การพัฒนาแผ่นปิดแผลในช่องปากของยาไทรแอมซิโนโลนอะเซตโตไนด์ด้วย ไฮดรอกซีโพรพิลเมทิล เซลลูโลส โดยทดสอบคุณสมบัติการยึดติดและการปล่อยยาในห้องปฏิบัติการ

นางสาวเปรมฤดี ศรีสุนทร

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HPMC BASED MUCOADHESIVE FOR DELIVERY OF TRIAMCINOLONE ACETONIDE: MUCOADHESION AND DRUG RELEASE PROPERTIES, AN *IN VITRO* STUDY

Miss Premrudee Srisuntorn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Oral Medicine
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Thesis Title	HPMC BASED MUCOADHESIVE FOR DELIVERY OF TRIAMCINOLONE ACETONIDE: MUCOADHESION AND DRUG RELEASE PROPERTIES, AN *IN VITRO* STUDY
By	Miss Premrudee Srisuntorn
Field of Study	Oral Medicine
Thesis Advisor	Assistant Professor Kanokporn Bhalang, Ph.D.
Thesis Co-Advisor	Assistant Professor Pratanporn Arirachakaran, Ph.D.

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

.....Dean of the Faculty of Dentistry
(Assistant Professor Suchit Poolthong, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Pornpan Piboonratanakit, Ph.D.)

.....Thesis Advisor
(Assistant Professor Kanokporn Bhalang, Ph.D.)

.....Thesis Co-Advisor
(Assistant Professor Pratanporn Arirachakaran, Ph.D.)

.....Examiner
(Associate Professor Chairat Wiwatwarrapan)

.....External Examiner
(Assistant Professor Paramaporn Chiewpattanakul Kaewmanee, Ph.D.)

เปรมฤดี ศรีสุนทร : การพัฒนาแผ่นปิดแผลในช่องปากของยาไทรแอมซิโนโลนอะเซตโตไนด์ด้วย ไฮดรอกซีโพรพิลเมทิลเซลลูโลส โดยทดสอบคุณสมบัติการยึดติดและการปล่อยยาในห้องปฏิบัติการ (HPMC BASED MUCOADHESIVE FOR DELIVERY OF TRIAMCINOLONE ACETONIDE: MUCOADHESION AND DRUG RELEASE PROPERTIES, AN *IN VITRO* STUDY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทญ. ดร.กนกพร พะลัง, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ทญ. ดร.ประทานพร อารีราชการัณย์, 63 หน้า.

ยาไทรแอมซิโนโลน อะเซตโตไนด์ถือเป็นยาตัวแรกที่ใช้รักษารอยโรคที่เกี่ยวข้องกับการอักเสบภายในช่องปาก โดยที่ความเข้มข้นร้อยละ 0.1 เป็นความเข้มข้นที่ยาที่ประสิทธิภาพทางการรักษาดีที่สุด ส่วนการรักษาการอักเสบภายในช่องปากนั้นจะใช้ยาทาเฉพาะที่ แต่พบว่ายาทามีความสามารถยึดติดอยู่บริเวณรอยโรคต่ำ จึงมีการพัฒนาสารโพลีเมอร์เพิ่มการยึดติดกับเนื้อเยื่อ ในการศึกษานี้ใช้ไฮดรอกซีโพรพิลเมทิลเซลลูโลส ซึ่งเป็นโพลีเมอร์ที่นิยมใช้ทั่วไปในอาหารและยา เพื่อใช้เป็นสารเพิ่มการยึดติดช่วยให้ยาสัมผัสกับเนื้อเยื่อที่มีการอักเสบได้นานขึ้น

วัตถุประสงค์การวิจัย เพื่อพัฒนาแผ่นปิดแผลในช่องปากของยาไทรแอมซิโนโลน อะเซตโตไนด์ที่ความเข้มข้นร้อยละ 0.1 ด้วยไฮดรอกซีโพรพิลเมทิลเซลลูโลส โดยการทดสอบคุณสมบัติการละลาย การดูดน้ำ การยึดติดและการปล่อยยาเปรียบเทียบกับแผ่นปิดแผลตามท้องตลาดในห้องปฏิบัติการ

ผลการวิจัย พบว่าแผ่นปิดแผลในช่องปากที่ได้พัฒนาที่ความเข้มข้นร้อยละ 3 และ 2 ของไฮดรอกซีโพรพิลเมทิลเซลลูโลส ละลายช้ากว่าแผ่นปิดแผลที่ขายตามท้องตลาดแตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 โดยแผ่นที่ละลายช้าสุดคือ ร้อยละ 3 ละลายที่ (7.11 ± 0.68) ชั่วโมง ในทุกความเข้มข้นของแผ่นปิดแผลที่พัฒนามีคุณสมบัติในการดูดน้ำมากกว่าแผ่นปิดแผลที่ขายตามท้องตลาดในช่วงเวลา 1 และ 5 นาที โดยที่ความเข้มข้นของแผ่นปิดแผลร้อยละ 3 และ 2 ดูดน้ำมากกว่าแผ่นปิดแผลที่ขายตามท้องตลาดที่ 10 และ 30 นาที แตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 แต่ในทุกกลุ่มตัวอย่างแผ่นปิดแผลไม่มีความแตกต่างทางสถิติในด้านการยึดติด อีกทั้งไม่มีความแตกต่างกันในด้านการปล่อยยาระหว่างแผ่นปิดแผลที่พัฒนากับแผลปิดแผลที่ขายตามท้องตลาดในทุกๆ เวลาที่ทดสอบ โดยที่แผ่นความเข้มข้นร้อยละ 3 ปล่อยยาได้มากที่สุดในกลุ่ม แผ่นความเข้มข้นร้อยละ 3 ปล่อยยาได้มากกว่าแผ่นตามท้องตลาดอย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 ที่เวลา 2 ชั่วโมง และแผ่นปิดแผลที่ความเข้มข้นร้อยละ 3 ปล่อยยามากกว่าแผ่นร้อยละ 1 ที่เวลา 2, 4 และ 6 ชั่วโมง อย่างมีนัยสำคัญทางสถิติที่ระดับ 0.05 โดยทุกการทดสอบทำในห้องปฏิบัติการ

สรุปผลวิจัย แผ่นปิดแผลในช่องปากที่ได้พัฒนามีคุณสมบัติที่ดีเมื่อเปรียบเทียบกับแผ่นปิดแผลที่ขายตามท้องตลาด โดยเฉพาะแผ่นที่มีความเข้มข้นของไฮดรอกซีโพรพิลเมทิลเซลลูโลสร้อยละ 3 ที่ละลายช้าและมีปริมาณการปล่อยยาได้มากกว่าในช่วง 2 ถึง 10 ชั่วโมง สามารถช่วยเพิ่มประสิทธิภาพในการรักษาได้ โดยสามารถปรับปรุงคุณสมบัติบางประการให้ดีขึ้นได้ในอนาคต

ภาควิชา เวชศาสตร์ช่องปาก	ลายมือชื่อนิสิต .....
สาขาวิชา เวชศาสตร์ช่องปาก	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ปีการศึกษา 2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5775814032 : MAJOR ORAL MEDICINE
KEYWORDS: HYDROXYPROPYLMETHYLCELLULOSE (HPMC) / TRIAMCINOLONE ACETONIDE / MUCO-ADHESIVE / MUCO-ADHESION / BUCCAL PATCH

PREMRUDEE SRISUNTORN: HPMC BASED MUCOADHESIVE FOR DELIVERY OF TRIAMCINOLONE ACETONIDE: MUCOADHESION AND DRUG RELEASE PROPERTIES, AN *IN VITRO* STUDY. ADVISOR: ASST. PROF. KANOKPORN BHALANG, Ph.D., CO-ADVISOR: ASST. PROF. PRATANPORN ARIRACHAKARAN, Ph.D., 63 pp.

Triamcinolone acetonide (TA) is the first-line drug for treating inflammatory oral lesions, 0.1% being the most effective concentration. However, conventional topical TA applications are poorly retained on the oral mucosa. Hydroxypropyl methylcellulose (HPMC) polymer patches are widely used as buccal mucosa drug delivery systems, as they enhance a drug's ability to adhere to the oral mucosa and reduces the frequency and amount of drug application.

The objective of this study was to prepare a new HPMC-based buccal muco-adhesive polymer patch for the delivery of 0.1% TA. The solubility, water absorption, muco-adhesion and *in vitro* drug release study using high-performance liquid chromatography (HPLC) were compared with a commercial product.

The results revealed that the 3% and 2% HPMC patches had significantly lower dissolution rates, a favorable property, compared with that of the commercial product ($p<0.05$). The 3% HPMC group demonstrated the highest dissolution time of (7.11 ± 0.68) hours. Every concentration of the newly developed muco-adhesive polymer patches had higher water absorption than that of the commercial patches at 1 and 5 minutes. In addition, the 3% and 2% HPMC patches demonstrated significantly higher water absorption compared with the commercial patches at 10 and 30 minutes. There was no significant difference in muco-adhesion between the developed patches with commercial product. All HPMC groups did not show significantly higher drug release compared with the commercial product group at every time point. 3% HPMC group had the highest drug release profiles. 3% HPMC had significantly higher than the commercial product at 2 hours ($p<0.05$). The 3% HPMC group had significantly higher drug release than 1% HPMC at 2,4 and 6 hours ($p<0.05$).

We demonstrated the potential of a buccal muco-adhesive polymer patch as an alternative treatment for oral ulcerations. The buccal mucosa patches had a higher dissolution time compared with the commercial product. The 3% HPMC had lower dissolving and higher drug release at 2 to 10 hours. The newly developed muco-adhesive polymer patches had improved properties pertaining to drug application. Further study is needed to improve some of the properties of the oral patches and to implement a clinical study.

Department:	Oral Medicine	Student's Signature	.....
Field of Study:	Oral Medicine	Advisor's Signature	.....
Academic Year:	2016	Co-Advisor's Signature	.....

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Introduction

During the past several years, advances in pharmaceutical technology of drug formulations and innovative routes of administration have been investigated. recurrent aphthous ulcer (RAU) and oral lichen planus (OLP) are common painful mucosal conditions affecting the oral cavity (1). The diagnosis of RAU is based on patient history and clinical manifestations. There is no specific diagnostic test. The underlying etiology remains unclear, and no curative treatment is available (2). OLP is a chronic inflammatory cell-mediated immunological dysregulation. OLP is likely of multifactorial origin or non-specific etiology (3). The lesions are often asymptomatic but atrophic, erosive and ulcerative forms of OLP can cause burning sensation or severe pain (3). The diagnosis of OLP is based on the histopathological results of biopsied specimens.

Corticosteroids are frequently used for the treatment of RAU and OLP by most clinicians. Triamcinolone acetonide (TA) is the first line of drug for the treatment of RAU. It can be administered in the form of orabase or mouthwash with concentrations ranging from 0.05-0.5%, applied 3-5 times per day (2). It is particularly indicated in patients with small lesions. A study reported that the most effective concentration to be was 0.1% (2). Although these forms provide high drug levels in the oral cavity, they can be washed out easily from the applied region due to the physiological removing mechanisms like washing effect of the saliva, swallowing and

tongue movement. Therefore, the therapeutic drug level decreases (4-7). The conventional topical drug of TA applications are poorly retained on the oral mucosa. Muco-adhesive polymers have extensively been employed in buccal drug delivery systems. They enhance drug's ability to adhere onto oral mucosa. They can be easily dispersed throughout the mucosa, and are able to form contact with the mucosal membrane and providing high patient compliance due to their non-irritable characteristics (8) . Polymers that have been investigated are polyacrylates, ethylene vinyl alcohol, polyethylene oxide, poly alcohol, poly (N-acryloylpyrrolidine), polyoxyethylenes, self-cross-linked gelatin, sodium alginate, natural gum sand cellulose ethers like methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose (HPMC), and sodium carboxymethylcellulose (9, 10) .

