.2503	-1.4430
	4-5% MTZ	-7.14667*	.35376	.000	-8.5503	-5.7430
	4-10% MTZ	-4.92833*	.35376	.000	-6.3320	-3.5247
	4-20% MTZ	-1.68500*	.35376	.008	-3.0886	-.2814

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

Table f36 The result of One-Way ANOVA of spreadability test of **ER_SP_vW-3** base at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.234	2	2.117	2.442	.121
Within Groups	13.005	15	.867		
Total	17.239	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.54500	.53758	.608	-.9139	2.0039
	3-20% MTZ	1.18667	.53758	.121	-.2722	2.6455
3-10% MTZ	3-5% MTZ	-.54500	.53758	.608	-2.0039	.9139
	3-20% MTZ	.64167	.53758	.506	-.8172	2.1005
3-20% MTZ	3-5% MTZ	-1.18667	.53758	.121	-2.6455	.2722
	3-10% MTZ	-.64167	.53758	.506	-2.1005	.8172

Table f37 The result of One-Way ANOVA of spreadability test of **ER_SP_vW-4** base at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.700	2	.350	.674	.524
Within Groups	7.786	15	.519		
Total	8.486	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.38500	.41596	.659	-.7438	1.5138
	3-20% MTZ	.44500	.41596	.576	-.6838	1.5738
3-10% MTZ	3-5% MTZ	-.38500	.41596	.659	-1.5138	.7438
	3-20% MTZ	.06000	.41596	.990	-1.0688	1.1888
3-20% MTZ	3-5% MTZ	-.44500	.41596	.576	-1.5738	.6838
	3-10% MTZ	-.06000	.41596	.990	-1.1888	1.0688

Table f38 The result of One-Way ANOVA of spreadability test of **$ER_{SL}P_vW$-1** base at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.185	2	1.093	.554	.586
Within Groups	29.588	15	1.973		
Total	31.773	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.60167	.81086	.763	-1.5988	2.8022
	3-20% MTZ	.82500	.81086	.606	-1.3755	3.0255
3-10% MTZ	3-5% MTZ	-.60167	.81086	.763	-2.8022	1.5988
	3-20% MTZ	.22333	.81086	.963	-1.9772	2.4238
3-20% MTZ	3-5% MTZ	-.82500	.81086	.606	-3.0255	1.3755
	3-10% MTZ	-.22333	.81086	.963	-2.4238	1.9772

Table f39 The result of One-Way ANOVA of spreadability test of **$ER_{SL}P_vW$-2** base at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.735	2	1.867	1.226	.321
Within Groups	22.853	15	1.524		
Total	26.588	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.99333	.71264	.401	-.9406	2.9273
	3-20% MTZ	.93667	.71264	.442	-.9973	2.8706
3-10% MTZ	3-5% MTZ	-.99333	.71264	.401	-2.9273	.9406
	3-20% MTZ	-.05667	.71264	.997	-1.9906	1.8773
3-20% MTZ	3-5% MTZ	-.93667	.71264	.442	-2.8706	.9973
	3-10% MTZ	.05667	.71264	.997	-1.8773	1.9906

Table f40 The result of One-Way ANOVA of spreadability test of **ER_SP_vW-3** base containing 5% MTZ at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.181	2	1.591	.910	.424
Within Groups	26.222	15	1.748		
Total	29.403	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.12667	.76335	.986	-1.9449	2.1982
	3-20% MTZ	.94833	.76335	.480	-1.1232	3.0199
3-10% MTZ	3-5% MTZ	-.12667	.76335	.986	-2.1982	1.9449
	3-20% MTZ	.82167	.76335	.572	-1.2499	2.8932
3-20% MTZ	3-5% MTZ	-.94833	.76335	.480	-3.0199	1.1232
	3-10% MTZ	-.82167	.76335	.572	-2.8932	1.2499

