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DEVELOPMENT OF *IN SITU* GEL FOR ARTIFICIAL SALIVA AND SATISFACTION STUDY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Cosmetic Science
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Thesis Title	DEVELOPMENT OF *IN SITU* GEL FOR ARTIFICIAL SALIVA AND SATISFACTION STUDY
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Field of Study	Cosmetic Science
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ภาวะปากแห้ง คือการที่ร่างกายไม่ผลิตน้ำลาย หรือผลิตได้น้อยลง ส่งผลให้ใช้ชีวิตประจำวันได้ยาก ทั้งการพูด การกลืนอาหาร การรับรู้รสชาติ รวมทั้งการนอนหลับ โดยเมื่อเกิดภาวะปากแห้ง ผู้ป่วยมักจะได้รับการแนะนำให้ ดื่มน้ำ หรือเคี้ยวน้ำแข็งเพื่อช่วยเพิ่มความชุ่มชื่นภายในช่องปาก นอกจากนี้ยังมีการแนะนำให้ใช้สารกระตุ้นหรือทดแทนน้ำลาย เช่น การอมลูกอมที่มีรสเปรี้ยว การเคี้ยวหมากฝรั่ง หรือการใช้น้ำลายเทียม โดยในประเทศไทยการเข้าถึงผลิตภัณฑ์น้ำลายเทียมยังไม่ทั่วถึง เนื่องจากยังไม่มีผลิตภัณฑ์น้ำลายเทียมจำหน่ายในท้องตลาด ทำให้โรงพยาบาลหลายแห่งได้มีการผลิตน้ำลายเทียมในรูปแบบสารละลาย เพื่อจ่ายให้แก่ผู้ป่วยที่มีปัญหาปากแห้ง แต่เนื่องจากรสชาติที่ไม่ดี และการคงอยู่ในช่องปากที่สั้น ทำให้ผู้ป่วยไม่ให้ความร่วมมือในการใช้ผลิตภัณฑ์ดังกล่าว งานวิจัยชิ้นนี้จึงได้พัฒนาตำรับเจลก่อตัวเองจากเจลแลนกัม และ ไฮดรอกซีเอทิล เซลลูโลสเพื่อใช้เป็นน้ำลายเทียม โดยได้ทำการทดสอบความคงตัวของตำรับ จากนั้นจึงทำการทดสอบตำรับที่มีความคงตัวในหัวข้อระยะเวลาในการเกิดเจล และความสามารถในการยึดเกาะเนื้อเยื่อกระพุ้งแก้มหมูของตำรับที่มีความคงตัว และทำการศึกษาความพึงพอใจของอาสาสมัครที่มีภาวะปากแห้งต่อน้ำลายเทียมแบบเจลก่อตัวเอง

ผลการทดลองพบว่าน้ำลายเทียมแบบเจลก่อตัวเองที่มีส่วนประกอบของเจลแลนกัม 0.1 และ 0.15 เปอร์เซ็นต์ และไฮดรอกซีเอทิล เซลลูโลส 0.15 และ 0.3 เปอร์เซ็นต์มีความคงตัว และความเข้มข้นของเจลแลนกัมมีผลต่อระยะเวลาในการเกิดเจล โดยเมื่อเพิ่มความเข้มข้นของเจลแลนกัม ระยะเวลาในการเกิดเจลจะลดลง น้ำลายเทียมแบบเจลก่อตัวเองที่ประกอบด้วยเจลแลน กัม 0.15 เปอร์เซ็นต์ และไฮดรอกซีเอทิล เซลลูโลส 0.3 เปอร์เซ็นต์มีความสามารถในการยึดเกาะเนื้อเยื่อกระพุ้งแก้มหมูได้ดีที่สุด และได้รับการคัดเลือกเพื่อศึกษาความพึงพอใจของอาสาสมัครที่มีภาวะปากแห้งต่อน้ำลายเทียมแบบเจลก่อตัวเอง และผลจากการศึกษาในอาสาสมัครแสดงให้เห็นว่า น้ำลายเทียมแบบเจลก่อตัวเองสามารถลดความแห้งภายในช่องปากลงได้ และอาสาสมัครส่วนใหญ่พึงพอใจในผลิตภัณฑ์น้ำลายเทียมแบบเจลก่อตัวเองนี้

ภาควิชา วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาศาสตร์เครื่องสำอาง ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2560 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: GELLAN GUM / IN SITU GEL / ARITIFICIAL SALIVA / HYDROXYETHYL CELLULOSE / MUCOADHESIVE

RAWI TISHYADHIGAMA: DEVELOPMENT OF *IN SITU* GEL FOR ARTIFICIAL SALIVA AND SATISFACTION STUDY. ADVISOR: ASST. PROF. JUTARAT KITSONGSERMTHON, Ph.D., CO-ADVISOR: ASSOC. PROF. ANGKANA TANTITUVANONT, Ph.D., 130 pp.

Xerostomia, the subjective complaint about the dry mouth, may lead to the difficulty in daily living including speaking, swallowing, taste acuity and sleeping. Patients are often advised to drink water or chew ice to moisturize the oral cavity and also recommended to use stimulant or saliva substitute, such as sour candies, chewing gum and artificial saliva. In Thailand, there is no artificial saliva products available in the market and this makes it difficult to access the artificial saliva. Hence, artificial saliva have been developed in the form of solution in many hospitals to dispense for xerostomia patients in the hospitals. Because of its bad taste and short duration of action, patients did not cooperate to use such products. In this study, *in situ* gel-forming artificial saliva containing gellan gum and hydroxyethyl cellulose was developed. Physical stability of *in situ* gel-forming artificial saliva was investigated. The stable formulations were investigated for gelation time and mucoadhesion of *in situ* gel-forming artificial saliva on porcine buccal mucosa. The highest mucoadhesive formulation was selected for satisfaction study in 15 volunteers with dry mouth.

The result found that *in situ* gel-forming artificial saliva containing 0.1% and 0.15%w/v of gellan gum and 0.15% and 0.3% of HEC were stable. The concentration of gellan gum affected the gelation time of *in situ* gel-forming artificial saliva, by increasing gellan gum concentration decreases gelation time. The *in situ* gel-forming artificial saliva, which contained 0.15%w/v of gellan gum and 0.3%w/v of HEC had the highest mucoadhesive property and was selected for satisfaction study. The clinical outcome showed that use of this *in situ* gel-forming artificial saliva could reduce dryness of mouth and almost volunteers were satisfied with this formulation.

Department:	Pharmaceutics and Industrial Pharmacy	Student's Signature	.....
		Advisor's Signature	.....
Field of Study:	Cosmetic Science	Co-Advisor's Signature	.....
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Finally, this achievement will be given to my parents, my dad, who always trust in me. And my beloved mother, who passed away, the perfect woman who exemplifies my path of life.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AR grade	Analytical reagents grade
°C	Degree Celsius
cm^2	Square centimeter
CMC	Carboxymethylcellulose
cPs	Centipoises
°	Degree
DI water	De-ionized water
DEAE	Dimethylaminoethyl
EC	Ethyl cellulose
ESS	Electrolyte stock solution
g	Gram (s)
HEC	Hydroxyethyl cellulose
HPMC	Hydroxypropyl methyl cellulose
Lot no.	Lot number
LCST	Lower critical solution temperature
ml (s)	Milliliter
ml/min	Milliliter per min
mm	Millimater
mmol/L	Millimole per liter
MP	Methyl paraben
N	Newton (s)
PAA	Poly-(acrylic acid)
PAAm	Polyacrylamide
PC	Polycarbonate
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
p-value	Probability value

q.s.	Quantum satis
rpm.	Revolutions per minute
SB	Sodium benzoate
S.D.	Standard deviation
SCMC	Sodium carboxymethylcellulose
TMJ	Temporo-mandibular joint
UCST	Upper critical solution temperature
USP grade	United States Pharmacopeia grade
v/v	Volume by volume
VAS	Visual analogue scales
w/v	Weight by volume

CHAPTER I
INTRODUCTION

Xerostomia is a condition that reduction or loss of salivary flow and the changes in composition of saliva, resulting in oral dryness (Plankhurst et al., 1996). The condition is, in most cases, the result of salivary gland hypofunction of which there are many causes. The most common causes are medication induced, radiation treatment of the head and neck region, Sjögren's syndrome, and other systemic diseases such as asthma, psychiatric diseases, hematological diseases, thyroid diseases, diabetes mellitus, rheumatic diseases, particularly hypertension, and eating disorder (Dost and Farah, 2013; Kelly et al., 2004; Villa et al., 2015). Xerostomia causes an increased risk of dental caries, periodontitis, infection, mucostitis and gingivitis (Plankhurst et al., 1996), and may lead to problems with some or all of the following: speaking, mastication, swallowing, taste acuity and sleeping (Hamlet et al., 1997).

Suggestions for relieving symptoms related to dry mouth include the use of water, crushed ice, chewing gums, hard lozenges, mint, candies, artificial saliva and avoidance of irritating dentifrices and crunchy and hard foods (Stewart et al., 1998; Villa et al., 2015) Saliva stimulants such as pilocarpine are available but patients commonly indicate dissatisfaction due to little relief of symptoms, poor taste, short duration of action, and inconvenience of use (Stewart et al., 1998; Kelly et al., 2004).