Muco-adhesive HPMC is commonly known as hypromellose. It is non-toxic and is used in a wide variety of pharmaceutical and food preparations. HPMC is a cellulose ether and is one of the most common hydrophilic carriers used in controlled oral drug delivery systems, due to its swelling ability when in contact with water or fluid (11-16). It is produced by synthetic modification of the naturally occurring polymer cellulose and is considered safe for normal consumption in humans. Its applications as a viscolizing agent, coating polymer, bio-adhesive, binder in the process of granulation and in modified release formulations have been well documented (17). Muco-adhesive of carbopol/poloxamer/HPMC film formulation has been tested. They found the adhesive force increased with increasing HPMC content

in the film, and release of TA from TA-loaded carbopol/poloxamer/HPMC polymer film *in vitro* increased with increasing loading content of the drug (13) . A preparation with 2.5% HPMC K 100M, at viscosity range of 80-120 cps, has been reported as the most appropriate formulation for buccal application because it was suitable mechanical properties, as well as exhibits high cohesion and bio-adhesion (11).

In a past study, gel formulations containing TA were prepared by using poloxamer 407, carbopol 934, chitosan and HPMC. All the developed formulations were compared with commercial product containing 0.1% TA (Kenacort-A Orabase®). It was observed that the bio-adhesive properties of the formulations depend on the bio-adhesive polymer concentration and molecular weight of chitosan. The bio-adhesive performance of the chitosan based formulations was improved with HPMC. Texture profile analysis (TPA) results indicated that mechanical properties of the developed gels were more favorable than the commercial product (4).

In another previous study, buccal muco-adhesive polymer base of 1% HPMC with 0.06 mm thickness without TA has been reported as the best formulation ($p<0.05$). It showed higher muco-adhesive force and limited water absorption which were 333.33% of weight at 10 minutes. The patches were averagely dissolved 203.8 minutes (18). In development of buccal healing film containing TA, a study revealed that the proper components were 2% methycellulose, 0.1% glycerine and 3% HPMC (19). As today in 2015, oral paste formulation of TA containing 60% plastibase, 3.3%

pectin, 6.6% gelatin and 30% carboxymethylcellulose showed similar characteristics compared with reference formulation (Adcortyl®) for the treatment of RAU (20).

In accordance with the market, imported commercial product had about 29.16 – 40.83 average THB per patch. The developed patches were cheaper than the commercial patches because HPMC and TA without package were lower costs. If the patches demonstrated an acceptable *in vitro* properties, they could be used to produce a buccal muco-adhesive polymer patch as an alternative treatment for oral ulcers.

Up to the present*, In vivo* study has been performed on healthy volunteers to check the acceptability of the patch of diclofenac sodium. They did not cause any irritation and dryness of mouth but they were found to be slightly bitter taste of the drug (21). This results displayed the buccal patch form was interesting.

The main objective of this study was to prepare a HPMC based muco-adhesive for delivery of TA that possesses appropriate dissolving time, water absorption, muco-adhesion and drug release properties compared with the commercial patches.

Review of literatures

Human oral mucosa

Oral cavity is lined by mucous membrane consisting of a stratified squamous epithelium, which may or may not be keratinized. There is some varieties in the type of epithelium present. As a consequence, several regions are usually different from one another. Stratified squamous epithelium can be classified in four types:

1. Masticatory mucosa consists of collagenous connective tissue with keratinized epithelium involving gingival and palatal areas.

2. Lining mucosa is non-keratinized epithelium found in most areas of oral mucosa except the dorsal surface of the tongue, gingival and palatal areas.

3. Specialized mucosa found on the dorsal surface of the tongue, which may or may not be keratinized epithelium.

4. Transitional zone mucosa is thin and keratinized and found the vermillion zone.

The underlying connective tissue layer, or lamina propria is separated from the epithelium by a basement membrane which is similar in structure and composition to the dermis of the skin. For long-term drug delivery, the buccal area is the most appropriate because it consists of dense smooth muscle and non-

keratinized epithelium. Buccal mucosa is drier than other areas of the oral cavity(22) . Lesions are commonly found in this area. As well. However, the muco-adhesion can also be applied at other affected area of oral mucosa up to design of buccal patch. The patch dissolves by itself in time, so do not peel it off. If fungal infection was found, will treat the infection before (23).

Mucus layer

Mucus layer is a translucent and viscid secretion which forms a thin, continuous gel blanket on the mucosal epithelial surface. The mean thickness of this layer varies from about 50 to 450 μm in humans (8, 24) which is determined by the balance between the rate of secretion and the rate of degradation and shedding, and is site dependent (25). This layer may actually play a role in adhesion and act as a lubricant. Mucus layer is composed chiefly of mucins and inorganic salts. Mucins contain approximately 95% water, 0.5-5% glycoproteins and lipids, 1% mineral salts and up to 1% free proteins. Mucin glycoproteins are high molecular weight proteins that can be attached to oligosaccharide units. The mucus layer, which covers the epithelial surface, has various function including protection resulting particularly from its hydrophobicity, barrier involve absorption of drugs or other substrates, as it influences the bioavailability of drugs, adhesion (mucus has strong cohesional properties and firmly binds to the epithelial cell surface as a continuous gel layer), and lubrication to keep the mucosal membrane moist in the oral cavity (24, 26, 27).

Hydroxypropyl methylcellulose (HPMC)

Muco-adhesive HPMC is commonly known as 'hypromellose', is non-toxic used in a wide variety of pharmaceutical and food preparations. This polymer is produced by the synthetic modification of naturally occurring polymer cellulose and is safe for human use (28) . HPMC is a cellulose ether water-soluble polymers and is one of the most common hydrophilic carriers used in controlled oral drug delivery systems, due to its ability to swell when contacted to fluid. These polymers work well with soluble and insoluble drugs and at high and low dosage levels (28). HPMC offers a wide range of properties that would enhance adhesion to the mucosa, which turn increases the contact time of the drug with oral mucosa (28). The uses of HPMC as a thickening agent and a bio-adhesive are well documented .HPMC polymers are nonionic, are stable pH range 3.0–11.0 and enzyme resistant (29) so they minimize interaction surrounding environment as oral cavity. Moreover, HPMC promotes a strong and tight gel formation compared to other cellulosics. As a result, drug-release rates have been sustained longer with HPMC than with equivalent levels of methylcellulose (MC), hydroxyethylcellulose (HEC), or carboxymethylcellulose (CMC) (28). For these reasons, HPMC is very often the polymer of choice over other cellulosics. The nomenclature of HPMC concentration identifies the chemistry and viscosity of the product in millipascal-seconds (mPa·s) measured at 2% concentration in water at 20°C. Increasing gel temperature causes loss hydrating water that leads to a decrease in relative viscosity (29). Several different suffixes in the nomenclature are

also used to identify special products that represent their properties. To improve HPMC's properties, glycerin has been added to the formula because of its moisturizing and emollient properties in this study (30) .

The addition of glycerin into the formulation make the patch softer and improve elasticity (31) . The research study on design and evaluation of buccal patch of diclofenac sodium showed that polyvinyl alcohol (PVA) patches can be used for fast release whereas HPMC patches can be used for the sustained release of the drug (21).

Triamcinolone Acetonide (TA)

Triamcinolone acetonide is a long acting synthetic glucocorticoid similar to a natural hormone produced by adrenal glands (32). It is a medium to high potency corticosteroid, a fluorinated prednisolone derivative and considered an intermediate-acting glucocorticoid. It is effective in the treatment of dermatoses, asthma and allergic rhinitis and is used in the treatment of the signs and symptoms of many oral inflammatory conditions, including RAU (20). It can be administered on oral mucosa as an oral base when the lesions are localized in nature, or in oral rinse form when the lesions are diffuse or numerous in the oral cavity. Clinicians frequently use corticosteroids for the treatment of RAU and OLP (20, 33). TA is the first-line drug for treating RAU, and can be administered in the form of orabase or mouthwash with concentrations ranging from 0.05–0.5%, applied 3-5 times per day (2). It is par-

ticularly indicated in patients with small and mild oral lesions. The most effective concentration was reported to be 0.1% (2). In order to promote wound healing, it must be applied directly onto the lesions, keeping it in direct contact as long as possible, with no eating or drinking for 20 minutes after application. For oral rinse, it should be used for the indicated period of time without swallowing (2). Although the conventional topical drug forms provide high drug levels in the oral cavity, they also have limitations, including low retention on the oral mucosa (4, 5, 7, 34).They can be easily displaced from the applied region due to the washing effect of saliva, swallowing, and tongue movements. These effects decrease therapeutic drug levels, so, developing a muco-adhesive patch as a drug carrier, would enhance drug delivery systems by increasing drug's ability to adhere and drug delivery onto oral mucosa (35).

Muco-adhesive systems

Muco-adhesive polymers play an important role in drug delivery. Muco-adhesive systems are derived from natural or synthetic substances and can also adhere to specific sites of oral mucosa. These polymers can be used as carriers for drug delivery because of the adhesion between polymers and mucosal surfaces, which increases the contact time of the drug with the mucosal surface. Thus, drug release and absorption are increased on the mucosa. Muco-adhesive polymers have

extensively been employed in buccal drug delivery systems to enhance the drugs' ability to adhere onto oral mucosa (24, 35, 36).

Bio-adhesion term has the same meaning as muco-adhesive systems, defined in 1986, as attachment of a synthetic or natural macromolecules to mucus or an epithelial surface (4, 5, 24). Many bio-adhesives are made by synthetic or natural polymers. Different types of chemical bonds such as covalent bonds, hydrogen bonds, ionic bonds and van der Waals bonds, can develop bio-adhesion between polymer and biological surface (4, 34, 36).

Mechanisms of muco-adhesion are generally divided into two steps: the contact stage and the consolidation stage. The first stage is characterized by the contact between the muco-adhesive polymer based patches and the mucosa, with spreading and swelling of the formulation(35) . After contact is established, penetration of the muco-adhesive into the mucosal surface or penetration of the chains of the muco-adhesive with those of mucosa takes place (Figure 1). Muco-adhesion can be explained based on molecular interactions. However, several theories have suggested that it. The wetting theory described the ability of a bio-adhesive polymer directly depended on spreading coefficients (27). Contact angle should be equal or close to zero to provide adequate the spread ability for increasing adhesion. Diffusion theory described the penetration depended on the diffusion coefficient, flexibility of polymer chains, mobility and contact time. The penetration of polymer chains into the mucus network or mucosa surface increases

adhesive strength (35). Also electronic and adsorption theories described adhesion occurring by electron transfer and/or chemical interactions, hydrophobic, hydrogen bonding, and van der Waals interactions between adhesive polymer and mucosa (37) . The interaction between two molecules is consists of attraction and repulsion which arises from van der Waals forces, electrostatic attractions, hydrogen bonding and hydrophobic interactions. Repulsive interactions occur because of electrostatic and steric repulsion. For muco-adhesion to occur, the attractive interaction should be larger than the non-specific repulsion (24, 35, 36).