Table f41 The result of One-Way ANOVA of spreadability test of **ER_SP_vW-4** base containing 5% MTZ at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.214	2	.107	.141	.870
Within Groups	11.404	15	.760		
Total	11.618	17			

Multiple Comparisons

Dependent Variable: spread
Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.13383	.50340	.965	-1.2323	1.5000
	3-20% MTZ	.26717	.50340	.870	-1.0990	1.6333
3-10% MTZ	3-5% MTZ	-.13383	.50340	.965	-1.5000	1.2323
	3-20% MTZ	.13333	.50340	.966	-1.2328	1.4995
3-20% MTZ	3-5% MTZ	-.26717	.50340	.870	-1.6333	1.0990
	3-10% MTZ	-.13333	.50340	.966	-1.4995	1.2328

Table f42 The result of One-Way ANOVA of spreadability test of **$ER_{SL}P_vW$-1** base containing 5% MTZ at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.919	2	4.960	3.303	.065
Within Groups	22.521	15	1.501		
Total	32.440	17			

Multiple Comparisons

Dependent Variable: spread

Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	.72500	.70744	.602	-1.1948	2.6448
	3-20% MTZ	1.80667	.70744	.067	-.1132	3.7265
3-10% MTZ	3-5% MTZ	-.72500	.70744	.602	-2.6448	1.1948
	3-20% MTZ	1.08167	.70744	.337	-.8382	3.0015
3-20% MTZ	3-5% MTZ	-1.80667	.70744	.067	-3.7265	.1132
	3-10% MTZ	-1.08167	.70744	.337	-3.0015	.8382

Table f43 The result of One-Way ANOVA of spreadability test of **$ER_{SL}P_vW$-2** base containing 5% MTZ at 0, 1 and 6 months

ANOVA

spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.987	2	4.994	2.413	.123
Within Groups	31.037	15	2.069		
Total	41.024	17			

Multiple Comparisons

Dependent Variable: spread

Scheffe

(I) forspread	(J) forspread	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-5% MTZ	3-10% MTZ	1.56833	.83049	.202	-.6854	3.8221
	3-20% MTZ	1.59167	.83049	.193	-.6621	3.8454
3-10% MTZ	3-5% MTZ	-1.56833	.83049	.202	-3.8221	.6854
	3-20% MTZ	.02333	.83049	1.000	-2.2304	2.2771
3-20% MTZ	3-5% MTZ	-1.59167	.83049	.193	-3.8454	.6621
	3-10% MTZ	-.02333	.83049	1.000	-2.2771	2.2304

Table f44 The result of one-way ANOVA of adhesive test of thermo-reversible gel with different of concentration of Aerosil® (0.5%, 1.5% and 3%w/w)

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.080	2	.040	926.353	.000
Within Groups	.001	15	.000		
Total	.080	17			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ae0.5	Ae 2	.13562*	.00378	.000	.1254	.1459
	Ae 5	.14585*	.00378	.000	.1356	.1561
Ae 2	Ae0.5	-.13562*	.00378	.000	-.1459	-.1254
	Ae 5	.01024	.00378	.051	.0000	.0205
Ae 5	Ae0.5	-.14585*	.00378	.000	-.1561	-.1356
	Ae 2	-.01024	.00378	.051	-.0205	.0000

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

Table f45 The result of one-way ANOVA of adhesive test of each $\mathbf{P_{L20}}$ containing PVA, CMC and HPMC

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	2	.000	.936	.414
Within Groups	.006	15	.000		
Total	.007	17			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HPMC	PVA	-.01167	.01151	.609	-.0429	.0196
	CMC	-.01500	.01151	.448	-.0462	.0162
PVA	HPMC	.01167	.01151	.609	-.0196	.0429
	CMC	-.00333	.01151	.959	-.0346	.0279
CMC	HPMC	.01500	.01151	.448	-.0162	.0462
	PVA	.00333	.01151	.959	-.0279	.0346

Table f46 The result of paired-sample T test of adhesive test of 5% and 10% of polyethylene