In Thailand, access to artificial saliva products is not thorough because no artificial saliva products are commercially available. Many hospitals such as in King Chulalongkorn Hospital have produced artificial saliva in the form of solution to dispense for xerostomia patients in the hospital. However, the preliminary information showed that the patients did not cooperate to use artificial saliva, because of its bad taste and short duration of action. Researchers had the concept to development of *in situ* gel-forming artificial saliva using a characteristic polymer, which forms gel when contacting with saliva, for more convenient to use and long duration action.

In situ gels are drug delivery systems that are present in the solution before it administrated into the body and, after the administration, it will undergo *in situ* gelation

and form a gel, triggered either by physiological factors such as electrolyte content, temperature and pH. As the convenience of administration of *in situ* gel-forming systems, they have been investigated for drug delivery have been reported (Nirmal, Bakliwal, & Pawar, 2010).

Various natural, semi-natural and synthetic polymers are used for development of *in situ* gel-forming drug delivery systems. One of the most widely used of polymers is gellan gum, due to its clear hydrogels in the presence of cations. The gellan gum gelation process is temperature-dependent. It becomes a clear solution by heating to 70 ℃ and triggered by ions to forms gels. Since, there are inorganic constitutes as composition of body fluids that can trigger gellan gum to forms gels, various pharmaceutical formulations have been studied for ophthalmic, nasal, oral, buccal, and vaginal administration (El-Kamel & El-Khatib, 2006).

In the present study, the formulation of *in situ* gel-forming artificial saliva containing gellan gum as gelling agent and hydroxyethyl cellulose (HEC) as mucoadhesive polymer and thickening agent was developed. Then, physical stability of *in situ* gel-forming artificial saliva was investigated. The stable formulations were investigated for gelation time and mucoadhesion of *in situ* gel-forming artificial saliva on porcine buccal mucosa. The highest mucoadhesive formulation was selected for satisfaction study in 15 volunteers with dry mouth.

The objectives of this study were as follows:

1. To study the effect of concentration of gellan gum on the gelation of *in situ* gel-forming artificial saliva when contact the electrolyte in oral cavity.
2. To study the effect of concentration of HEC on mucoadhesion between porcine buccal mucosa and *in situ* gel-forming artificial saliva.
3. To study the satisfaction of *in situ* gel-forming artificial saliva in xerostomia patients.

CHAPTER II

LITERATURE REVIEW

2.1 Saliva

Saliva is an oral fluid, secreted by three paired major salivary glands including the parotid gland, sublingual gland and submandibular gland (Edgar, Dawes, & O'Mullane, 2012; Han, Suarez-Durall, & Mulligan, 2015; Humphrey & Williamson, 2001). It also contains the secretions from the minor salivary glands that are found at the tongue, lower lip, cheeks, and palate (Roth & Calmes, 1981). Saliva contains 99% of water and 1% organic and inorganic constituents. The main inorganic ions are calcium, potassium, sodium, chloride, phosphate and bicarbonate, contributing to the salinity of saliva (Almståhl & Wikström, 2003). The amount of inorganic constituents of unstimulated saliva is shown in Table 2.1 (Edgar et al., 2012). Saliva is slightly acidic, with pH 6-7 at normal stage, pH 5.3 at low flow, and pH 7.8 at peak flow (Edgar et al., 2012; Humphrey & Williamson, 2001).

Table 2.1 The amount of inorganic constituents of unstimulated saliva (Edgar et al., 2012).

Inorganic constituents	Mean ± S.D.
Sodium (mmol/L)	5.76 ± 3.43
Potassium (mmol/L)	19.47 ± 2.18
Calcium (mmol/L)	1.32 ± 0.24
Magnesium (mmol/L)	0.20 ± 0.08
Chloride (mmol/L)	16.40 ± 2.08
Bicarbonate (mmol/L)	5.47 ± 2.46
Phosphate (mmol/L)	5.9 ± 1.91
Thiocyanate (mmol/L)	0.70 ± 0.42
Fluoride (mmol/L)	1.37 ± 0.76

Approximately 0.5-1.5 lites of saliva is secreted per day in healthy adult. Normal salivary flow rate is 0.3-0.4 ml/min when unstimulated, 1.5-2.0 ml/min when stimulated and 7 ml/min at the maximum stimulated flow rate (Edgar et al., 2012; Humphrey & Williamson, 2001).

The presence of saliva is critical for preservation and maintenance of oral health, teeth and mucosa, due to its 5 major properties: (1) protection and lubrication, (2) clearance and buffering action, (3) maintenance the integrity of the teeth, (4) antibacterial property, and (5) taste and digestion (Edgar et al., 2012; Han et al., 2015; Humphrey & Williamson, 2001).

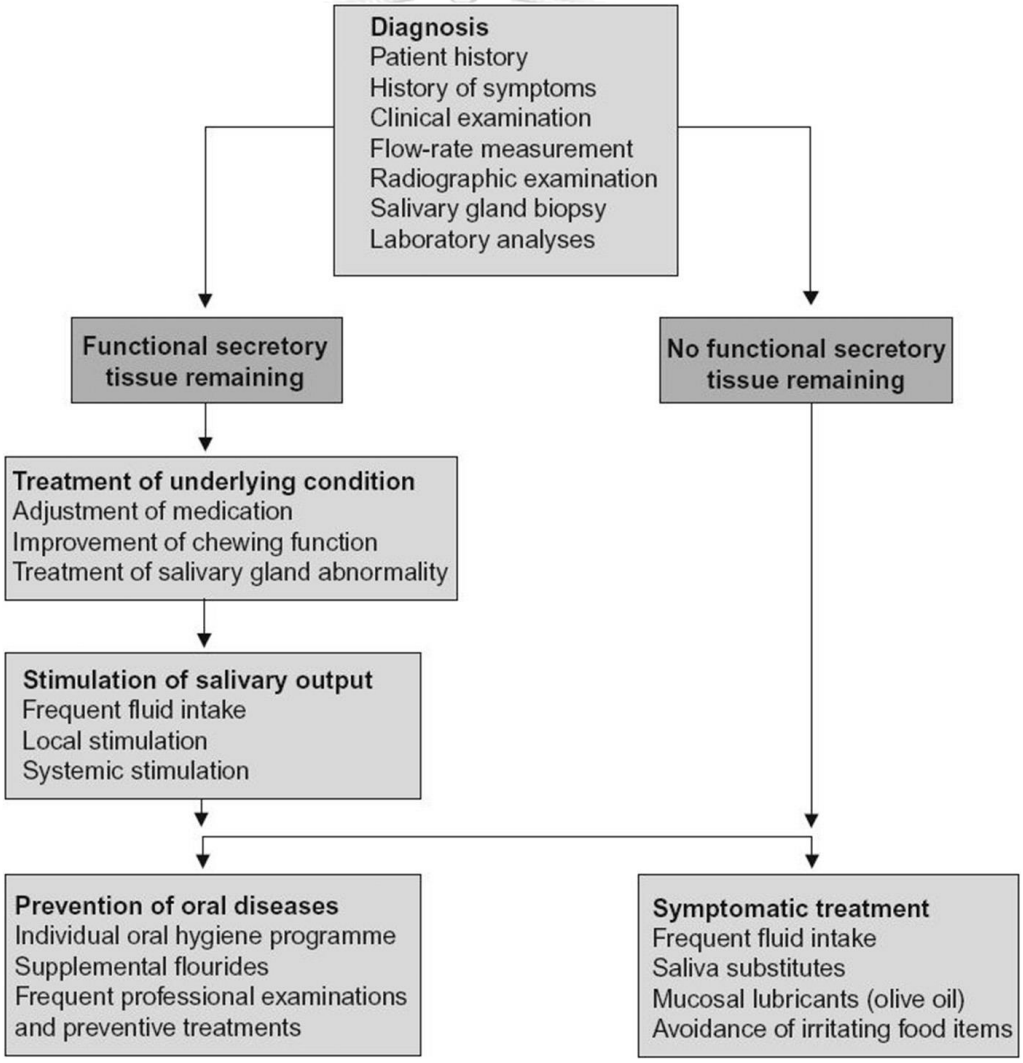
2.2 Xerostomia

The definition of xerostomia is the subjective sensation of dry mouth (Dost & Farah, 2013; Stewart et al., 1998; Villa et al., 2015; Visvanathan & Nix, 2010). It occurs because of a reduction or loss in salivary gland functions, often with a stimultaneous change in the composition of the saliva (Kelly et al., 2004; Visvanathan & Nix, 2010). The conditions that cause of xerostomia are radiotherapy of the head and neck region, use of certain medications, Sjögren's syndrome, type II diabetes (Dost & Farah, 2013; Kelly et al., 2004; Stewart et al., 1998; Visvanathan & Nix, 2010). Other factors include depression, anxiety, stress, and malnutrition (Villa et al., 2015). Xerostomia leads to changes in oral pH and microflora (Mossman & Henkin, 1978). The most complaints of patients anguish from xerostomia include the generalized oral discomfort, difficulty with mastication, swallowing, speech, the wearing of dentures, polydipsia, polyuria (Hamlet et al., 1997), dysphagia, dysgeusia (Dost & Farah, 2013), trauma and wound of oral mucosa, poor oral hygine, and a burning sensation of the oral mucosa (Edgar et al., 2012). It may also results in an increased risk of *Candida* infection, dental caries, periodontal disease, and non-carious tooth loss (Dost & Farah, 2013; Edgar et al., 2012; Kelly et al., 2004; Villa et al., 2015).