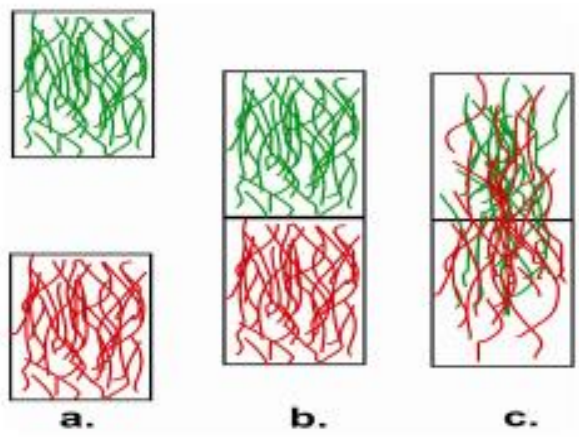


Figure 1. The muco-adhesive polymer chains and mucosa surface (a), The contact between the muco-adhesive polymer chains and the mucosa (b), Penetration of polymer chains into the mucus network or mucosa surface (c) (37)**.**

There are several advantages of oral muco-adhesive drug delivery systems (35) . They prolong the retention time of the dosage form at the site of absorption, hence increases the bioavailability. They have excellent accessibility and can be

administered to unconscious patients. They have rapid absorption because of enormous blood supply, good blood flow rates and controlled drug release. They permit localization of the drug, and the drug is protected from degradation. They have high drug loading capacity which can improve patient respondent and compliance (21, 27). It may possibly be used as an alternative treatment for oral lesion in laboratory testing.

Factors that can affecting muco-adhesion are physical characteristics of the patches such as thickness, weight, dissolution, elasticity and appearance. They should be evaluated to determine suitable concentrations and amounts of substrates. Others factors that influence muco-adhesion and the drug delivery generally involve (35):

Polymer-related factors

Molecular weight: High molecular weight results in superior adhesive property. Muco-adhesiveness improves with increasing molecular weight. Moreover, molecular weight plays an important role in water absorption. The water absorption rates following oral administrations of low molecular weight is much greater than high molecular weight (27).

Hydrophilicity: Desired polymers must have hydrophilic functional groups, such as hydroxyl and carboxyl. These groups allow hydrogen bonding with mucosa, swelling in aqueous media, thereby allowing maximal muco-adhesion. Flexibility of the polymer is important to improve hydrogen bond. In addition, swollen polymers

have the maximum distance between their chains leading to increased chain flexibility and efficient penetration of polymer (24, 38).

Charge of the muco-adhesive polymer: Cationic and anionic polymers bind more effectively than neutral polymers. Especially, lipophilic molecules are more permeable across cellular barriers because of the oil-water partition coefficient. Thus, lipophilic molecules can store drugs and slowly release them and would be more flexible for penetrating into the mucosa. However there is no significant about the influence of the charges on muco-adhesion but pH affects muco-adhesion as it can influence the ionized or un-ionized forms of the polymers that it is pH of polymer–substrate interface issue (27, 35).

Flexibility, cross-linking density and concentration of polymer: The increased chain interpenetration is attributed to the increased structural flexibility of the polymer. Sufficient swelling of the polymer increase flexibility. However, a critical degree of hydration of the muco-adhesive polymer exists optimum swelling. Additionally, proper increased cross-linking density of the polymer will increase muco-adhesive property. The increased cross-linking density is attributed to the increased concentration or molecular weight of the polymer. In general, the more concentrated polymer will increases muco-adhesion due to a longer penetrating chain length. Therefore, the suitable polymer provides high muco-adhesion but if the polymer is over loaded concentration, it will increases cross-linking density until loss of flexibility and muco-adhesion, respectively (27, 35).

Environmental factors

Salivary turnover: High turnover limits the residence time of the muco-adhesive on the mucosal layer. No matter how high the muco-adhesive strength, they can be easily detached from the surface due to salivary turnover (27).

pH: The pH can influence the formal charge on the surface of the mucus as well as that of certain ionizable muco-adhesive polymers. Some studies have shown that the pH of the medium is important for the degree of hydration of cross-linked polymers, showing consistently increased hydration from pH 4 through pH 7, and a decreased hydration at alkaline pH levels (39) . It can measure with litmus paper to control this factor. Because METHOCEL products are nonionic, the viscosity of their solutions is generally stable over a wider pH range than are the viscosities of polymers that are ionic in nature (28).

Initial contact time and contact force: Contact time and force between the polymer and mucosa surface. Muco-adhesive strength increases when the contact time or force increases (27).

Physical and chemical properties of substrate agents

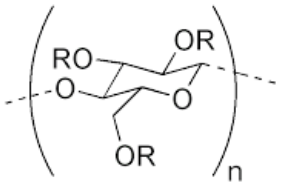
1. Hydroxypropyl methylcellulose (HPMC) (40)

Synonyms cellulose, hydroxypropyl methyl ether, methylcellulose propylene glycol ether, methyl hydroxypropylcellulose, culminal MHPC, Methocel

Molecular formula $CH_2CH(OH)CH_3$

Molecular weight Depend on type of HPMC, in this study used Methocel F4M (average molecular weight of 8.6 x10^4 Da)

Structure They have the polymeric backbone of cellulose, a natural carbohydrate that contains a basic repeating structure of anhydroglucose units.



R = H or CH_3 or $CH_2CH(OH)CH_3$

Properties (41)

Appearance: White to off-white or cream colored fine to coarse powder with odorless and tasteless. It is nonionic, minimize interaction problems when used inacidic, basic, or other electrolytic systems.

Gelation temperature (2% aqueous solution): 48-70°C (54 °C for Methocel F4M) (28)

Bulk density: 0.5-0.70 g/cm^3

Real specific gravity: 1.26-1.31

Surface tension (0.1% solution at 20 °C): 45-55 mN/m (dyn/cm)

Interfacial tension (0.1% solution versus paraffin oil at 25 °C): 17-29 mN/m (dyn/cm)

The texture and the strength of gel produced by METHOCEL products varies with the type, viscosity grade, and concentration of METHOCEL used. In general, the strength of the gel increases with increasing molecular weight. However, gel strength may level off at molecular weights greater than approximately 150 kDa (28)

pH value (0.1% solution at 20 ºC): 5.5-8.0

pH stability: 2-11

Application (41)

- Pharmaceuticals**:** To be used as densifiers, emulsifying and dispersing agents for ointment and creams. It's used as an adhesive coating agent for a tablet which control released matrix and eye drop reagent for hard contact lens. In a past of study, examining the effect of substitution on release rate from hydrophilic matrix tablets, high viscosity grade results in the slowest release compared to other polymers of similar molecular weight.
- Plastic bandages: Food & Whip-topping as effective emulsification reagent, adhesive, thickening reagent as well as can be used as packing materials.
- Construction and industry: To be used as ingredient of compounds, tile cements and grouts, etc.

Package, storage and transportation

It is kept closed box. Direct sunshine, raining, and moisture must be avoided

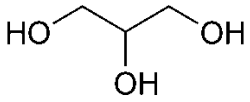
2. Glycerol (42)

Synonyms glycerin, glycerine, propanetriol, 1,2,3-trihydroxypropane.

Molecular formula $C_3H_8O_3$

Molecular weight 92.09 kDa

Structure



Properties (43)

Appearance: A colorless, odorless, viscous liquid with a sweet taste, derived from both natural and petrochemical feedstocks

Density: 1.261 g/cm^3

Viscosity: 1.5 Pa.s

Melting point : 18.2 °C

Boiling point : 290 °C

Food energy: 4.32 kcal/g

Flash Point: 160 1 °C (closed cup)

Surface tension: 64.00 mN/m (dyn/cm)

Application (43)

- Food: It is used as flavoring and coloring product and a solvent.
- Drugs and cosmetics: In personal care products, glycerol serves as an emollient, plasticizer, antimicrobial preservative, humectant, solvent, and

lubricant in an enormous variety of products including toothpaste, mouthwashes, skin care products, shaving cream, hair care products and soaps.

- Wrapping and packaging material: As a plasticizer used in heat casings and special types of papers such as glassine and grease proof paper.
- Tobacco: To retain moisture and prevent drying out of tobacco. It is also used in the processing of chewing tobacco to add sweetness, retain moisture and prevent drying out of tobacco.

Package, storage and transportation

It can be decomposed when heated; thus, it should be kept in a closed and sealed container (43).

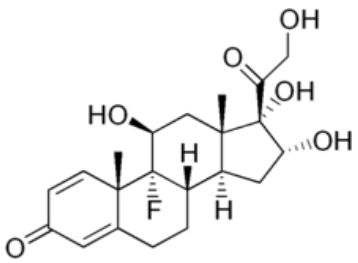
3. Triamcinolone acetonide (44-48)

Synonyms 9α-Fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione 16,17-acetonide, 9α-Fluoro-16α-hydroxy prednisolone 16α,17α-acetonide

Molecular formula $C_{24}H_{31}FO_6$

Molecular weight 434.49 kDa

Structure



Properties (49)

Appearance: White to off-white crystalline powder

Density: 1.33 g/cm^3

Melting point: 274-278 ℃

Boiling point: 576.9 ℃ at 760 mmHg

Refractive index: 1.588

Flash point: 302.7℃

Vapour pressure: 1.04E-15 mmHg at 25 ℃

Solubility: Water, alcohol, dimethyl sulfoxide, propylene glycol, acetone and chloroform.

Application

A synthetic glucocorticoid with anti-inflammatory and immunomodulating properties. The mechanism of action of TA is as a corticosteroid hormone receptor agonist. TA is free alcohol or in esterified form, orally, intramuscularly, local injection, inhalation, or applied topically in the management of various disorders in which corticosteroids are indicated (49).

Utilization forms

- Spray, cream, mouthwash, ointment, paste and tablet (49).

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) or high-pressure liquid chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. It is used to analyze non-volatile organic and semi-volatile organic compounds. Chromatography is an analytical technique based on the separation of molecules due to differences in their structure and/or composition (50). The assay can be described to two phases of its function, called stationary phase or column and mobile phase or solvent. A HPLC system is basically composed of 1) mobile phase 2) a pump 3) an injector or autosampler 4) a column and column oven and 5) detector, as shown in Figure 2.

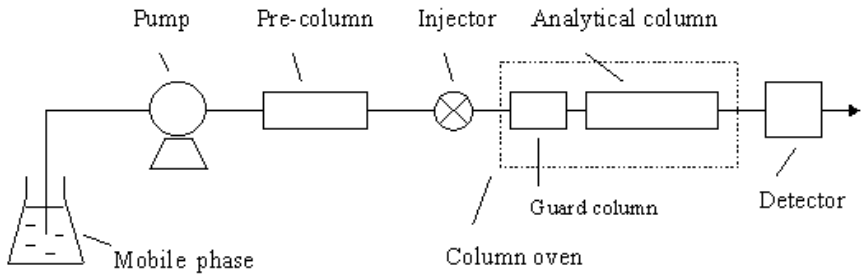


Figure 2. HPLC System (51)

The stationary phase designates is substance in a column and the mobile phase is liquid solvent flowing over the substance into column. Under a certain dynamic condition, each component in a sample has a different distribution

equilibrium depending on solubility in the phases and/or molecular size, structure and chemical property. First step, injector/ autosampler injects a sample into the system. As a result, the components move at different speeds over the stationary phase and are thereby separated from each other. This is the principle behind HPLC. The column is made of a stainless steel or resin tube which is packed with spherical solid particles. Mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from a sample injector, located near the column inlet. The injected sample enters the column with the mobile phase and the components in the sample migrate through it, passing between the stationary and mobile phases. Compounds move in the column only when they are in the mobile phase. Compounds that tend to be distributed in the mobile phase therefore migrate faster through the column while compounds that tend to be distributed in the stationary phase migrate slower. In this way, each component is separated on the column and sequentially elutes from the outlet. Each compound eluting from the column is detected by a detector connected to the outlet of the column.