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Adhesive	12	-.727	.007

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Adhesive	1.30083	.53900	.15559	.95837	1.64330	8.360	11	.000

Table f47 The result of paired-sample T test of adhesive test of 1.5% and 3% of Aerosil in 5% polyethylene

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Adhesive	12	-1.000	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Adhesive	1.42500	.52746	.15226	1.08987	1.76013	9.359	11	.000

Table f48 The result of one-way ANOVA of adhesive test when increasing of EC and changed the ratio of RS/RL

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.457	.715
Within Groups	.001	20	.000		
Total	.001	23			

Multiple Comparisons

Dependent Variable: Spread
Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
EC12.5	EC17.5RL0	.00230	.00396	.952	-.0098	.0144
	RL2.5	-.00186	.00396	.974	-.0139	.0102
	RL5	-.00152	.00396	.985	-.0136	.0106
EC17.5RL0	EC12.5	-.00230	.00396	.952	-.0144	.0098
	RL2.5	-.00416	.00396	.777	-.0162	.0079
	RL5	-.00382	.00396	.818	-.0159	.0083
RL2.5	EC12.5	.00186	.00396	.974	-.0102	.0139
	EC17.5RL0	.00416	.00396	.777	-.0079	.0162
	RL5	.00034	.00396	1.000	-.0117	.0124
RL5	EC12.5	.00152	.00396	.985	-.0106	.0136
	EC17.5RL0	.00382	.00396	.818	-.0083	.0159
	RL2.5	-.00034	.00396	1.000	-.0124	.0117

Table f49 The result of paired-sample T test of adhesive test $\mathbf{ER_SP_L}$ and $\mathbf{ER_SP_LP_v}$

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	.694	.012

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	1.24895	.50869	.14685	.92574	1.57216	8.505	11	.000

Table f50 The result of paired-sample T test of adhesive test with 20% and 25% of WS in $\mathbf{ER_SP_v}$ formulation

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Formulation & Spread	12	-.054	.867

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Formulation - Spread	.96167	.51607	.14898	.63377	1.28956	6.455	11	.000

Table f51 The result of One-Way ANOVA of weight loss of $\mathbf{ER_SP_vW\text{-}3}$ and $\mathbf{ER_SP_vW\text{-}4}$ base containing 5, 10, 20 and 40 % MTZ

ANOVA

weightloss

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2366.501	9	262.945	96.959	.000
Within Groups	54.238	20	2.712		
Total	2420.740	29			