2.3 Management and treatment of xerostomia

Management and treatment of xerostomia aim to reduce the undersired symptoms and increase salivary flow. In order to determine the efficacy of the management of xerostomia, a precise diagnosis of the cause and severity level of xerostomia are the most important requisite for its choices of treatment (Han et al., 2015). An example of treatment process, as described by Närhi et al. shows in Figure 2.1 (Närhi, Meurman, & Ainamo, 1999).

Figure 2.1 Diagnosis and treatment of salivary gland hypofunction and xerostomia (Närhi et al., 1999)



The cost versus efficacy of the selected treatment must be evaluated, and a consideration of the side effects of the selected treatment is also necessary. Recommendations for relieving symptoms related to dry mouth include the use of

water, crushed ice, chewing gums, hard lozenges, mints, candies, and artificial saliva (Stewart et al., 1998).

2.3.1 General recommendations

Patients should be advised about fluid intake, at least 2 liters per day, since the regular sips of water have shown to be helpful. Mouth spray containing water and glycerin can also be useful for relief of dry mouth in day time and use of a room humidifier for adding moisture to the environment at night may give some relief during sleep (Han et al., 2015). Patients should be stimulated to increase their fluid intake during meals and avoid irritating substances such as smoking, alcohol and caffeine intake (Visvanathan & Nix, 2010).

Anxiety and stress are recognized as causes of xerostomia. The consultation may be required in this context, and should be properly diagnosed and managed (Villa et al., 2015).

2.3.2 Modifiable behaviors

Patients can improve the temporary causes of dry mouth by changing behaviors which causative factors to patients dry mouth condition including avoiding intake of irritants such as alcohol, caffeine and spicy foods. Patients with long-term smoking habits and alcohol behaviors may need the help of behavioral psychologists to cease them from the offending substances (Han et al., 2015).

2.3.3 Medication substitution and adjustment of dosage regimen

Xerostomia from medication is usually reversible, so reducing the dosage of the medications, ceasing the drug therapy, and potentially replacing the medications with less xerogenic alternatives may cause the salivary flow back to normal (Han et al., 2015; Villa et al., 2015).

2.3.4 Systemic sialogogues

The systemic drugs of choices for use as a salivary stimulant, such as pilocarpine and cevimeline, are approved by the United States Food and Drug

Administration for treatment of dry mouth. Their efficacy depends on the presence of salivary gland function (Villa et al., 2015). Pilocarpine and cevimeline provide a similar effect in patients with dry mouth. They are used only in patients, who still have residual function left. Both drugs also have poor side effects including cutaneous emesis, vasodilatation, excessive sweating, increased urinary frequency, diarrhea, nausea, hypotension, persistent hiccup, bronchoconstriction, bradycardia, and vision problems (Kelly et al., 2004; Villa et al., 2015).

2.3.5 Other treatments

Commonly recommended treatments for the management of xerostomia include candies, chewing gums, saliva substitutes or stimulants. The main concept of saliva substitute is to provide long-lasting moisture in oral cavity. However, solutions, sprays or gels formulations may need to be used frequently during the day depending on their adhesiveness or lasting ability. Since the buffering action of saliva and concentrations of calcium and phosphate in saliva play an important role in tooth demineralization and remineralization processes (Li, Wang, Joiner, & Chang, 2014), xerostomia patients benefited by using products containing calcium and phosphate to maintain the tooth enamel (Featherstone, 2008). Sugar-free chewing gums, flavored with sweetener such as xylitol or sorbitol are available. There is no evidence that chewing gum are better or worse effect than use of artificial saliva, as chewing gums are effective only in patients who is still remaining salivary gland functions. However, chewing gums can be a problem for the elderly, especially those who have arthritis, which affecting the temporomandibular joint (TMJ) or wear removable denture.

2.4 In situ gels

In situ is a Latin word that means “in process”. *In situ* gels are environment-sensitive drug delivery systems that present in the form of solution before it administrated into the body and after the administration, it will undergo *in situ* gelation

and form a gel. There are three mechanisms widely describing the used of biomaterials for triggering the *in situ* gel formation: physical changes in physiological stimuli (e.g. temperature and pH), biomaterials (e.g. diffusion and swelling), and chemical reactions (e.g. ion activation) (Chaudhary & Verma, 2014; Karavasili & Fatouros, 2016; Nirmal et al., 2010).

2.4.1 *In situ* formation based on physiological stimuli

2.4.1.1 Thermally triggered in situ gel systems

Temperature-sensitive hydrogels are type of environmentally sensitive polymer systems which widely studied in drug delivery research. There are three categories of temperature-sensitive hydrogels including negatively thermo-sensitive, positively thermo-sensitive, and thermally reversible gels as shown in Table 2.2 (Patil, Kadam, Bandgar, & Patil, 2015; Wu et al., 2018).

Table 2.2 Classification of thermally triggered *in situ* gel systems (Patil et al., 2015)

Types of Hydrogels	Characteristics	Polymers
Negatively thermo-sensitive	Polymer solution have a lower critical solution temperature (LCST) and undergo micellization upon heating above the LCST.	Poly-(N-isopropylacrylamide) (PNIPAAm)
Positively thermo-sensitive	Polymer solution have an upper critical solution temperature (UCST) and undergo micellization upon cooling below the UCST.	Poly-(acrylic acid) (PAA) and Polyacrylamide (PAAm) or Poly-(acrylamide-co-butyl methacrylate)
Thermally reversible	Gelation can be reversed by changing temperature	Pluronics®, Tetronics®, Poloxamers®

Polymer solution undergoes micellization in temperature-dependent manner and, later, the gel is formed by micellar packing as Figure 2.2 (Karavasili & Fatouros, 2016).

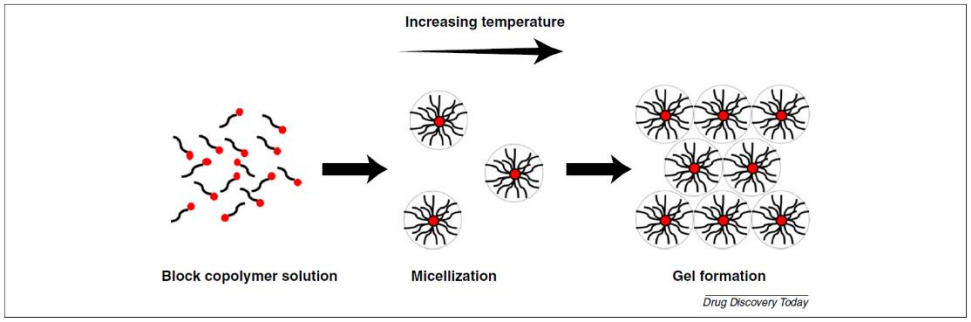


Figure 2.2 The mechanism of *in situ* gelation of a thermo-responsive polymer as a function of temperature (Karavasili & Fatouros, 2016).

2.4.1.2 pH-triggered in situ gel systems

The pH- sensitive polymers response to changes in environmental pH by containing acidic or basic groups that can accept or release protons in their structures. Polymeric hydrogels undergo rapidly transition to the viscous gel, when the external pH increases for polymer containing weakly acidic groups (anionic) but decreases in case of weakly basic groups (cationic) due to uncoiled polymer chains by neutralizing leads to gel expansion. Polymers used in pH-triggered *in situ* gel systems are Carbopol® and its derivatives (Patil et al., 2015; Wu et al., 2018).

2.4.2 *In situ* formation based on physical mechanism

2.4.2.1 Swelling

In situ formation may also occur when water from surrounding environment is absorbed by the gelling agents and then gel expands. Polymers used in swelling systems are glycerol mono-oleate, etc (Patil et al., 2015).

2.4.2.2 Diffusion

The sol- gel transition occurs when the solvent of polymer solution diffuses out to surrounding tissue and, after that, water or fluid in the body, which does not dissolve the polymer, diffuses to replace the solvent and results in precipitation or solidification of polymer matrix. Polymers used in diffusion systems are N- methyl pyrrolidone, etc (Setthajindalert & Phaechamud, 2012).

2.4.3 *In situ* formation based on chemical reactions

2.4.3.1 Ion-activated in situ gel system

Polymers may undergo phase transition in presence of various ions due to the interaction with functional groups of polymer chains. Figure 2. 3 shows the gelation mechanism of polysaccharides. The development of ionic interactions between cations and functional groups in polymer structure results in the formation of a three- dimensional network in the gel structure. Polymers used in ion- activated *in situ* gel system are gellan gum, sodium alginate, pectin, etc (Karavasili & Fatouros, 2016; Wu et al., 2018).

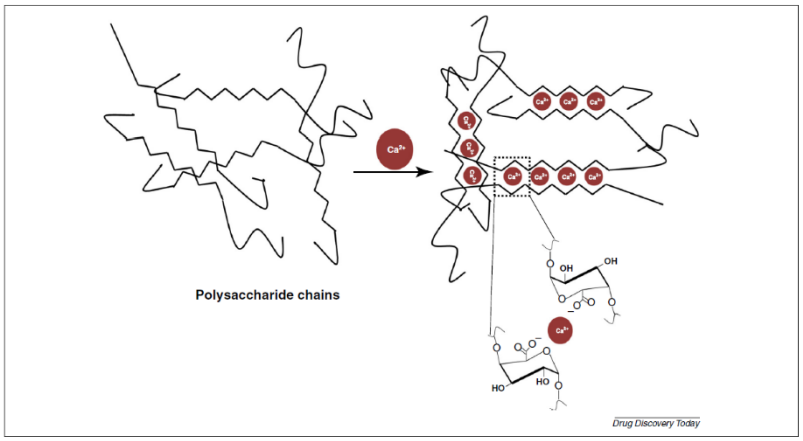


Figure 2.3 Ion-induced *in situ* gelation of anionic polysaccharides (e.g. pectin) in the presence of divalent cations (Karavasili & Fatouros, 2016).