When the separation process is monitored by the recorder, a graph is obtained. This graph is called a chromatogram. The time required for a compound to elute that called retention time. The relationship between compound concentration or amount and peak area depend on the characteristics of the compound. Retention

time is used as an index for qualitative determination and peak surface area as an index for quantitative determination. The retention time of the target compounds and the concentration for each unit of peak area are based on data obtained by analyzing a sample with a known reference standards. Normally, reference standards are highly purified target compounds that in this study is 0.1% of TA.

HPLC is a versatile technique that has been used for separation tool for biomedical and pharmaceutical analysis. It is a separating the components of complex biological sample assay and detecting purposes medical (51, 52).

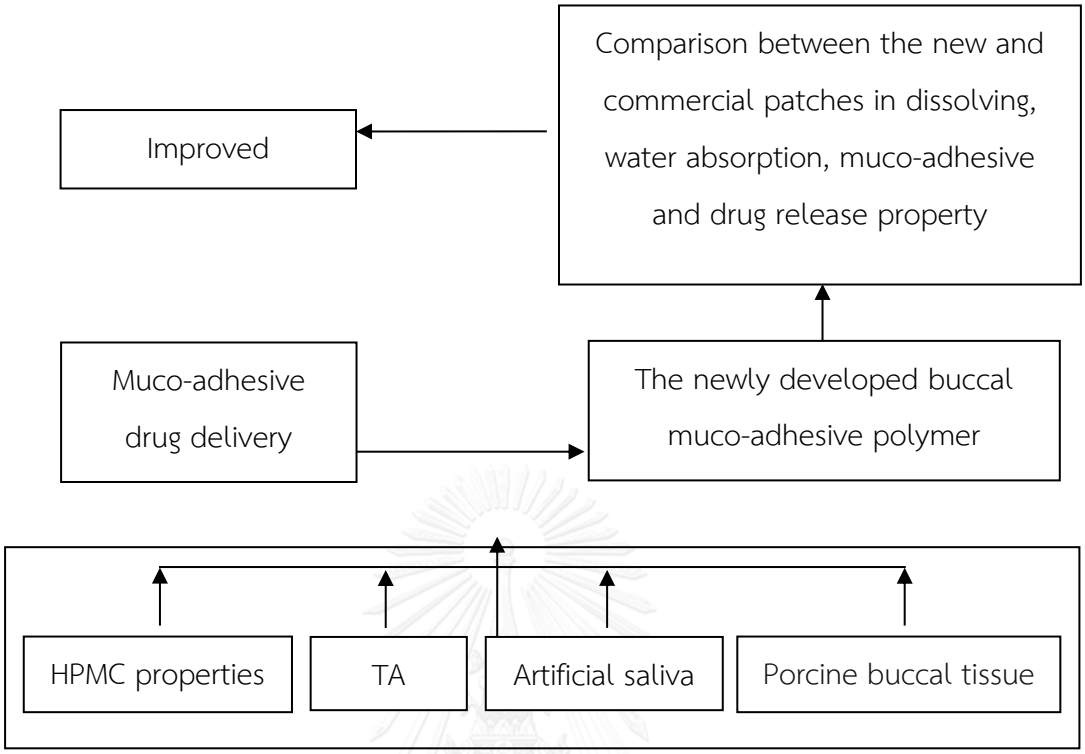
Research question

Can a newly developed HPMC based muco-adhesive deliver TA at the same level as the commercially available patches?

Research objectives

1. To prepare a new HPMC based muco-adhesive for delivery of TA.
2. To test the developed HPMC based muco-adhesive properties: dissolving, water absorption, muco-adhesive and drug release properties compared with commercial patches.

Conceptual framework



Research hypothesis

HPMC based muco-adhesive has drug property release that are comparable with commercial patches.

Materials and Methods

Materials

1. Petri dish diameter 9 cm.

2. Water bath with thermostat

3. Micropipette

4. Digital balance (Sartorius, SPC Calibration Center, Thailand)

5. Incubator (model ES-20, Bisan, Latvia)

6. Texture analyzer with mucoadhesive rig at department of food technology, Faculty of science, Chulalongkorn University (TA.XT plus, Stable Micro Systems, UK)

7. High Performance Liquid Chromatography (Shimadzu Corporation, Japan) at Pharmaceutical technology service center (PTSC) analyzed at Faculty of pharmaceutical science, Chulalongkorn University

8. Porcine buccal tissue (Slaughterhouse Nakhon Pathom, Thailand)

Chemical agents

1. Hydroxypropyl methylcellulose (Methocel F4M, Namsiang group Co.Ltd., Thailand)

2. HPMC commercial patches (Traful Direct, Daiichi Sankyo Healthcare Co.Ltd., Japan)

3. Triamcinolone acetonide (S.Tong Chemicals Co., Ltd., Thailand)

4. Glycerin (Glycerin 99.5%USP/BP, Siam Absolute Chemicals Co.Ltd.,Thailand)

5. Artificial saliva pH 7.0 (KCl, NaCl, $MgCl_2$, Ca_3PO_4,Na_3PO_4) were prepared from department of Biochemistry Sciences Faculty of Dentistry, Chulalongkorn University)

Preparation of buccal patches

HPMC was dissolved in 60 mL of distilled water at a concentration of 1, 2, or 3% (mass/volume). Glycerin (0.1%) was added to the preparations in a beaker. All of the solutions were poured into clean, dry glass petri dishes and the resulting clear viscous solutions left at room temperature until all air bubbles disappeared. The

resultant films were dried in an oven at 55 ºC for 48 hours, loaded with 0.1% TA dissolved in a distilled water and ethanol (65:35) solution, soaked in the TA solution and dried for 1 d each at -20 ºC, 4 ºC, and 25 ºC. Finally, the films were left at room temperature for 48±1 hours to allow the residual solvent to evaporate and cut into 9-mm diameter patches (18).

Preparation of porcine buccal tissue

Buccal tissue from 3-4-year-old pigs was obtained from a local slaughterhouse. Each piece of tissue was washed with deionized water to remove undigested food from the surface. Sixty specimens, thickness 3–5-mm, were prepared and placed in a 0.9% sodium chloride (NaCl) solution at 8 ℃ and used within 6 hours (19).

Preparation of artificial saliva (53)

Simulated saliva fluid (SSF, pH 7) was used to substitute human saliva. The ingredients of SSF are presented (Table 1). The SSF was prepared as follows:

1. Dissolved potassium dihydrogenphosphate (KH_2PO_4)0.738 g with potassium chloride (KCl) 1.114 g, sodium chloride (NaCl) 0.381 g and calcium chloride dihydrate ($CaCl_2.2H_2O$) 0.0213 g in 500 mL of distilled water in a beaker.
2. 2.2 g porcine mucin was poured into item 1 and stirred until it was completely dissolved.

3. Then, solution is mixed together and adjusted to pH7 using potassium chloride (KOH).
4. The solution was added to distilled water until dissolved and 1000 mL is the final volume.

Table 1. Ingredients of simulated saliva fluid

Ingredients	Weight or volume
KCl	1.114 g
NaCl	0.381g
$CaCl_2.2H_2O$	0.0213 g
KH_2PO_4	0.738 g
Porcine mucin	2.2 g
Distilled water	1,000 mL

Dissolution assay

Each buccal patch (n=5 for each HPMC concentration) was soaked in a beaker containing 20 mL SSF at the room temperature. Each beaker, containing a magnetic stirrer, was placed on a stirring machine and the stirrer rotated at 90 rpm using an environment shaker-incubator. The solutions were collected after the patches had completely dissolved, and the time required for dissolution was recorded (18).

Water absorption assay

The porcine buccal tissues were soaked in SSF at pH 7 at 37°C for 60 min, then dried with filter paper. Five buccal patches for each concentration of HPMC were used and their weights recorded. The buccal patches were placed on the porcine buccal tissues and the patch weights recorded at 1, 5, 10, and 30 minutes (18) using a 3 decimal place digital balance (Sartorius, SPC Calibration Center, Thailand).The percentage of weight absorption were calculated using equation 1.

$$\frac{W_2 - W_1}{W_1} \times 100 \quad (1)$$

Where W_1 was the dried patch weight and W_2 was the patch weight after placed on the porcine buccal tissues. This experiment was conducted five times and the results expressed as mean ± SE.

Muco-adhesive study

Testing of the buccal patches were carried out using a texture analyzer (TA.XT plus, Stable Micro Systems, UK), equipped with a 50-N load cell and a bio-adhesive holder. Patches were attached to a cylindrical probe (10 mm in diameter) with double-sided adhesive tape. They were equilibrated in SSF at pH 7 (37±0.5°C) for 15 minutes before being placed on the platform of the bio-adhesive holder. The probe with buccal patches was moved downward to attach the tissue with a specified

contact force of 0.2 N and a contact time of 30 seconds before withdrawal at a speed of 10 mm/s. By using the texture analyzer, the maximum force needed to separate the probe from the tissue (maximum detachment force, F_{max}) could be derived directly.In this study calculated the area under the curve (AUC) from force-distance plot as the work of muco-adhesion (11). Each experiment was carried out five times.

***In vitro* drug release validation** (54)

The chromatographic peaks of triamcinolone acetonide specificity reference standard at 20 µg/mL concentration (54). The HPLC assay allowed for detection of TA at a retention time of 5.8 minutes (Figure 3). Linearity of the standard calibration curves were obtained with five triamcinolone acetonide reference standard solutions. The concentrations used were 0.1, 0.05, 0.02, 0.008, and 0.005 percentage of TA. Each solution was injected three times in the chromatographic system. The linearity was estimated by linear regression analysis by the least square regression method. The correlation coefficient was calculated (Figure 4) (Equation 2).

$$y = 10^{8}x + 503165 \qquad R^2 = 0.9989 \qquad (2)$$

Where y = Absorbance area of TA by UV detector

x = concentration of drug releasing

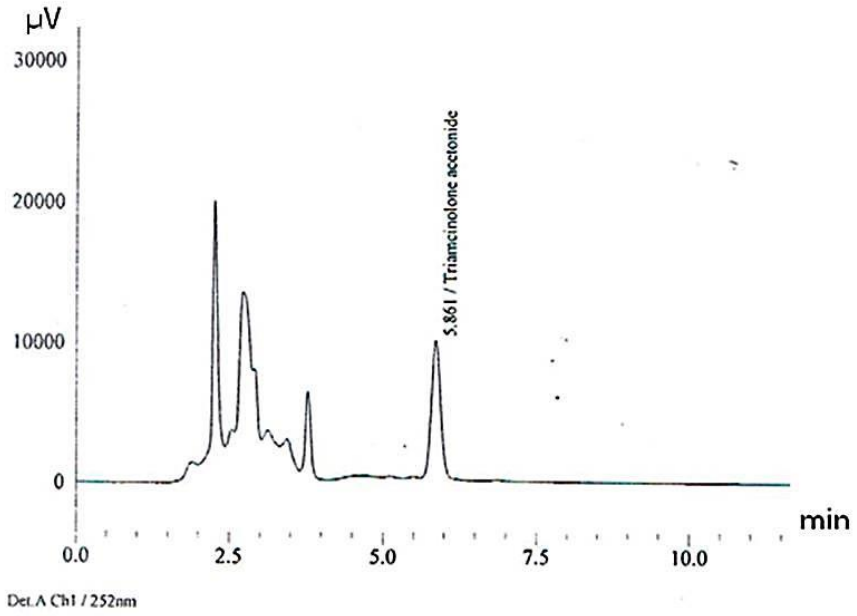


Figure 3. A chromatogram of triamcinolone acetonide and peak area were obtained from HPLC.