Multiple Comparisons

Dependent Variable: weightloss

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-0%	3-5%	.69650	1.34460	1.000	-5.5433	6.9363
	3-10%	16.71118*	1.34460	.000	10.4714	22.9510
	3-20%	24.61933*	1.34460	.000	18.3796	30.8591
	3-40%	21.41800*	1.34460	.000	15.1782	27.6578
	4-0%	1.11667	1.34460	1.000	-5.1231	7.3564
	4-5%	9.05717*	1.34460	.001	2.8174	15.2969
	4-10%	10.04467*	1.34460	.000	3.8049	16.2844
	4-20%	17.27733*	1.34460	.000	11.0376	23.5171
	4-40%	21.41800*	1.34460	.000	15.1782	27.6578
3-5%	3-0%	-.69650	1.34460	1.000	-6.9363	5.5433
	3-10%	16.01468*	1.34460	.000	9.7749	22.2545
	3-20%	23.92283*	1.34460	.000	17.6831	30.1626
	3-40%	20.72150*	1.34460	.000	14.4817	26.9613
	4-0%	.42017	1.34460	1.000	-5.8196	6.6599
	4-5%	8.36067*	1.34460	.003	2.1209	14.6004
	4-10%	9.34817*	1.34460	.001	3.1084	15.5879
	4-20%	16.58083*	1.34460	.000	10.3411	22.8206
	4-40%	20.72150*	1.34460	.000	14.4817	26.9613
3-10%	3-0%	-16.71118*	1.34460	.000	-22.9510	-10.4714
	3-5%	-16.01468*	1.34460	.000	-22.2545	-9.7749
	3-20%	7.90815*	1.34460	.006	1.6684	14.1479
	3-40%	4.70682	1.34460	.269	-1.5330	10.9466
	4-0%	-15.59452*	1.34460	.000	-21.8343	-9.3547
	4-5%	-7.65402*	1.34460	.008	-13.8938	-1.4142
	4-10%	-6.66652*	1.34460	.029	-12.9063	-.4267
	4-20%	.56615	1.34460	1.000	-5.6736	6.8059
	4-40%	4.70682	1.34460	.269	-1.5330	10.9466
3-20%	3-0%	-24.61933*	1.34460	.000	-30.8591	-18.3796
	3-5%	-23.92283*	1.34460	.000	-30.1626	-17.6831
	3-10%	-7.90815*	1.34460	.006	-14.1479	-1.6684
	3-40%	-3.20133	1.34460	.759	-9.4411	3.0384
	4-0%	-23.50267*	1.34460	.000	-29.7424	-17.2629
	4-5%	-15.56217*	1.34460	.000	-21.8019	-9.3224
	4-10%	-14.57467*	1.34460	.000	-20.8144	-8.3349
	4-20%	-7.34200*	1.34460	.012	-13.5818	-1.1022
	4-40%	-3.20133	1.34460	.759	-9.4411	3.0384
3-40%	3-0%	-21.41800*	1.34460	.000	-27.6578	-15.1782
	3-5%	-20.72150*	1.34460	.000	-26.9613	-14.4817
	3-10%	-4.70682	1.34460	.269	-10.9466	1.5330
	3-20%	3.20133	1.34460	.759	-3.0384	9.4411
	4-0%	-20.30133*	1.34460	.000	-26.5411	-14.0616
	4-5%	-12.36083*	1.34460	.000	-18.6006	-6.1211
	4-10%	-11.37333*	1.34460	.000	-17.6131	-5.1336
	4-20%	-4.14067	1.34460	.435	-10.3804	2.0991
	4-40%	.00000	1.34460	1.000	-6.2398	6.2398
4-0%	3-0%	-1.11667	1.34460	1.000	-7.3564	5.1231
	3-5%	-.42017	1.34460	1.000	-6.6599	5.8196
	3-10%	15.59452*	1.34460	.000	9.3547	21.8343
	3-20%	23.50267*	1.34460	.000	17.2629	29.7424
	3-40%	20.30133*	1.34460	.000	14.0616	26.5411
	4-5%	7.94050*	1.34460	.006	1.7007	14.1803
	4-10%	8.92800*	1.34460	.001	2.6882	15.1678
	4-20%	16.16067*	1.34460	.000	9.9209	22.4004
	4-40%	20.30133*	1.34460	.000	14.0616	26.5411
4-5%	3-0%	-9.05717*	1.34460	.001	-15.2969	-2.8174
	3-5%	-8.36067*	1.34460	.003	-14.6004	-2.1209
	3-10%	7.65402*	1.34460	.008	1.4142	13.8938
	3-20%	15.56217*	1.34460	.000	9.3224	21.8019
	3-40%	12.36083*	1.34460	.000	6.1211	18.6006
	4-0%	-7.94050*	1.34460	.006	-14.1803	-1.7007
	4-10%	.98750	1.34460	1.000	-5.2523	7.2273
	4-20%	8.22017*	1.34460	.004	1.9804	14.4599
	4-40%	12.36083*	1.34460	.000	6.1211	18.6006
4-10%	3-0%	-10.04467*	1.34460	.000	-16.2844	-3.8049
	3-5%	-9.34817*	1.34460	.001	-15.5879	-3.1084
	3-10%	6.66652*	1.34460	.029	.4267	12.9063
	3-20%	14.57467*	1.34460	.000	8.3349	20.8144
	3-40%	11.37333*	1.34460	.000	5.1336	17.6131
	4-0%	-8.92800*	1.34460	.001	-15.1678	-2.6882
	4-5%	-.98750	1.34460	1.000	-7.2273	5.2523
	4-20%	7.23267*	1.34460	.014	.9929	13.4724
	4-40%	11.37333*	1.34460	.000	5.1336	17.6131
4-20%	3-0%	-17.27733*	1.34460	.000	-23.5171	-11.0376
	3-5%	-16.58083*	1.34460	.000	-22.8206	-10.3411
	3-10%	-.56615	1.34460	1.000	-6.8059	5.6736
	3-20%	7.34200*	1.34460	.012	1.1022	13.5818
	3-40%	4.14067	1.34460	.435	-2.0991	10.3804
	4-0%	-16.16067*	1.34460	.000	-22.4004	-9.9209
	4-5%	-8.22017*	1.34460	.004	-14.4599	-1.9804
	4-10%	-7.23267*	1.34460	.014	-13.4724	-.9929
	4-40%	4.14067	1.34460	.435	-2.0991	10.3804
4-40%	3-0%	-21.41800*	1.34460	.000	-27.6578	-15.1782
	3-5%	-20.72150*	1.34460	.000	-26.9613	-14.4817
	3-10%	-4.70682	1.34460	.269	-10.9466	1.5330
	3-20%	3.20133	1.34460	.759	-3.0384	9.4411
	3-40%	.00000	1.34460	1.000	-6.2398	6.2398
	4-0%	-20.30133*	1.34460	.000	-26.5411	-14.0616
	4-5%	-12.36083*	1.34460	.000	-18.6006	-6.1211
	4-10%	-11.37333*	1.34460	.000	-17.6131	-5.1336
	4-20%	-4.14067	1.34460	.435	-10.3804	2.0991