2.5 Gellan gum

Gellan gum is an extracellular bacterial polysaccharide produced by *Sphingomonas elodea* (ATCC31461), previouly known as *Pseudomonas elodea* or *Auromonas elodea*, which CP Kelco company (San Diego, USA) discovered its commercial potential. The gum is produced by fermented medium, consists of carbon source, nitrogen source, and inorganic salts, with this organism. The fermentation is carried out under sterile conditions with rigid control of pH, temperature, aeration, and agitation. When fermentation is complete, the viscous fermented broth is pasteurized to destroy the viable cells. The fermented broth is then refined to obtain the polysaccharide in the acylated native form or the deacylated form (Sanderson, 1990). Gellan gum is formerly known as polysaccharide S-60, a linear anionic polymer with a repeated tetrasaccharide sequence which consists of b-D-glucose, b-D-glucuronate and a-L-rhamnose in the molar ratios of 2:1:1 units containing one carboxyl side group as Figure 2.4 (Yamamoto & Cunha, 2007).

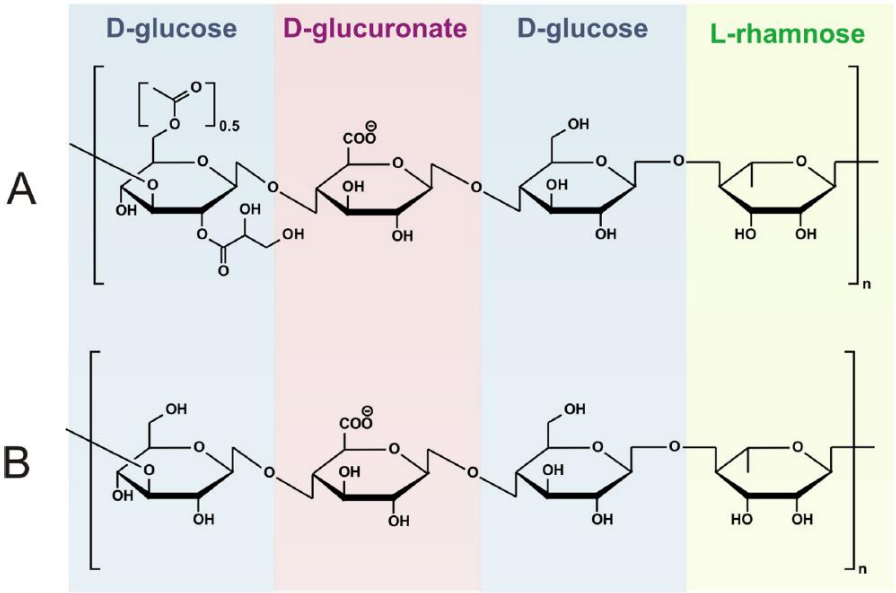


Figure 2.4 The structure of (A) acylated native and (B) deacylated form of gellan gum (Osmalek, Froelich, & Tasarek, 2014).

Gellan gum forms clear hydrogels in the presence of mono-, di- and trivalent cations. The traditional mechanism proposed for the sol-gel transition of gellan gum, as Figure 2.5, is also temperature-dependent based on coil-to-helix transition. When

the solution is heated to at least 70 °C to obtain a clear water solution and is converted to double helix transition when cooled down (Osmalek et al., 2014), followed by helix to helix aggregation depending on the presence of cations, which involves weak interactions such as hydrogen bond ionic bond and Van der Waals force as shown in Figure 2. 6. Gel-promoting ions can reduce the impact of electrostatic repulsions between helices due to carboxyl groups in the chains, which augment the development of a gel network (Bradbeer, Hancocks, Spyropoulos, & Norton, 2015).

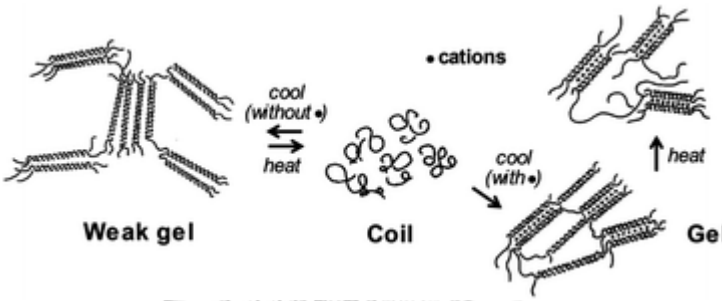


Figure 2. 5 Gelation mechanism of gellan gum (Ferris, Gilmore, Wallace, & in het Panhuis, 2013)

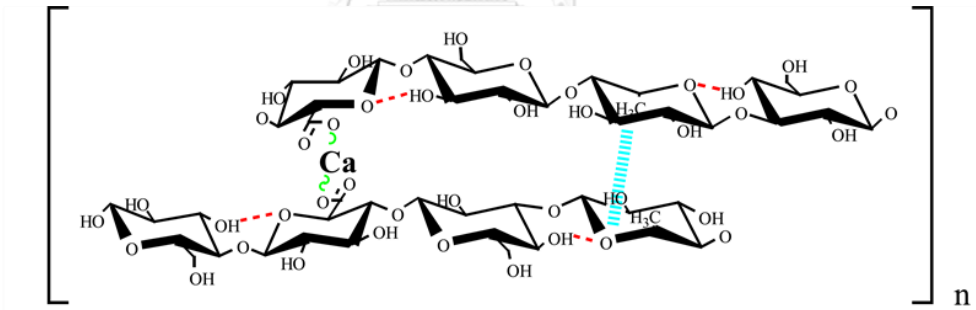


Figure 2.6 Gelation mechanism of deacetylated gellan gum in aqueous solution (Tako et al., 2016).

Gellan gum has been developed as a drug delivery system due to its specific gel-forming properties in different media. Various drug delivery systems based on gellan gum have been investigated for ophthalmic (Balasubramaniam, Kant, & Pandit, 2003; El-Kamel, Al-Dosari, & Al-Jenoobi, 2006; Hîncu et al., 2007; Kesavan, Nath, & JK, 2010; Liu et al., 2010; Meseguer, Buri, Plazonnet, Rozier, & Gurny, 1996), nasal (Cao et al., 2009; Cao, Zhang, & Jiang, 2007; Jansson, Hägerström, Fransén, Edsman, & Björk, 2005; Mahajan & Gattani, 2009), oral (Kubo et al., 2003; Rajinikanth et al., 2007)(Kubo,

Miyazaki, & Attwood, 2003; Rajinikanth, Balasubramaniam, & Mishra, 2007), buccal (Remuñán-López, Portero, Vila-Jato, & Alonso, 1998), rectal (Gupta & Sharma, 2009) and vaginal administration (El-Kamel & El-Khatib, 2006). In addition, materials based on gellan gum are investigated in many field such as wound healing (Cencetti, Bellini, Longinotti, Martinelli, & Matricardi, 2011; Shin, Olsen, & Khademhosseini, 2012), bone repair (Chang et al., 2010), gene delivery (Goyal et al., 2011), dental care (Chang, Huang, Yang, Kuo, & Lee, 2012) and biosensor synthesis (Wen, Yang, Hu, Chen, & Jia, 2008).

2.6 Bioadhesion

The definition of bioadhesion can be describes as a phenomenon of the intermolecular interactions between the polymer and the biological substrate surfaces. These bioadhesive polymers can adhere to the biological surface for an extended period of time (Roy, Pal, Anis, Pramanik, & Prabhakar, 2012; Yu, Andrews, & Jones, 2014). In addition, in case that adherent substrate surface is a mucosal surface, bioadhesion is specifically referred to as mucoadhesion (Yu et al., 2014). Since 1947, the mucoadhesive polymers have been used for the development of pharmaceutical formulations, when the penicillin delivery system was established for the oral mucosa using tragacanth and dental adhesive powders (Harding, Davis, Deacon, & Fiebrig, 1999; Scrivener & Schantz, 1947). After that Roy et al. were use sodium carboxymethylcellulose (SCMC) and petrolatum for the formulations. Ensuing research resulted in the development of mucoadhesive delivery systems which consisted of pectin, gelatin and SCMC (Roy et al., 2012).

Mucoadhesion is a complex process and is not completely understood (Salamat-Miller, Chittchang, & Johnston, 2005; Yu et al., 2014). Additionally, it has been shown that anionic polymers are usually have more bioadhesion with mucosa than cationic or uncharged polymer (Salamat-Miller et al., 2005). Several theories have been suggested to explain mucoadhesion, remarkably, the adsorption theory, the diffusion-interpenetration theory, the electronic transfer theory, the fracture theory,

the wetting theory (Salamat-Miller et al., 2005), and the mechanical interlocking theory (Yu et al., 2014).