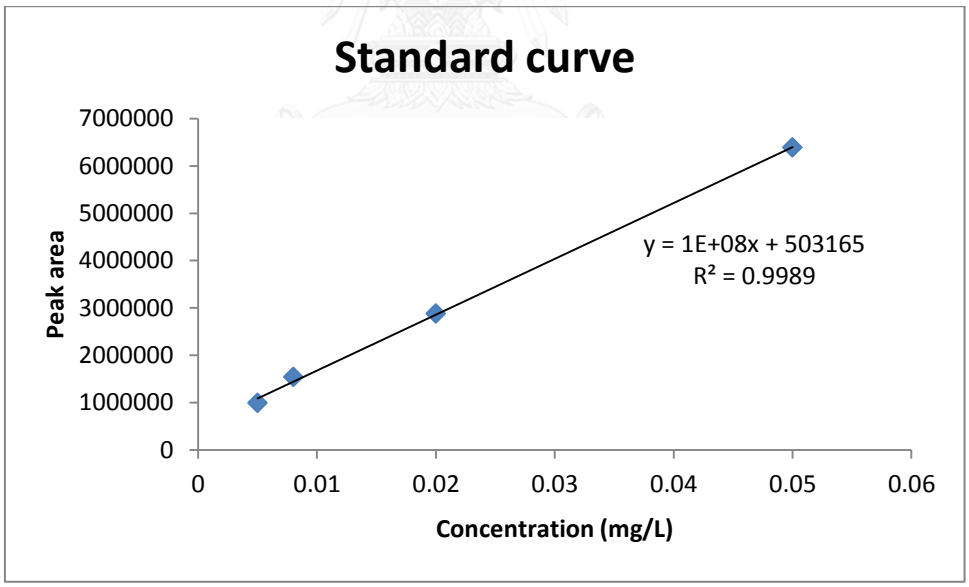


Figure 4. A standard curve of know concentrations of triamcinolone acetonide and peak area were obtained from HPLC.

***In vitro* drug release study using HPLC**

One patch was soak into a centrifuge tube of SSF. All centrifuge tube were shook by shaker in room temperature. Then the drug release solution 500 µl each tube were got off from tube at 2, 4, 6, 8 and up to 10 hours. 500 µl of the 2 ml sample volume were drawn and replaced with 500 µl of SSF every time an analysis was conducted, 0.5/2 of sample were removed each time an analysis was conducted. To compensate for the diluting effect, the drug release value for each sample was adjusted according to the modified relationship (equation 3) (55).

$$A_{adj}(h) = A(h) + 0.5/2 \times \sum_{n=0}^{n=h-2} A \qquad (3)$$

Where $A_{adj}(h)$ = adjusted amount of drug releasing at hour

A (h) = analyzed amount of drug releasing at hour

All samples were analyzed by chromatographic system from Shimadzu products (Shimadzu Corporation, Japan) consisted of pump (model LC-10ADvp), autosampler (model SIL-10Avp) and UV absorbance detector (model SPD-10Avp). The separation was performed by a Inertsil ODS-3, 5 um, 250 x 4.6 mm ID (GL Sciences, Japan) analytical column. The mobile phase was methanol-water-phosphoric acid (75/25/0.5, v/v). Mobile phase degassed by aspiration for 5 min prior to use. The flow-rate was 1.0 mL/min and the temperature was ambient. The eluate was monitored by UV absorbance at 252 nm(56). Each *in vitro* study were performed in five times.

Statistical analysis

The values were analyzed with Kruskal-Wallis H test for all sample group. Then, pairwise comparisons were performed using Mann-Whitney test. The level of significance was 0.05 for all statistical analyses.

Results and Discussion

Study of dissolving property

The mean dissolution times of the patches prepared using different HPMC concentrations and a commercial product are illustrated in Table 2 (Appendix). It was found that the 3% HPMC group demonstrated the highest dissolution time of 7.11 ± 0.68 hours. The 2% HPMC 5.06 ± 0.39 hours, commercial product (3.81 ± 0.45) hours, and 1% HPMC (2.92 ± 0.69) hours groups displayed decreasing dissolution times compared with the 3% HPMC group.

The results revealed that the 3% and 2% HPMC patches had significantly lower dissolution rates (Figure 5), a favorable property, compared with that of the commercial product ($p<0.05$). These HPMC patches could thus remain in the oral cavity for a longer time. Our results support a previous finding that increasing HPMC concentration significantly increased dissolution time ($p<0.05$) (8). However, human saliva contains digestive enzymes that SSF does not, indicating that future in situ

studies are needed to determine the actual dissolution rate of the newly developed muco-adhesive polymer patches in the oral cavity.

The texture and strength of patch formulation varies with the type, viscosity and concentration of HPMC used. In general, the strength of the patch increases with increasing molecular weight. However, the patch strength may decrease at molecular weights more than approximately 150,000 (approx. 100 mPa·s for a 2% aqueous solution) (28). Additives will also affect the patch strength.

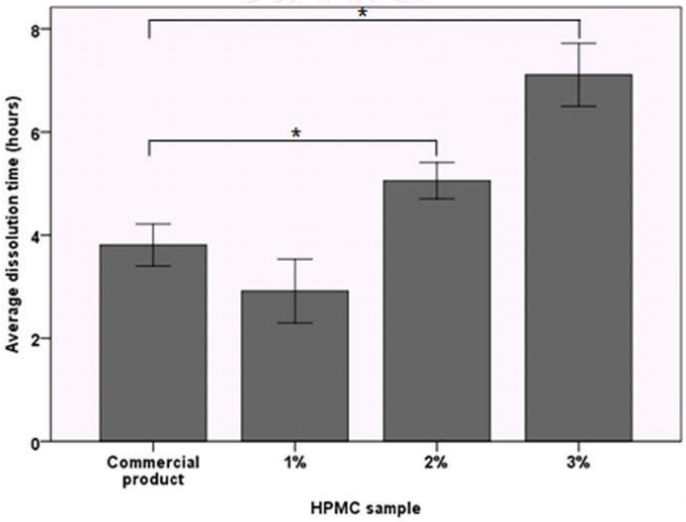


Figure 5. Dissolution times of the different HPMC sample and commercial product groups. * Indicates a significant difference from commercial product ($p<0.05$).

Water absorption

The water absorption results are shown in Table 3 (Appendix) and Figure 6. Every concentration of the newly developed muco-adhesive polymer patches had higher water absorption than the commercial patche at 1 and 5 minutes ($p<0.05$).1% HPMC

group demonstrated the highest water absorption 156.51± 53.50 percentage at 1 minute and 236.29±113.63 percentage at 5 minutes. In addition, the 3% and 2% HPMC patches demonstrated significantly higher water absorption compared with the commercial patches at 10 and 30 minutes ($p<0.05$). 3% HPMC group demonstrated the highest water absorption 331.65± 50.64 percentage at 10 minutes and 359.62±72.85 percentage at 30 minutes. The commercial product had the lowest water absorption at each time point, which would result in minimal changes in the concentration of a loaded drug. Although we found no clear relationship between HPMC concentration and water absorption, a prior study reported that HPMC percentage had an inverse relationship with water absorption (18)

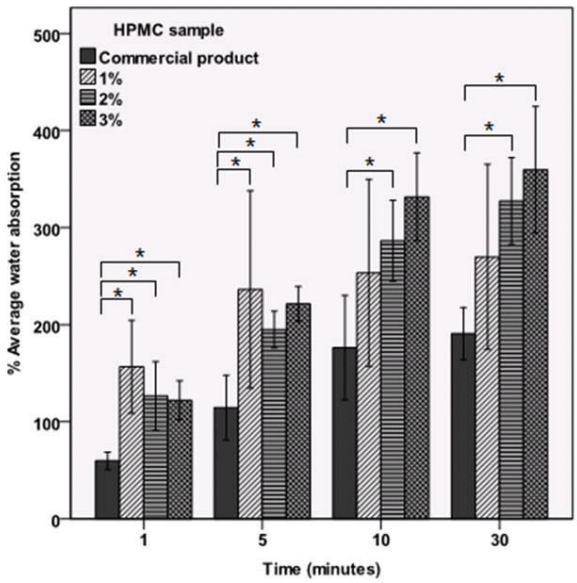


Figure 6. Water absorption of patches with different HPMC concentrations at different time point. * indicates a significant difference compared with control ($p<0.05$).

Although the 3% HPMC patches had the longest dissolution time, they also demonstrated high water absorption. The patch dissolution rate indicates the length of time that the patch will remain in the oral cavity, while water absorption plays an important role in muco-adhesion. When either excess hydration of the patches or the buccal tissue was wet, decreases their muco-adhesiveness so the adhesion between the patch and mucosa was no longer strengthening (27). We observed swelling of the patches during the absorption test. The patches in each group began to swell almost immediately. We assayed the absorption as the percentage of patch weight change at 1, 5, 10, and 30 minutes after immersion in SSF. At 1 and 5 minutes, the 1% HPMC had higher water absorption because they had more structural flexible of polymer allowed water to penetrate into the polymer. After 30 minutes, the 1% HPMC had lower water absorption because they were possibly saturated with water. Then the patches began to detach from the porcine buccal tissue. A previous study indicated that molecular weight plays a more important role in water absorption

than does the hydrophilicity of a polymer (22). Low-molecular-weight polymers can penetrate the mucosa layer well and the optimum molecular weight is between 10 and 4,000 kDa (27). Our patches have an average molecular weight of 86 kDa (28) which is in the optimum molecular weight range. To obtain suitable water absorption, the molecular weight of the polymer should be adjusted to the optimum range. Moreover, glycerin in formula is an humectant that is able to absorb water. As moisturizers that contain glycerine produce long-lasting moisturizing by

binding and holding water and, at high concentrations of glycerine, minimize water loss (57) .

Muco-adhesive force

The muco-adhesive force assay was performed using an instrument that measured the maximum detachment force (F_{max}) (Table 4 Appendix). No significant differences in muco-adhesive force and work of adhesion were found between the different HPMC concentration and the commercial product groups ($p>0.05$). The muco-adhesiveness of the patches was determined (Figure 7, 8). No significant difference in muco-adhesiveness was found between the groups ($p>0.05$). However, the 1% HPMC group had the highest detachment force 0.37±0.30 N. This finding could stem from the flexibility of the polymer chains and their high water absorption. The 2% HPMC, commercial product, and 3% HPMC groups demonstrated decreasing muco-adhesive force compared with the 1% HPMC group. The same trend was found In a prior study, where the 4% HPMC group demonstrated the lowest detachment stress per area and the 1% HPMC group had the highest detachment stress per area (18). Our observations indicated that there was sufficient adhesion between the patches and the dried porcine buccal mucosa. Adhesion decreased after artificial saliva was included in order to mimic the oral cavity. Although not significantly different between the 1% and 2% HPMC groups, all HPMC concentration groups had

a higher detachment force than the commercial product. But all HPMC concentration groups had a lower adhesion area than the commercial product. From the result could discuss because 1% and 2% HPMC groups had more structural flexible of polymer, so increased penetrating into the mucosa. 3% HPMC was longer penetrating chain length and increased cross-linking density was attributed to the increased concentration of polymer but over cross-linking density decreased structural flexible of polymer. Higher water absorption could alter the drug concentration and a critical degree of hydration of the muco-adhesive polymer affects optimum swelling and thus, bio-adhesion (58-60). An acceptable polymer should have sufficient water absorption to increase the penetration of the polymer chains into the mucosal network. Penetration enhancers are substrates added to the patch formulation to improve adhesiveness. These enhancers can be used alone or in combination to improve the bioavailability of a loaded drug without increasing its toxicity (27). Some enhancers are enzyme inhibitors, such as aprotinin, bestatin, and puromycin. These inhibitors effectively reduce proteolytic enzyme activity in the saliva (27).