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

Table f52 The result of One-Way ANOVA of release rate of 5% MTZ from **ER_SP_vW-3, ER_SP_vW-4**, **$ER_{SL}P_vW$-1** and **$ER_{SL}P_vW$-2**

ANOVA

Spread

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.010	3	.003	1.047	.423
Within Groups	.026	8	.003		
Total	.036	11			

Multiple Comparisons

Dependent Variable: Spread

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PG3	PG4	-.05307	.04660	.736	-.2158	.1097
	PGL1	-.04370	.04660	.829	-.2065	.1191
	PGL2	.01680	.04660	.987	-.1460	.1796
PG4	PG3	.05307	.04660	.736	-.1097	.2158
	PGL1	.00937	.04660	.998	-.1534	.1721
	PGL2	.06987	.04660	.553	-.0929	.2326
PGL1	PG3	.04370	.04660	.829	-.1191	.2065
	PG4	-.00937	.04660	.998	-.1721	.1534
	PGL2	.06050	.04660	.655	-.1023	.2233
PGL2	PG3	-.01680	.04660	.987	-.1796	.1460
	PG4	-.06987	.04660	.553	-.2326	.0929
	PGL1	-.06050	.04660	.655	-.2233	.1023

Table f53 The result of One-Way ANOVA of release rate of 5, 10, 20 and 40% MTZ from **ER_SP_vW-3** and **ER_SP_vW-4**

ANOVA

Releaserate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	216.896	5	43.379	4965.947	.000
Within Groups	.105	12	.009		
Total	217.001	17			