2.6.1 Adsorption theory

It involves the secondary interaction between surface of polymer and mucosa. The initial interfacial bonding forces are ascribed to non-covalent forces such as electrostatic attraction, Van der Waals' force, hydrogen bond and hydrophobic interaction, resulting in semi-permanent interactions. These secondary chemical bonds mostly depending on polymer properties (Salamat-Miller et al., 2005).

2.6.2 Diffusion-interpenetration theory

It involves the entanglement and permeation between the mucus and the polymer chains. Initial step, the mucus and the bioadhesive polymer chains contact was created by weak physical forces, such as attraction and electrostatic forces, due to the mobility of the polymer chains. Then, bioadhesive polymer chains permeate into mucus layer to achieve mucoadhesion through more bond formation (Salamat-Miller et al., 2005; Yu et al., 2014).

2.6.3 Electronic transfer theory

The transfer of electrons between two different substrates results in a double-layer electron configuration at the interface of mucus and polymer due to the different electronic properties of the polymer and mucus glycoprotein. (Salamat-Miller et al., 2005; Yu et al., 2014).

2.6.4 Fracture theory

The fracture strength involves the force required to detach the polymer from the mucus surface. Depending on the occurred location, fractures may be classified into polymer-mucus fracture, polymer fracture, and mucus fracture. Fracture theory not only presents the measurement of the adhesion between the polymer surface and the mucus surface, however, it is also used to

investigate the strength of intermolecular interactions within the mucus or polymer (Yu et al., 2014).

2.6.5 Wetting theory

It explains the liquid or low viscosity mucoadhesive system. The spreadability in the system measured by the liquid- solid contact angle determines the interaction. Furthermore, there are two forces that have play roles in liquid-solid contact angle. The adhesive force between a solid and liquid allows the drop to spread across the surfaces. The cohesive force, in the other hand, causes the drop to ball up and avoid contact to the surface. To determine whether the wetting of the surface is favorable, the contact surface less than 90° will allow the liquid to spread out more. However, if the contact surface is less than 90° the molecules of the liquid maintain their shape and less spread out and the wetting surface is less favorable and the droplet will avoid the surface (Salamat-Miller et al., 2005; Yu et al., 2014).

2.6.6 Mechanical interlocking theory

It involves the adhesion between liquid and a rough surface or a surface riched in pores. Adhesion occurs by adhesive polymer filling the voids or pores of the surfaces and holding together by mechanical interlocking (Yu et al., 2014).

In general, adhesive polymers can be classified by source, aqueous solubility, charge and potential bioadhesive forces, as listed in Table 2.3.

Table 2.3 Classification of adhesive polymers (Salamat-Miller et al., 2005)

Categories	Examples
Sources	
Semi-natural/natural	Agarose, chitosan, gelatin
	Hyaluronic acid
	Various gums (guar, xanthan, gellan, carragenan, and pectin)
Synthetic	Cellulose derivatives [carboxymethylcellulose (CMC), hydroxyethyl cellulose (HEC) and hydroxypropyl methyl cellulose (HPMC)]
	Poly(acrylic acid)-based polymers [polyacrylates, poly(alkylcyanoacrylate) and poly(isobutylcyanoacrylate)]
	Others [Polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), and thiolated polymers]
Aqueous solubility	
Water-soluble	HEC, HPMC (cold water), SCMC, sodium alginate
Water-insoluble	Chitosan (soluble in dilute aqueous acids), ethyl cellulose (EC), polycarbonate (PC)
Charge	
Cationic	Aminodextran, chitosan, dimethylaminoethyl (DEAE)-dextran, trimethylated chitosan
Anionic	Chitosan-Ethylene diamine tetra-acetic acid, CMC, pectin, sodium alginate and xanthan gum
Non-ionic	Hydroxyethyl starch, poly(ethylene oxide), PVA and PVP
Potential bioadhesive forces	
Covalent	Cyanoacrylate
Hydrogen bond	Acrylates, PC and PVA
Electrostatic interaction	Chitosan

2.7 Hydroxyethyl cellulose (HEC)

HEC is a white, odorless, tasteless powder that, as in 1% aqueous solution, it is non-ionic and has a pH of 6.5-8.5. Figure 2.7 represents the chemical structure of HEC. It is soluble in hot and cold water, 70% soluble in alcohol, and generally insoluble in organic solvents. Being non-ionic in character, HEC does not react with polyvalent cations, and, in solution, is generally unaffected by moderate shifts in pH. HEC is compatible with sodium chloride (0.5-26%), alum (2.0%), ammonium sulfate (10.0%), atropine sulfate, pilocarpine- hydrochloride, detreomycin, zinc sulfate, potassium iodide, and some anionic and amphoteric surfactants (12.5%) depending on specific concentrations. This polymer has well-performanced abilities including, suspending, emulsifying, binding, thickening, stabilizing, and it also provide good protection action by retaining water and forming a film. HEC is used in different kinds of industrial fields such as thickening paints, thickener in cement mortar, finishing of textile and sizing agent in paper making. To prepare of HEC in industry level, cellulose pulp or pure cellulose is treated with sodium hydroxide solution. Cellulose is swollen and converted into active alkaline cellulose. Once the active alkaline cellulose reacts with gaseous ethylene oxide, the HEC is produced by esterification reaction. During the esterification reaction, hydroxyl groups in cellulose are replaced the hydrogen atoms, which result in the consequence of the polymer's water stability (Abdel-Halim, 2014; Santos, 1986). HEC has demonstrated synergistic effect on viscosity when combined with an equal amount of an anionic cellulose derivatives. The result showed that HEC (viscosity of 1800 cps) combined with cellulose gum (viscosity of 1500 cps) had an actual viscosity of 3200 cps when the expected viscosity was 1650 cps (Rufe, 1975).

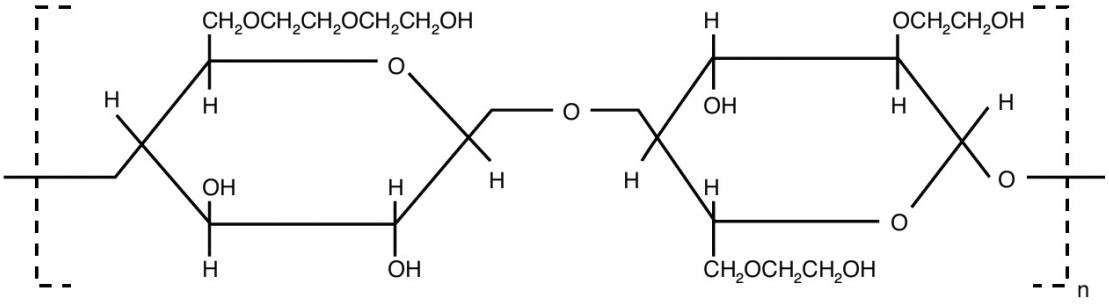


Figure 2.7 Hydroxyethyl cellulose structure (Abdel-Halim, 2014)

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and instruments

3.1.1 Materials

1. Brilliant blue
2. Calcium chloride dehydrate, AR grade (Merck, Germany, Lot no. A0211082 043)
3. De-ionized water (DI water)
4. Gellan gum, low acyl, Food grade (Kelcogel®, CPKelco, USA)
5. Hydroxyethyl cellulose (HEC), USP grade
6. Magnesium chloride hexahydrate cryst., USP grade (Merck, Germany, Lot no.A1106632 728)
7. Methyl paraben, USP grade (S. Tong Chemical Co., Ltd., Thailand, Lot no. GBG 0001718)
8. Porcine buccal mucosa
9. Potassium chloride, USP grade (S. Tong Chemical Co., Ltd., Thailand, Lot no.1004110286)
10. Potassium phosphate, USP grade (S. Tong Chemical Co., Ltd., Thailand)
11. Sodium benzoate, USP grade
12. Sodium chloride, USP grade (S. Tong Chemical Co., Ltd., Thailand, Lot no.K41012000)
13. Sodium hydroxide, AR grade (Merck, Germany, Lot no.B1233898 546)
14. Sorbitol, USP grade (S. Tong Chemical Co., Ltd., Thailand, Lot no.C3C01)

15. Xylitol, Food grade (Chemipan Corporation Co., Ltd., Thailand, Lot no.117050506)

3.1.2 Instruments

1. Analytical balance (Model AG285, Mettler Toledo, Switzerland)
2. Analytical balance (Model APG403-S, Mettler Toledo, Switzerland)
3. Cooling incubator (Model KB720, Binder, Germany)
4. Magnetic stirrers (Model RCT basic, IKA®-Werke, Germany)
5. pH meter (Model SevenCompact™pH/Ion S220, Mettler Toledo, Switzerland)
6. pH meter (Model SevenEasy™ pH S20, Mettler Toledo, Switzerland)
7. Universal Testing Machine (Model EZ-S, SHIMADZU, Japan)
8. Viscometer (Model DV-II+, Brookfield Engineering Labs., Inc., USA)
   - Spindle LV2 (62)
   - Spindle LV3 (63)
9. Viscometer (Model DV2T, Brookfield Engineering Labs., Inc., USA)
   - Sample cup (CPA-44YZ)
   - Spindle (CPA-41Z)
10. Viscometer (Model SV-10, A&D Company, Limited, Japan)
    - Small volume sample container (AX-SV-34)

3.2 Preliminary study of gellan gum concentrations

3.2.1 Preparation of electrolyte stock solution

The electrolyte stock solution was prepared. The solution components are shown in Table 3.1. All of components were dissolved in DI water and the volume were adjusted to 100 ml by using volumetric flask. The concentration

of electrolyte solution was 10 times of normal human saliva's electrolyte concentration (Edgar et al., 2012).