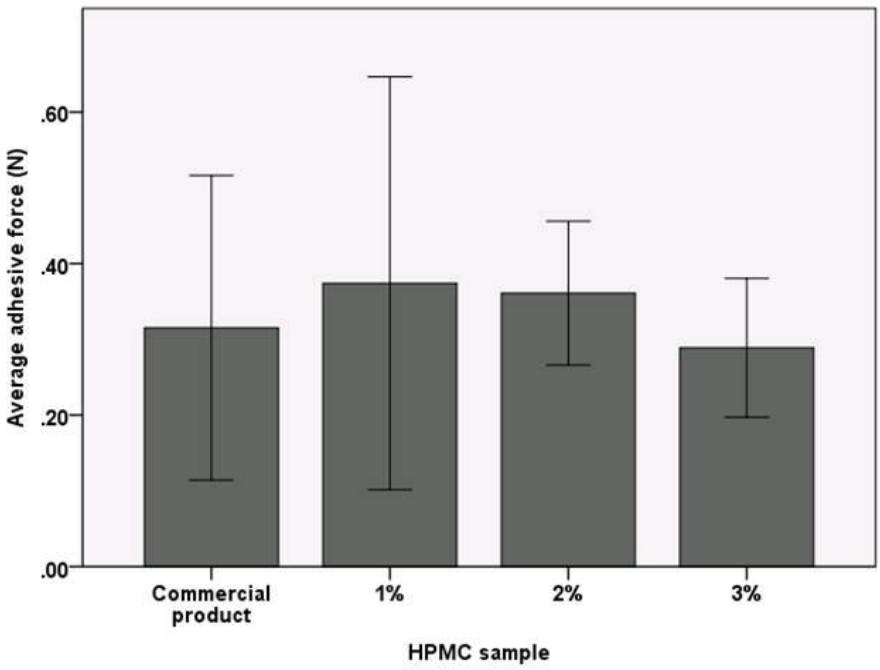


Figure 7. Detachment force of the patches with different HPMC samples and commercial product.

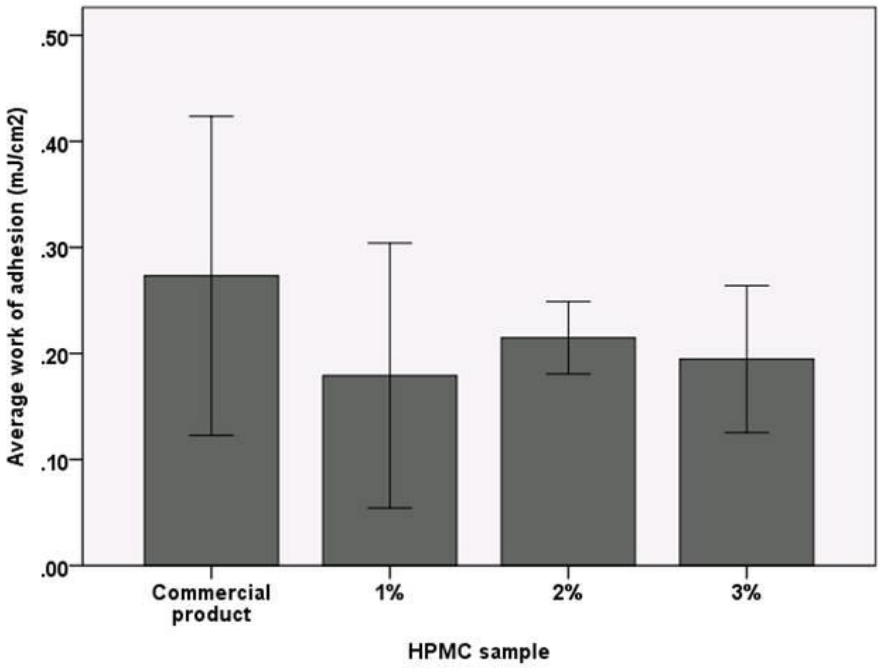


Figure 8. Adhesion area of the different HPMC samples and commercial product groups.

High-Performance Liquid Chromatography (HPLC) analysis of in vitro drug release

All HPMC concentration groups did not show significantly higher drug release at every time point compared with the commercial product group (Table 5 Appendix, Figure 9). 3% HPMC group had the highest drug release profiles. 3% HPMC had significantly higher than the commercial product at 2 hours. The commercial product group had lower drug release than the 3%, followed by 2% and 1% of HPMC, respectively. The 3% HPMC group had significantly higher drug release than 1% HPMC at 2, 4 and 6 hours. After 4 hours, we found that the 1% HPMC patches were completely dissolved, and total drug was released from the patches, and after 8 hours, the 2% HPMC patches were completely dissolved, resulting in total drug release. The 3% HPMC and commercial patches required 10 hours or more to completely dissolve. From this results, we found did not the same as study of dissolving property because analysis of *in vitro* drug release study did not use the stirring machine.

We found that dissolution did not significantly affect drug release by the commercial product. An acceptable patches had higher and prolong drug releasing, moreover they could still attach on buccal tissue without dissolving. Our results indicated that TA was still released from the 3% HPMC patches after 8 to 10 hours.

From the results, the conventional topical of TA were applied 2-3 times per 24 hours, so the 3% HPMC were an acceptable patches.

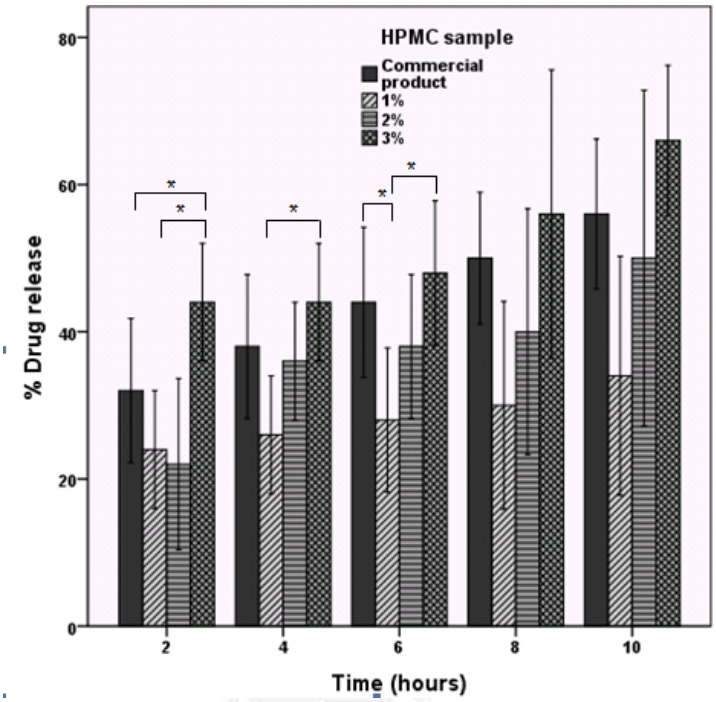


Figure 9. In vitro triamcinolone acetonide release profiles of the HPMC patches and commercial product. * indicates a significant difference compared with control ($p<0.05$)

Thus, to achieve the optimal treatment level, higher drug concentrations should be loaded into the buccal patches. Furthermore, HPMC concentration and the particle size of polymer can greatly influence the patches properties. Increasing the polymer concentration or smaller particle size decrease in drug-release rate. HPMC polymers with smaller particle size have more surface area relative to equivalent weights with larger particle size. Because the greater surface area provides for better polymer-water contact, thus increasing complete polymer hydration and

gelation occurs. This leads to the more effective formation of the protective gel barrier of the patches so critical to muco-adhesive drug delivery system. For this reason, increasing the polymer concentration does not result in decreases in drug-release rate because drug release does not only result from polymer erosion, but also from drug diffusion through the hydrated polymer layers and polymer particle size. If polymer concentration is too low, complete patch formation will be formed that decreased the patch properties. The smaller polymer particle size was found in premium form of identify special product. This effect of slower release for higher polymer levels causes from the longer period of time required to reach the disentanglement in muco-adhesive drug delivery system. An increase in polymer level tends to decrease the sensitivity of the formulation to minor variations. The TA were dissolved complete in H_2O and ethanol. but in this study, the patches were tested in SSF. So drug releasing that were investigated by HPLC, were less than in completely dissolved solution.

This study revealed that the newly developed polymer patches (3% HPMC) could be an alternative treatment for oral ulcerations because this formulation had a higher amount of drug released from 2–10 hours compared with the commercial product. Furthermore, the 3% HPMC had higher drug release than 1% and 2% HPMC. We could not explain *to* cut-point definitely for the best concentration because no one was the best all. 3% HPMC were chosen because they had higher and prolong drug release. An increase in polymer level tends to decrease the sensitivity of the

formulation to minor variations (28).The patches could hold at mucosa more than 8 hours that adequate for oral ulcer treatment compared with conventional topical drug.

However, this a new developed muco-adhesive polymer patches should not be used by the following persons considered to have infectious lesion. Persons considered to have infection who have white plaque, which are easily wipe off by rubbing with gauze that candida infection is suspected. Having yellow pus at the affected area or having systemic symptoms such as fever, malaise or swelling of lymph nodes that viral infection is suspected can be exacerbated by the steroids. A future study should investigate the interaction between HPMC and TA by FT-IR spectra or X-ray diffraction. Higher solubility of the drug generally leads to faster release. In addition, selection of HPMC polymer type, molecular weight, and viscosity will improve the newly developed oral patches. There are polymer combinations other than HPMC with good texture and muco-adhesiveness such as chitosan, polyacrylic acid, and pectin that can be used as a vehicle for oral patches. A previous study found that the adhesive force of carbopol/poloxamer/HPMC films increased with increased HPMC content in the film, and the release of TA from TA-loaded carbopol/poloxamer/HPMC polymer films *in vitro* increased with increased drug loading (4). A study of gel formulations of polaxamer 407, carbopol 934, chitosan, and HPMC with TA compared with a commercial product containing 0.1% TA (Kenacort-A Orabase®) observed that the bio-adhesiveness of the formulations

depended on the bio-adhesive polymer concentration and molecular weight of chitosan. The bio-adhesive performance of the chitosan-based formulations was improved with the inclusion of HPMC. Texture profile analysis (TPA) results indicated that the mechanical properties of the developed gels were improved compared with the commercial product (13).

Conclusions

The buccal muco-adhesive polymer patches fabricated from HPMC for the delivery of 0.1% TA demonstrated significantly an acceptable *in vitro* dissolution time. The 3% HPMC group had higher drug release than the commercial product. However, the HPMC patches had higher water absorption than the commercial product. There was no significant difference in muco-adhesion between the patches with different HPMC concentrations and the commercial product. Therefore, HPMC could be used to produce a buccal muco-adhesive polymer patch as an alternative treatment for oral ulcers. In laboratory testing these patches are comparable to a commercial patch and could lead to a better response to drug treatment. Further study is needed to improve the water absorption and muco-adhesive properties of the oral patches.

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APPENDIX

Table 2. Dissolution times of the different HPMC concentration and commercial product with mean and SD

HPMC sample	No.	Dissolution time (h)			
		Commercial product	1% HPMC	2% HPMC	3% HPMC
	1	3.25	3.36	4.43	6.12
	2	3.38	3.43	5.02	7.14
	3	4.06	3.47	5.05	7.19
	4	4.16	2.15	5.4	7.05
	5	4.19	2.17	5.38	8.05
	Mean	3.81	2.92	5.06	7.11
	SD	0.45	0.69	0.39	0.68

Table 3. Percentage of water absorption with different HPMC concentration and a commercial product at different time point.