Multiple Comparisons

Dependent Variable: Releaserate

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
3-10	3-20	5.41657*	.07631	.000	5.1158	5.7173
	3-40	9.14180*	.07631	.000	8.8411	9.4425
	4-10	1.33033*	.07631	.000	1.0296	1.6311
	4-20	5.33893*	.07631	.000	5.0382	5.6397
	4-40	9.04997*	.07631	.000	8.7492	9.3507
3-20	3-10	-5.41657*	.07631	.000	-5.7173	-5.1158
	3-40	3.72523*	.07631	.000	3.4245	4.0260
	4-10	-4.08623*	.07631	.000	-4.3870	-3.7855
	4-20	-.07763	.07631	.953	-.3784	.2231
	4-40	3.63340*	.07631	.000	3.3327	3.9341
3-40	3-10	-9.14180*	.07631	.000	-9.4425	-8.8411
	3-20	-3.72523*	.07631	.000	-4.0260	-3.4245
	4-10	-7.81147*	.07631	.000	-8.1122	-7.5107
	4-20	-3.80287*	.07631	.000	-4.1036	-3.5021
	4-40	-.09183	.07631	.910	-.3926	.2089
4-10	3-10	-1.33033*	.07631	.000	-1.6311	-1.0296
	3-20	4.08623*	.07631	.000	3.7855	4.3870
	3-40	7.81147*	.07631	.000	7.5107	8.1122
	4-20	4.00860*	.07631	.000	3.7079	4.3093
	4-40	7.71963*	.07631	.000	7.4189	8.0204
4-20	3-10	-5.33893*	.07631	.000	-5.6397	-5.0382
	3-20	.07763	.07631	.953	-.2231	.3784
	3-40	3.80287*	.07631	.000	3.5021	4.1036
	4-10	-4.00860*	.07631	.000	-4.3093	-3.7079
	4-40	3.71103*	.07631	.000	3.4103	4.0118
4-40	3-10	-9.04997*	.07631	.000	-9.3507	-8.7492
	3-20	-3.63340*	.07631	.000	-3.9341	-3.3327
	3-40	.09183	.07631	.910	-.2089	.3926
	4-10	-7.71963*	.07631	.000	-8.0204	-7.4189
	4-20	-3.71103*	.07631	.000	-4.0118	-3.4103

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

Table f54 The result of One-Way ANOVA of inhibition clear zone of **ER_SP_vW-3**, **ER_SP_vW-4**, **$ER_{SL}P_vW$-1** and **$ER_{SL}P_vW$-2**

ANOVA

Diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	3	.001	1.000	.479
Within Groups	.005	4	.001		
Total	.009	7			

Multiple Comparisons

Dependent Variable: Diameter

Scheffe

(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RS/RL 25:0	RS/RL 22.5:2.5	.05000	.03536	.615	-.1072	.2072
	RS/RL 20:5	.05000	.03536	.615	-.1072	.2072
	EC15	.05000	.03536	.615	-.1072	.2072
RS/RL 22.5:2.5	RS/RL 25:0	-.05000	.03536	.615	-.2072	.1072
	RS/RL 20:5	.00000	.03536	1.000	-.1572	.1572
	EC15	.00000	.03536	1.000	-.1572	.1572
RS/RL 20:5	RS/RL 25:0	-.05000	.03536	.615	-.2072	.1072
	RS/RL 22.5:2.5	.00000	.03536	1.000	-.1572	.1572
	EC15	.00000	.03536	1.000	-.1572	.1572
EC15	RS/RL 25:0	-.05000	.03536	.615	-.2072	.1072
	RS/RL 22.5:2.5	.00000	.03536	1.000	-.1572	.1572
	RS/RL 20:5	.00000	.03536	1.000	-.1572	.1572

BIOGRAPHY

Mr. Alongkot Sangchanchai was born on June 10th, 1982 in Mukdahan, Thailand. He had received Prasit-Khunying Pattana Urirat's scholarship and graduated from Rangsit University in 2005 with a degree in Pharmacy (First Class Honours). During his study at Chulalongkorn University, he firstly had poster presentation on the topic of "Formulation Development of Periodontal Gel Base to control Metronidazole Release for Periodontitis Patients" at Asean Sciencetific Conference in Parmaceutical Technology 2008 at the University Sains Malaysia, and later had a presentation and publication at The 34th Congress on Science and Technology of Thailand, King Mongkut's Institute of Technology Ladkrabang on October 30th , 2008.