Table 3.1. Components of electrolyte stock solution (Edgar et al., 2012).

Component	Content
Calcium chloride	0.053 g
Magnesium chloride	0.005 g
Potassium chloride	0.221 g
Potassium phosphate	0.540 g
Sodium chloride	0.132 g
DI water	q.s. to 100 ml

3.2.2 Preparation of *in situ* gel-forming solution and appearance

The *in situ* gel-forming solution was prepared at various concentrations of gellan gum (0.025, 0.05, 0.075, 0.1%w/v). The formula is shown in Table 3.2. The artificial saliva was prepared by dissolving gellan gum in DI water, heating the solution to 70 °C, stirring by magnetic stirrer and then cooling down to 40 °C. After that, sorbitol, xylitol and electrolyte stock solution were added and mixed well. The *in situ* gel-forming solution was left at room temperature overnight before an appearance of the *in situ* gel-forming solution was observed. The concentration that gave free-flow solution was selected for *in situ* gel forming study.

Table 3.2. Components of *in situ* gel-forming solution

Component	Content(%)
Gellan gum	0.025, 0.05, 0.075, 0.1
Sorbitol	5
Xylitol	3
Electrolyte stock solution	10
DI water	q.s. to 100

3.2.3 *In situ* gel formation

0.1% and 0.15% of gellan gum *in situ* gel-forming solution were dropped on a glass slide and then electrolyte stock solution with 1% w/v Brilliant blue was dropped on the top as in Figure 3.1. The *in situ* gel was observed. The concentration that gave harder structure of gel was selected for further development of *in situ* gel artificial saliva.

in situ gel-forming solution

Electrolyte stock solution with 1%w/v Brilliant blue

Figure 3.1. *In situ* gel forming study

3.3 Effect of pH and Hydroxyethyl cellulose on gellan gum solution

3.3.1 Preparation of *in situ* gel-forming solution

Six formulation were composed of ingredients as listed in Table 3.3. A solution of 0.15% w/v gellan gum in DI water was heated to 70 °C and stirred.

For IG-5 and IG-6, HEC was added and mixed well with the heated solution. For *in situ* gel, when the mixture was cooled down to 40 ℃, calcium chloride stock solution was added to obtained 0.1% w/v in the final solution. pH was adjusted with diluted sodium hydroxide or diluted hydrochloric acid for IG-1, IG-2, and IG-3, and measured by MColorpHast™ pH-indicator strips. All of formulations were stored for 24 hours at cool place.

Table 3.3. Formulation of *in situ* gel-forming solutions

Formulation code	Gellan gum (%w/v)	HEC (%w/v)	Distillated water	Adjust pH to
IG-1	0.15	-	q.s. to 100	5
IG-2	0.15	-	q.s. to 100	6
IG-3	0.15	-	q.s. to 100	7
IG-4	0.15	-	q.s. to 100	-
IG-5	0.15	0.15	q.s. to 100	-
IG-6	0.15	0.30	q.s. to 100	-

3.3.2 Viscosity

The viscosity of both *in situ* gel-forming solution (without calcium chloride) and *in situ* gel (with calcium chloride) was determined by using Brookfield viscometer model DVII+ with spindle no. 61 and no. 63, respectively, at 140 rpm.

3.3.3 Gelling capacity

The gelling capacity of prepared *in situ* gel-forming solution was determined by modified method of previously study (Makwana, Patel and Parmar, 2016) placing a drop of formulation in a beaker containing 50 ml of

freshly prepared 1% w/v calcium chloride solution. Gel formation was observed and the time for gelation and the time taken for the formed gel to redissolve were recorded.

3.4 Preparation of *in situ* gel-forming artificial saliva

Six formulations were composed of ingredients as listed in Table 3.4. They were prepared by dissolving gellan gum in DI water, heating the solution to 70 °C, stirring by magnetic stirrer, HEC was added and mixed well with the heated solution and then cooling down to 40 °C. After that sorbitol, xylitol, electrolyte stock solution (ESS), sodium benzoate (SB) and methyl paraben (MP) were added and mixed well, as shown in Figure 3.1 All formulations were used for further experimental.

Table 3.4 Formulation of *in situ* gel-forming artificial saliva

Formulation	Gellan gum (g)	HEC (g)	Sorbitol (g)	Xylitol (g)	ESS (ml)	SB (g)	MP (g)	DI water (ml)
1	0.1	-	5	3	10	0.1	0.1	q.s. to 100
2	0.1	0.15	5	3	10	0.1	0.1	q.s. to 100
3	0.1	0.30	5	3	10	0.1	0.1	q.s. to 100
4	0.15	-	5	3	10	0.1	0.1	q.s. to 100
5	0.15	0.15	5	3	10	0.1	0.1	q.s. to 100
6	0.15	0.30	5	3	10	0.1	0.1	q.s. to 100

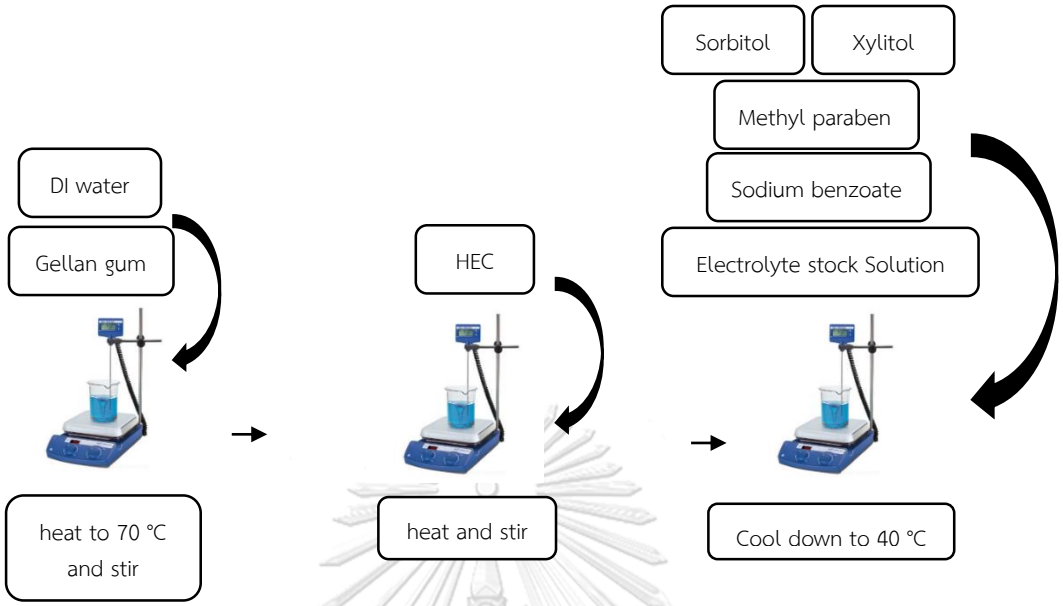


Figure 3.2 Preparation of *in situ* gel-forming artificial saliva

3.5 Physical stability of *in situ* gel-forming artificial saliva

To evaluate stability of *in situ* gel-forming artificial saliva, six formulations were kept in cooling chamber that was programmed the temperature at 4 °C, 48 hours and 45 °C, 48 hours as one cycle, 96 hours in total. The test was carried out for six cycles. At the end of each cycle, six formulations of *in situ* gel-forming artificial saliva were visually evaluated in term of clarity by placed in front of black background and triplicate measurements of pH by using SevenEasy™pH S20, Mettler Toledo and viscosity by using viscometer DV2T, Brookfield Engineering Labs., Inc., USA were performed. Formulations, which did not show significant difference to initial state were subjected to further experiments.

3.6 Simulated gelation time

To evaluate the simulated gelation time of *in situ* gel-forming artificial saliva, triplicate measurements of viscosity were performed by A&D viscometer model SV-10. The *in situ* gel-forming artificial saliva was diluted with electrolyte stock solution in ratio of 9:1 before measurement. The viscosity of all formulations was recorded every 15 minutes for 60 minutes. When viscosity was statically significant more than initial viscosity at p-value less than 0.05, the time was recorded as simulated gelation time.

3.7 Mucoadhesive test

3.7.1 Preparation of porcine buccal mucosa

Porcine buccal mucosa was obtained from 24-hour slaughtered pig. Mucosa was carefully removed using dissecting scissors and scalpel. All mucosa was stored at -20 °C until used, for a maximum of 3 days. Before used, the mucosa was rinsed and soaked in 10%v/v diluted electrolyte stock solution for 10 minutes, circular clear plastic sheet (diameter 10 mm) was attached on a basal of mucosa by cyanoacrylate glue and the mucosa was cut into circular pieces, 4-6 pieces per buccal side.