HPMC sample	No.	Dried weight (g)	% of water absorption			
			1 min	5 min	10 min	30 min
Commercial product	1	0.02	48.70	77.39	113.91	157.83
	2	0.02	52.63	80.26	110.96	165.35
	3	0.02	73.09	123.77	221.97	198.21
	4	0.02	66.09	123.91	238.26	200.43
	5	0.02	57.99	167.58	196.35	231.96
	Mean		59.70	114.58	176.29	190.76
	SD		9.93	27.22	60.18	29.90
1% HPMC	1	0.02	121.23	82.12	110.06	137.99
	2	0.00	187.50	222.92	250.00	308.33
	3	0.01	225.40	231.75	233.33	206.35
	4	0.01	158.98	402.56	412.82	420.51
	5	0.01	89.47	242.11	261.05	275.79
	Mean		156.51	236.29	253.45	269.79
	SD		53.50	113.63	107.74	106.81
2% HPMC	1	0.02	132.32	221.95	321.95	370.73
	2	0.01	189.29	203.57	285.71	292.14
	3	0.02	85.43	170.35	208.54	262.31
	4	0.02	123.31	201.84	323.31	377.91
	5	0.02	102.51	176.88	292.46	333.67
	Mean		126.57	194.92	286.40	327.35
	SD		36.52	21.11	46.71	49.87
3% HPMC	1	0.01	122.30	253.38	279.73	289.19
	2	0.01	109.22	225.53	297.16	309.93
	3	0.01	118.94	216.67	340.91	431.82
	4	0.01	100.87	211.30	329.57	322.61
	5	0.01	159.41	200.00	410.89	444.55
	Mean		122.15	221.38	331.65	359.62
	SD		22.46	20.14	50.64	72.85

Table 4. Muco-adhesive detachment force and work of adhesion with different HPMC concentration and a commercial product.

HPMC sample	No.	Maximum detachment force (N)	Work of adhesion (mJ/cm^2)
Commercial product	1	0.68	0.55
	2	0.18	0.14
	3	0.17	0.15
	4	0.39	0.31
	5	0.16	0.23
	Mean	0.32	0.27
	SD	0.23	0.17
1% HPMC	1	0.91	0.42
	2	0.27	0.13
	3	0.24	0.11
	4	0.31	0.16
	5	0.14	0.07
	Mean	0.37	0.12
	SD	0.31	0.04
2% HPMC	1	0.52	0.27
	2	0.40	0.21
	3	0.33	0.21
	4	0.30	0.22
	5	0.25	0.16
	Mean	0.36	0.22
	SD	0.11	0.04
3% HPMC	1	0.17	0.10
	2	0.32	0.22
	3	0.42	0.30
	4	0.34	0.22
	5	0.21	0.13
	Mean	0.29	0.19
	SD	0.10	0.08

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FACULTY OF PHARMACEUTICAL SCIENCES CHULALONGKORN UNIVERSITY BANGKOK 10330 THAILAND
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Form FS : 25-01E

Test Report Performa

REPORT OF ANALYSIS

Report No. RE 006/60

December 2, 2016

Sample No. 006/60

Product name	:	TA buccal patch
Active ingredient	:	Triamcinolone acetonide
Registration No.	:	-
Received date	:	November 30, 2016 Analysis date : November 30 , 2016 – December 2, 2016
Requested from	:	Premrudee Srisontorn, Faculty of Dentistry, Chulalongkorn University.
Manufacturer	:	-
Lot/ Batch No.	:	- Manufacturing date : - Expiry date : -
Objective	:	Miscellaneous
Description	:	Solution
Test method	:	HPLC method from client
Standard	:	-
Result	:	Attached document, 113 pages
Specification	:	-

Remarks :

1. *This test report certifies/accredits only the sample being tested*
2. *Partially reproduces this test report is prohibited without written approval of the center*
3. *This certificate is prohibited for any advertisement*
4. *Amount of samples after testing* ☑ *Run out*
   ☐ *Remain and to be returned with this test report*

Certified by : .....
(Vorasit Vongsutilers, Ph.D., R.Ph.)
Director
Date ..... Dec 7, 2016

Analyst : Sawika Suksaen
(Miss Sawika Suksaen)
Date ...2 December 2016

	แบบฟอร์มบันทึกผลการทดสอบ ห้องปฏิบัติการศูนย์บริการเทคโนโลยีเภสัชอุตสาหกรรม	WS : 25-14-01 ฉบับแก้ไขครั้งที่ : 2 วันที่เริ่มใช้ : 25 ธ.ค. 2556
Item : Miscellaneous.....HPLC.....		หน้า : 1 / 3

Product : ยา TA ที่ปล่อยจาก buccal patch	Lot No. : -	Sample ID : 006/60	Received Date : 30/11/59
Reg. No. : -	Mfg. Date : -	Exp. Date : -	
Label : Triamcinolone acetonide (TA)		Testing Date : 30/11/59 - 2/12/59	
Description : Solution		Quantity : Centifuge tube 5 หลอด, Microtube 100 หลอด	

Chromatographic system

HPLC	: Shimadzu, model LC-10AD$_{vp}$			Code : 3-01-07-00	
Column	: 4.6-mm x 25-cm ; C18 Inersil ODS , 5 µm			Code : 11	
Mobile phase	: Methanol : Water : Phosphoric acid (75 : 25 : 0.5)				
Flow rate	: 1.0 mL/min	Detection : 252 nm		Injection vol. : 10 µL	
Column Temp.	: - °C	Autosampler Temp : - °C		Pressure : 135 - 138 kgf/cm^2	
Retention time	: 5.8 Triamcinolone acetonide				

Procedure

Mobile phase : Methanol : Water : Phosphoric acid (75 : 25 : 0.5)

Note : ....2250..... mL of Methanol : ....750..... mL of Water : .....15..... mL of Phosphoric acid

Reagents

Name	Brand	Grade/Purity	Lot No.
Methanol	RCI Labscan	HPLC	16080258
Water	-	-	041116
Phosphoric acid	RCI Labscan	AR	15050250

Instrument (s) :-

Analyst : ศาริการ์	Date : 2/12/59	Approved : วิรัฐกร์	Date : 2/12/59

HPLC sample information

Calibration curve

Sample name	No inject	Peak#	Real time	Area	Height	Theoretical plate#
Control 0.1% TA	1	1	5.821	6360311	26592	7050
	2	1	5.822	6365895	25467	7037
	3	1	5.821	6339774	25858	7047
Control 0.05% TA	1	1	5.818	6383139	15893	7110
	2	1	5.810	6380789	15624	7097
	3	1	5.812	6387654	15654	7092
Control 0.02% TA	1	1	5.807	2868186	12353	7107
	2	1	5.809	2875591	12349	7116
	3	1	5.809	2881211	12461	7098
Control 0.008% TA	1	1	5.804	1542333	8168	7076
	2	1	5.810	1542814	8226	7102
	3	1	5.807	1542348	8144	7071
Control 0.005% TA	1	1	5.809	993629	6721	7087
	2	1	5.814	991379	6719	7068
	3	1	5.812	991710	6726	7049

Standard curve of triamcinolone acetonide

Commercial product

Sample name	Peak area	% TA analyzed	% TA adjusted
2 h (1)	3365450	0.03	
2 h (2)	2834100	0.02	
2 h (3)	5095900	0.05	
2 h (4)	3276050	0.03	
2 h (5)	3276050	0.03	
4 h (1)	2804200	0.02	0.03
4 h (2)	2817900	0.02	0.03
4 h (3)	4555200	0.04	0.05
4 h (4)	4747850	0.04	0.05
4 h (5)	3257500	0.03	0.03
6 h (1)	2776000	0.02	.04
6 h (2)	2590500	0.02	0.03
6 h (3)	4072650	0.04	0.06
6 h (4)	3683750	0.03	0.05
6 h (5)	3126000	0.03	0.04

Commercial product

Sample name	Peak area	% TA analyzed	% TA adjusted
8 h (1)	3028500	0.02	0.04
8 h (2)	3131000	0.02	0.04
8 h (3)	2981750	0.03	0.06
8 h (4)	1099050	0.03	0.05
8 h (5)	3257100	0.04	0.06
10 h (1)	2469350	0.02	0.04
10 h (2)	3099950	0.03	0.05
10 h (3)	2613950	0.02	0.06
10 h (4)	1120350	0.03	0.06
10 h (5)	2919300	0.04	0.07

1% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
2 h (1)	3028500	0.03	
2 h (2)	3131000	0.03	
2 h (3)	2981750	0.02	
2 h (4)	1099050	0.01	
2 h (5)	3257100	0.03	
4 h (1)	2469350	0.02	0.03
4 h (2)	3099950	0.026	0.03
4 h (3)	2613950	0.02	0.03
4 h (4)	1120350	0.006	0.01
4 h (5)	2919300	0.02	0.03
6 h (1)	1909500	0.01	0.03
6 h (2)	2780850	0.02	0.04
6 h (3)	2346900	0.02	0.03
6 h (4)	939850	0.004	0.01
6 h (5)	2568150	0.02	0.03

1% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
8 h (1)	1348850	0.01	0.02
8 h (2)	2682100	0.02	0.04
8 h (3)	2011050	0.02	0.03
8 h (4)	766150	0.003	0.01
8 h (5)	3369500	0.03	0.05
10 h (1)	1114700	0.01	0.02
10 h (2)	2423650	0.02	0.04
10 h (3)	3047950	0.03	0.05
10 h (4)	701500	0.002	0.01
10 h (5)	2714350	0.02	0.05

2% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
2 h(1)	3842200	0.03	
2 h (2)	3790550	0.03	
2 h (3)	3869750	0.03	
2 h (4)	2062300	0.02	
2 h (5)	3721200	0.03	
4 h (1)	3431150	0.03	.004
4 h (2)	3598900	0.03	0.04
4 h (3)	3931450	0.03	0.04
4 h (4)	1696000	0.01	0.02
4 h (5)	3361300	0.03	0.04
6 h (1)	2867950	0.02	0.04
6 h (2)	2863300	0.02	0.04
6 h (3)	3470050	0.03	0.05
6 h (4)	1499600	0.01	0.02
6 h (5)	2752500	0.02	0.04

2% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
8 h(1)	2739400	0.02	0.04
8 h (2)	2311850	0.02	0.04
8 h (3)	3130600	0.03	0.05
8 h (4)	995950	0.005	0.01
8 h (5)	4129850	0.04	0.06
10 h (1)	1843950	0.01	0.04
10 h (2)	2715850	0.02	0.05
10 h (3)	5960300	0.05	0.09
10 h (4)	1019050	0.01	0.02
10 h (5)	2467200	0.02	0.05

3% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
2 h(1)	4503300	0.04	
2 h (2)	3662050	0.03	
2 h (3)	5307000	0.05	
2 h (4)	5748300	0.05	
2 h (5)	5662750	0.05	
4 h (1)	4259850	0.04	0.05
4 h (2)	2980050	0.02	0.03
4 h (3)	4287350	0.04	0.05
4 h (4)	4086100	0.04	0.05
4 h (5)	3059550	0.03	0.04
6 h (1)	3633300	0.03	0.05
6 h (2)	2345450	0.02	0.03
6 h (3)	3100200	0.03	0.05
6 h (4)	3571300	0.03	0.05
6 h (5)	4590500	0.04	0.06

3% HPMC

Sample name	Peak area	% TA analyzed	% TA adjusted
8 h(1)	3094050	0.03	0.05
8 h (2)	1786950	0.01	0.03
8 h (3)	3622250	0.03	0.06
8 h (4)	2971200	0.02	0.05
8 h (5)	6277600	0.06	0.09
10 h (1)	3686000	0.03	0.07
10 h (2)	2835700	0.02	0.05
10 h (3)	2861750	0.02	0.06
10 h (4)	3422900	0.03	0.07
10 h (5)	4315100	0.04	0.08