3.7.2 Mucoadhesive measurement

To investigate mucoadhesion of *in situ* gel-forming artificial saliva by modified method from previously study (Cevher, Taha, Orlu, & Araman, 2008), the universal testing machine, equipped with 5-kg load cell, was used for tensile strength measurement. *In situ* gel-forming artificial saliva was weighed to 30 grams onto plastic petri dish and then placed on base of the instrument. The circular clear plastic sheet with porcine buccal mucosa on the top was attached to the upper movable cylinder probe of the instrument by using double-sided tape. The mucosa was lowered towards the surface of *in situ* gel-forming artificial saliva at constant speed until contacting the surface for 5

minutes then the probe was withdrawn upwards at 10 mm per min until separating from the surface. The tensile work (Newton, N) was recorded and *in situ* gel-forming artificial saliva in petri dish was reweighted. The formulation, which had the highest mucoadhesive force was subjected for satisfaction study.

3.8 Satisfaction study

A open-label, non-randomized, controlled trial was designed in order to study the satisfaction of 15 xerostomia patients on *in situ* gel-forming artificial saliva, at Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

This study protocol was approved by The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Bangkok, Thailand (Project 083.1/61). Thai male or female patients aged 18-70 years old with xerostomia using artificial saliva and no history of drug allergy were recruited. The volunteers were clearly informed about study protocol, asked to read the information sheet and signed the consent form. The exclusion criteria were a history of allergic reaction with ingredients of formulation, patients with immune system disorder, wish to terminate from study or violation of the study protocol.

In situ gel-forming artificial saliva was filled in 25-ml bottle with spray nozzle, as test product. The volunteers were asked to answer the questionnaire about dryness in their mouth and then used the *in situ* gel-forming artificial saliva spray for 4 positions, left buccal mucosa, right buccal mucosa, hard palate and tongue, 1 puff each. After left for 10 minutes, the volunteers were asked to answer the questionnaire about dryness in their mouth again and about product's satisfaction. The result of visual analogue scales (VAS) dry mouth questionnaire in mm unit was calculated by different between before and after use. The differences were examined using t-test. The scores for satisfaction of product were shown as median.

3.9 Statistical analysis

Data were presented in mean ± standard deviation (SD). Differences between treatments in 3.3.2 and 3.7.2 were examined using the one-way analysis of variance (ANOVA) with a Tukey's post-hoc test. The confidence interval for statistical analysis of these experiments was 95% which p-value less than 0.05 was considered as statistical difference.

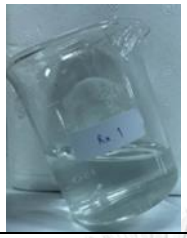
CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preliminary study of gellan gum concentrations

4.1.1 Preparation of *in situ* gel-forming solution and appearance

Table 4.1 shows the appearance of *in situ* gel-forming solution with various concentrations of gellan gum (0.025, 0.05, 0.075 and 0.1%w/v). All of formulations exhibited soft gel formation and the highest concentration of gellan gum provided the hardest gel. The traditional mechanism for the sol-gel transition of gellan gum is depended on a random coil (heated) to double helix transition (cooled), followed by aggregation of helix to helix, which related to weak interactions such as Van der Waals force and hydrogen bond. Strength of gel enhances by bonding of ionized carboxyl group (COO^-) in gellan gum structure and cation (Ca^{2+}, Na^+, K^+ and Mg^{2+}) in electrolyte stock solution. Gel-promoting ions can reduce the impact of electrostatic repulsions of ionized carboxyl group between the gellan gum helices when cooling, enhancing the development of a network. In addition, the use of divalent cations lead to occurrence of ionic bridges between carboxylic groups of neighbouring chains. (Bradbeer et al., 2015). The result was consistent with the previous study of Meng, Hong and Jin (2013), the strength of the gel increased with gellan gum concentration was reported. The appearance of 0.1%w/v gellan gum gel was a little bit softer than desired. Thus, 0.1 and 0.15%w/v gellan gum was selected for the *in situ* gel forming study (Meng, Hong, & Jin, 2013).

Table 4.1 Appearace of gellan gum *in situ* gel-forming solution

Gellan gum (%w/v)	**0.025**	**0.05**	**0.075**	**0.1**
Gel strength	+	++	+++	++++
pH	5.5	5.5	5.5	5.5
Appearance				

Gel strength: + very weak gel, ++ weak gel, +++ partly soft gel, ++++ soft gel

4.1.2 *In situ* gel formation

Figure 4.1 shows the appearance of *in situ* gel that immediately occurred when *in situ* gel-forming solution contacting with electrolyte stock solution. 0.15% w/v of gellan gum *in situ* gel formed harder and more obvious circular drop than that from 0.1% w/v. Due to the increased concentration of gellan gum, the bonding between ionized carboxyl groups and cation occurred more. Yamamoto and Cunha (2007) report that the gellan gum chains are closer to each other at higher concentrations, the probability of aggregation and the formation of junction zones are enhanced. However, both concentrations could form gel upon contacting with electrolyte. Thus, 0.1 and 0.15% w/v of gellan gum was selected to development for artificial saliva (Yamamoto & Cunha, 2007).

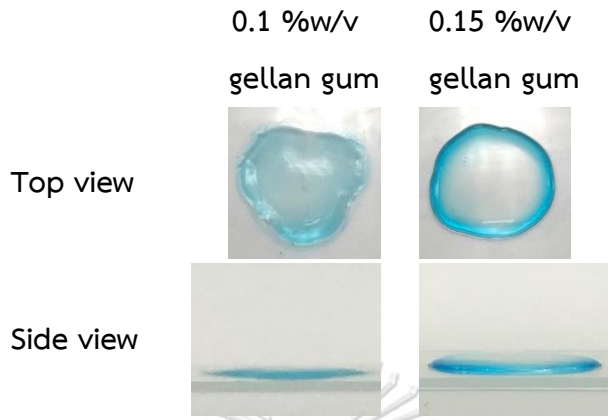


Figure 4.1 Appearance (top and side view) of gellan gum *in situ* gel formation with electrolyte stock solution at gellan gum concentration of 0.1 and 0.15 %w/v

4.2 Effect of pH and Hydroxyethyl cellulose on gellan gum solution

4.2.1 Viscosity

The viscosity of formulations at pH 5, 6 and 7 (IG-1, IG-2 and IG-3, respectively) before adding calcium chloride were lower than the viscosity after adding calcium chloride and this showed that the stronger gelation occurred in the presence of calcium ions. The viscosity of formulations at pH 5, 6 and 7 after gelation were significantly different among each other (p-value < 0.05) and the formulation at pH 6 (IG-2) exhibited the highest viscosity, as demonstrated in figure 4.2. The viscosity of gellan gum solution decreased as the pH increased, due to the electrostatic repulsion of ionized carboxyl groups (COO^-) between the chains of gellan gum. Thus, when the pH increased, the number of ionized carboxyl groups increased and all of them were dissociated to ionized form at pH 7 and above (Yamamoto and Cunha, 2007; Cao et al.,

2009). When calcium chloride was added for *in situ* gelation, cations could reduce the electrostatic repulsion and divalent cations could strengthen the gel network by bonding with two chains of carboxyl groups (Rakde, Galgatte, & Chaudhari, 2015; Tang, Lelievre, Tung, & Zeng, 1995; Yamamoto & Cunha, 2007). The more ionized carboxyl groups (by increase of pH), the stronger the gel network, as shown by higher viscosity of the gel at pH 6 than the viscosity of the gel at pH 5. This phenomenon was not observed for the gel at pH 7 which was adjusted to pH 7 by using sodium hydroxide. For the gel at pH 7, sodium ions were already in the solution, so sodium ions formed the ionic bond with carboxyl groups at better efficient rate than calcium. However, sodium ion had only one valence electron, it formed bond with only one carboxyl group on gellan gum chain (no interconnecting-chain network) and it resulted in forming weak gel. Thus, the further development of artificial saliva will use a native pH of gellan gum (pH 6) to get the strongest *in situ* gel.

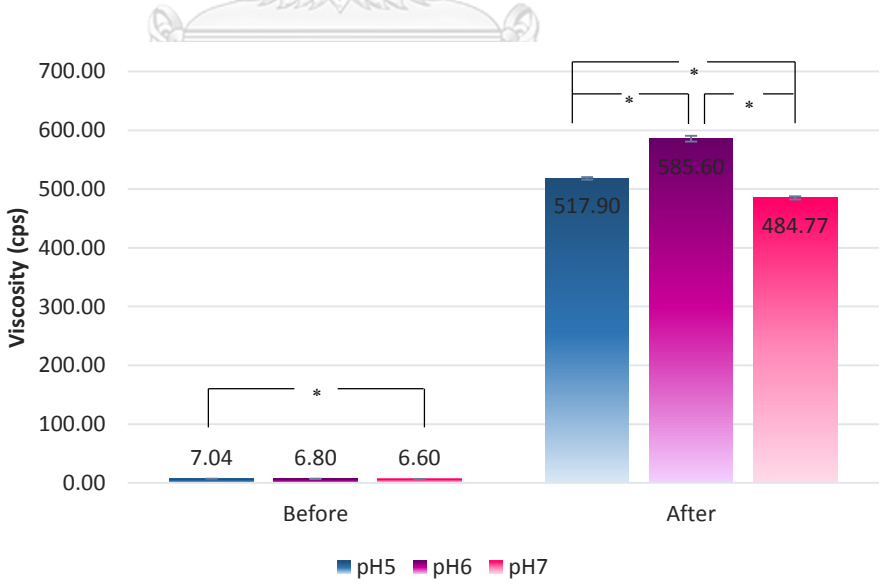


Figure 4.2 effect of pH before and after adding calcium chloride
*significant difference (p-value < 0.05)

Figure 4.3 shows the effect of HEC concentration on gellan gum gelation. The viscosity of formulations before gelation significantly increased (p-value < 0.05) when the concentration of HEC increased. However, after gelation, the significant differences of the viscosity was found only on the formulation with 0.30%w/v HEC (IG-6) (p-value < 0.05). HEC is non-ionic water soluble polymer with various functions, such as thickener and binder, widely used by the cosmetic and pharmaceutical industries (WHO., 1980). The viscosity of solutions dramatically increased when the concentration of HEC increased, due to its viscosity-enhancing property. Nevertheless, after gelation, the concentration of HEC had little effect on the gel, since calcium ions induced much stronger gel network. The development of *in situ* gelling system by using HEC for adjusting the viscosity of solutions, as in formulations, had less influence on the quality of occurred gel. Therefore, the further development of artificial saliva can use HEC in formulation as viscosity-inducing agent with less effect on *in situ* gel formation.