STATISTIC ANALYSIS

Dissolution assay

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Completed dissolve time	1%HPMC	5	4.00
	2%HPMC	5	13.00
	3%HPMC	5	18.00
	Commercial product	5	7.00
	Total	20	

Test Statistics[a,b]

	Completed dissolve time
Chi-Square	16.714
df	3
Asymp. Sig.	.001

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Mann-Whitney Test

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	1%HPMC	5	3.00	15.00
	2%HPMC	5	8.00	40.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	1%HPMC	5	3.00	15.00
	3%HPMC	5	8.00	40.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	1%HPMC	5	4.00	20.00
	Commercial product	5	7.00	35.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	5.000
Wilcoxon W	20.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.151[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	2%HPMC	5	3.00	15.00
	3%HPMC	5	8.00	40.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	2%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Completed dissolve time	3%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Completed dissolve time
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Water absorption 1 minute

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Weight diff%1 min	1%HPMC	5	14.60
	2%HPMC	5	12.60
	3%HPMC	5	11.80
	Commercial product	5	3.00
	Total	20	

Test Statistics[a,b]

	Weight diff%1min
Chi-Square	11.309
df	3
Asymp. Sig.	.010

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Mann-Whitney Test

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	1%HPMC	5	6.20	31.00
	2%HPMC	5	4.80	24.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	9.000
Wilcoxon W	24.000
Z	-.731
Asymp. Sig. (2-tailed)	.465
Exact Sig. [2*(1-tailed Sig.)]	.548[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	1%HPMC	5	6.40	32.00
	3%HPMC	5	4.60	23.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	8.000
Wilcoxon W	23.000
Z	-.940
Asymp. Sig. (2-tailed)	.347
Exact Sig. [2*(1-tailed Sig.)]	.421[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	1%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	2%HPMC	5	5.80	29.00
	3%HPMC	5	5.20	26.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	11.000
Wilcoxon W	26.000
Z	-.313
Asymp. Sig. (2-tailed)	.754
Exact Sig. [2*(1-tailed Sig.)]	.841[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	2%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%1min	3%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%1min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Water absorption 5 minutes

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Weight diff%5min	1%HPMC	5	14.60
	2%HPMC	5	10.00
	3%HPMC	5	13.80
	Commercial product	5	3.60
	Total	20	

Test Statistics[a,b]

	Weight diff%5min
Chi-Square	10.794
df	3
Asymp. Sig.	.013

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	1%HPMC	5	7.00	35.00
	2%HPMC	5	4.00	20.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	5.000
Wilcoxon W	20.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.151[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	1%HPMC	5	6.20	31.00
	3%HPMC	5	4.80	24.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	9.000
Wilcoxon W	24.000
Z	-.731
Asymp. Sig. (2-tailed)	.465
Exact Sig. [2*(1-tailed Sig.)]	.548[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	1%HPMC	5	7.40	37.00
	Commercial product	5	3.60	18.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	3.000
Wilcoxon W	18.000
Z	-1.984
Asymp. Sig. (2-tailed)	.047
Exact Sig. [2*(1-tailed Sig.)]	.056[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	2%HPMC	5	4.00	20.00
	3%HPMC	5	7.00	35.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	5.000
Wilcoxon W	20.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.151[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	2%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%5min	3%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%5min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Water absorption 10 minutes

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Weight diff%10min	1%HPMC	5	9.40
	2%HPMC	5	12.20
	3%HPMC	5	15.80
	Commercial product	5	4.60
	Total	20	

Test Statistics[a,b]

	Weight diff%10min
Chi-Square	9.571
df	3
Asymp. Sig.	.023

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	1%HPMC	5	4.60	23.00
	2%HPMC	5	6.40	32.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	8.000
Wilcoxon W	23.000
Z	-.940
Asymp. Sig. (2-tailed)	.347
Exact Sig. [2*(1-tailed Sig.)]	.421[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	1%HPMC	5	4.00	20.00
	3%HPMC	5	7.00	35.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	5.000
Wilcoxon W	20.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.151[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	1%HPMC	5	6.80	34.00
	Commercial product	5	4.20	21.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	6.000
Wilcoxon W	21.000
Z	-1.358
Asymp. Sig. (2-tailed)	.175
Exact Sig. [2*(1-tailed Sig.)]	.222[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	2%HPMC	5	4.20	21.00
	3%HPMC	5	6.80	34.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	6.000
Wilcoxon W	21.000
Z	-1.358
Asymp. Sig. (2-tailed)	.175
Exact Sig. [2*(1-tailed Sig.)]	.222[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	2%HPMC	5	7.60	38.00
	Commercial product	5	3.40	17.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	2.000
Wilcoxon W	17.000
Z	-2.193
Asymp. Sig. (2-tailed)	.028
Exact Sig. [2*(1-tailed Sig.)]	.032[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%10min	3%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%10min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Water absorption 30 minutes

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Weight diff%30min	1%HPMC	5	9.20
	2%HPMC	5	13.40
	3%HPMC	5	15.20
	Commercial product	5	4.20
	Total	20	

Test Statistics[a,b]

	Weight diff%30min
Chi-Square	10.269
df	3
Asymp. Sig.	.016

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	1%HPMC	5	4.60	23.00
	2%HPMC	5	6.40	32.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	8.000
Wilcoxon W	23.000
Z	-.940
Asymp. Sig. (2-tailed)	.347
Exact Sig. [2*(1-tailed Sig.)]	.421[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	1%HPMC	5	3.80	19.00
	3%HPMC	5	7.20	36.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	4.000
Wilcoxon W	19.000
Z	-1.776
Asymp. Sig. (2-tailed)	.076
Exact Sig. [2*(1-tailed Sig.)]	.095[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	1%HPMC	5	6.80	34.00
	Commercial product	5	4.20	21.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	6.000
Wilcoxon W	21.000
Z	-1.358
Asymp. Sig. (2-tailed)	.175
Exact Sig. [2*(1-tailed Sig.)]	.222[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	2%HPMC	5	5.00	25.00
	3%HPMC	5	6.00	30.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	10.000
Wilcoxon W	25.000
Z	-.522
Asymp. Sig. (2-tailed)	.602
Exact Sig. [2*(1-tailed Sig.)]	.690[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	2%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Ranks

	HPMC concentration	N	Mean Rank	Sum of Ranks
Weight diff%30min	3%HPMC	5	8.00	40.00
	Commercial product	5	3.00	15.00
	Total	10		

Test Statistics[b]

	Weight diff%30min
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009
Exact Sig. [2*(1-tailed Sig.)]	.008[a]

a. Not corrected for ties.

b. Grouping Variable: HPMC concentration

Adhesion

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Adhesive force(N)	1%HPMC	5	9.60
	2%HPMC	5	13.00
	3%HPMC	5	10.40
	Commercial product	5	9.00
	Total	20	

Test Statistics[a,b]

	Adhesive force(N)
Chi-Square	1.331
df	3
Asymp. Sig.	.722

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Work of adhesion

Kruskal-Wallis Test

Ranks

	HPMC concentration	N	Mean Rank
Area work of adhesion	1%HPMC	5	7.20
	2%HPMC	5	11.70
	3%HPMC	5	9.90
	Commercial product	5	13.20
	Total	20	

Test Statistics[a,b]

	Area work of adhesion
Chi-Square	2.856
df	3
Asymp. Sig.	.414

a. Kruskal Wallis Test

b. Grouping Variable: HPMC concentration

Drug release 2 hours

Kruskal-Wallis Test

Ranks

	HPMC sample	N	Mean Rank
% Drug release (2 h)	1% HPMC	5	4.60
	2% HPMC	5	11.20
	3% HPMC	5	16.80
	Commercial product	5	9.40
	Total	20	

Test Statistics[a,b]

	% Drug release (2 h)
Chi-Square	10.894
df	3
Asymp. Sig.	.012

Mann-Whitney Test

Ranks

	HPMC sample	N	Mean Rank	Sum of Ranks
% Drug release (2 h)	1% HPMC	5	3.00	15.00
	3% HPMC	5	8.00	40.00
	Total	10		

Test Statistics[b]

	% Drug release (2 h)
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009

Ranks

	HPMC sample	N	Mean Rank	Sum of Ranks
% Drug release (2 h)	3% HPMC	5	7.60	38.00
	Commercial product	5	3.40	17.00
	Total	10		

Test Statistics[b]

	% Drug release (2 h)
Mann-Whitney U	2.000
Wilcoxon W	17.000
Z	-2.200
Asymp. Sig. (2-tailed)	.028

Drug release 4 hours

Kruskal-Wallis Test

Ranks

	HPMC sample	N	Mean Rank
% Drug release (4 h)	1% HPMC	5	4.60
	2% HPMC	5	10.80
	3% HPMC	5	14.80
	Commercial product	5	11.80
	Total	20	

Test Statistics[a,b]

	% Drug release (4 h)
Chi-Square	7.869
df	3
Asymp. Sig.	.049

Mann-Whitney Test

Ranks

	HPMC sample	N	Mean Rank	Sum of Ranks
% Drug release (4 h)	1% HPMC	5	3.00	15.00
	3% HPMC	5	8.00	40.00
	Total	10		

Test Statistics[b]

	% Drug release (4 h)
Mann-Whitney U	.000
Wilcoxon W	15.000
Z	-2.611
Asymp. Sig. (2-tailed)	.009

Drug release 6 hours

Kruskal-Wallis Test

Ranks

	HPMC sample	N	Mean Rank
% Drug release (6 h)	1% HPMC	5	4.80
	2% HPMC	5	9.80
	3% HPMC	5	15.20
	Commercial product	5	12.20
	Total	20	

Test Statistics[a,b]

	% Drug release (6 h)
Chi-Square	8.280
df	3
Asymp. Sig.	.041

Mann-Whitney Test

Ranks

	HPMC sample	N	Mean Rank	Sum of Ranks
% Drug release (6 h)	1% HPMC	5	3.40	17.00
	3% HPMC	5	7.60	38.00
	Total	10		

Test Statistics[b]

	% Drug release (6 h)
Mann-Whitney U	2.000
Wilcoxon W	17.000
Z	-2.193
Asymp. Sig. (2-tailed)	.028

Ranks

	HPMC sample	N	Mean Rank	Sum of Ranks
% Drug release (6 h)	1% HPMC	5	3.60	18.00
	Commercial product	5	7.40	37.00
	Total	10		

Test Statistics[b]

	% Drug release (6 h)
Mann-Whitney U	3.000
Wilcoxon W	18.000
Z	-1.984
Asymp. Sig. (2-tailed)	.047

Drug release 8 hours

Kruskal-Wallis Test

Ranks

	HPMC sample	N	Mean Rank
% Drug release (8 h)	1% HPMC	5	5.40
	2% HPMC	5	9.40
	3% HPMC	5	14.00
	Commercial product	5	13.20
	Total	20	

Test Statistics[a,b]

	% Drug release (8 h)
Chi-Square	6.680
df	3
Asymp. Sig.	.083

Drug release 10 hours

Kruskal-Wallis Test

Ranks

	HPMC sample	N	Mean Rank
% Drug release (10 h)	1% HPMC	5	5.60
	2% HPMC	5	9.80
	3% HPMC	5	14.40
	Commercial product	5	12.20
	Total	20	

Test Statistics[a,b]

	% Drug release (10 h)
Chi-Square	6.086
df	3
Asymp. Sig.	.108

VITA

Education:

Dentistry, Thammasat University School of Dentistry, 2011

Work place:

Somdejprasangkharach XVII Hospital, Suphanburi in 2014

Sikhoraphum Hospital, Surin in 2012

Suka Hospital, Surin in 2011

Research:

The effect of oral epithelial cell on CCL 20 production in LPS induced gingival fibroblast, 2010