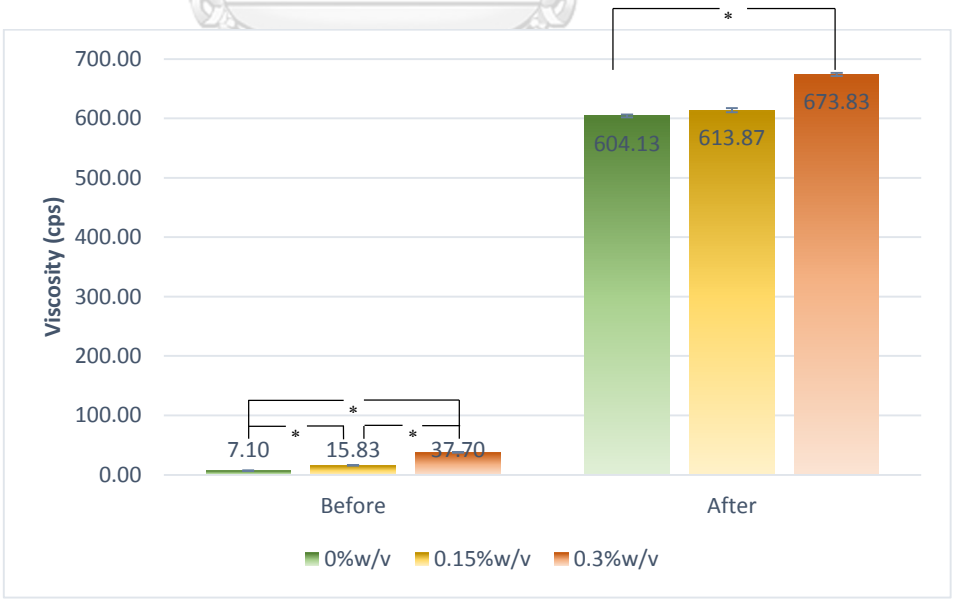


Figure 4.3 effect of HEC concentration before and after adding calcium chloride
*significant difference (p-value < 0.05)

4.2.2 Gelling capacity

Table 4.2 shows the gelation time and the residence time of gel of all formulations. All of formulations exhibited rapid sol-to-gel transformation and the gelation was occurred within 30 seconds. Gels from the acidic formulations (IG-1 and IG-2) were stable than that from the neutral formulation and they were more stable when HEC concentration in the formulations increased. Figure 4.4 shows the appearance of *in situ* gel that immediately occurred upon contacting with 1% w/v calcium chloride solution. Water-soluble color was added in the gellan gum solutions for better observation. Only formulation at pH7 (IG-3) formed unshaped gel. The formulations that contained HEC (IG-5 and IG-6) formed hard gel and the gel stability depended on concentration of HEC, due to the viscosity enhancing property of HEC. This results in that the in situ gel-forming solution had more viscous and formed more stable gel.

Table 4.2 Gelling capacity

Formulation	pH	HEC(%w/v)	Gelation time	Gel residence time
IG-1	5.00	-	+++	++
IG-2	6.00	-	+++	++
IG-3	7.00	-	+++	+
IG-4	6.00	-	+++	++
IG-5	5.96	0.15	+++	++
IG-6	6.08	0.30	+++	+++

Gelation time: +++ immediately within 30 seconds

Gel residence time (Gel redissolving time): + within 60 -120 min, ++ within 121 - 150 min, +++ within 151 -210 min

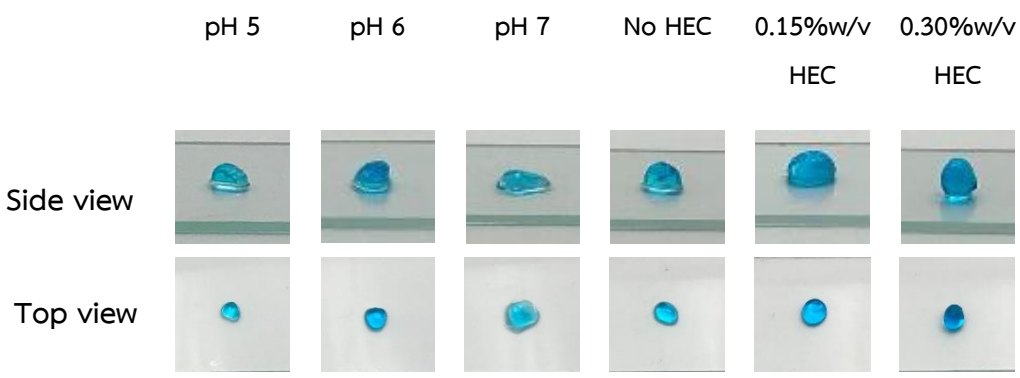


Figure 4.4. Appearance of *in situ* gels at pH 5, pH 6, pH 7 and the concentration of HEC at 0, 0.15, 0.03%w/v

4.3 Physical stability of *in situ* gel-forming artificial saliva

Figure 4.5 shows gel clarity of six formulation of artificial saliva, which clear and no different from initial appearance. Figure 4.6 and Figure 4.7 show pH values and viscosity, respectively, of six formulations of artificial saliva before and after Heating-cooling cycle for totally 6 cycles. The pH values of all formulation were found to be in the range from 5.74 ± 0.05 to 5.46 ± 0.10, which was expected since the artificial saliva was formulated with pH around 5.5 to 6, the native pH of gellan gum solution, which was suitable for gel formulation. Furthermore, the artificial saliva should be slightly acidic, for stimulating secretion of saliva in xerostomia patients. The viscosity of all formulations was designed for being sprayable which was in range of 0-400 cPs (The Dow Chemical Company). The viscosity values of all formulation also remained as similar as the beginning. Statistical differences in pH values and viscosity before and after Heating-cooling cycle were not detected. Gellan gum and HEC that used in these *in situ* gel-forming artificial saliva are resistant to heat (Blažková, Hrivíková, & Lapčík, 1990; Zhang, Ortiz, Goyal, & Kohn, 2014). Hence, all formulations were selected for further study.

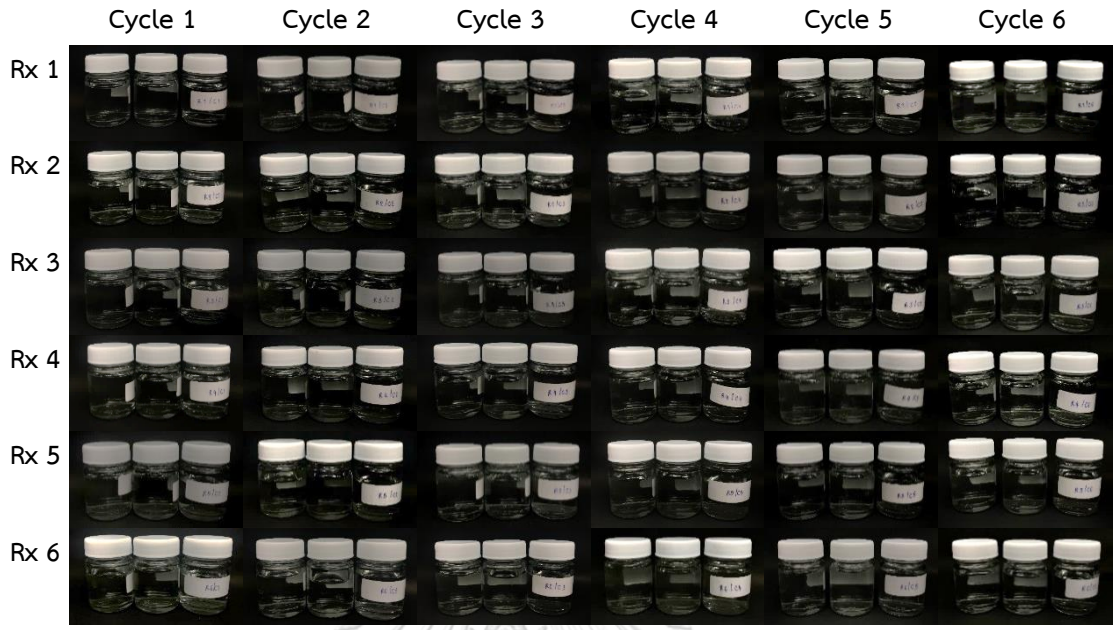


Figure 4.5 Gel clarity of six formulation of artificial saliva

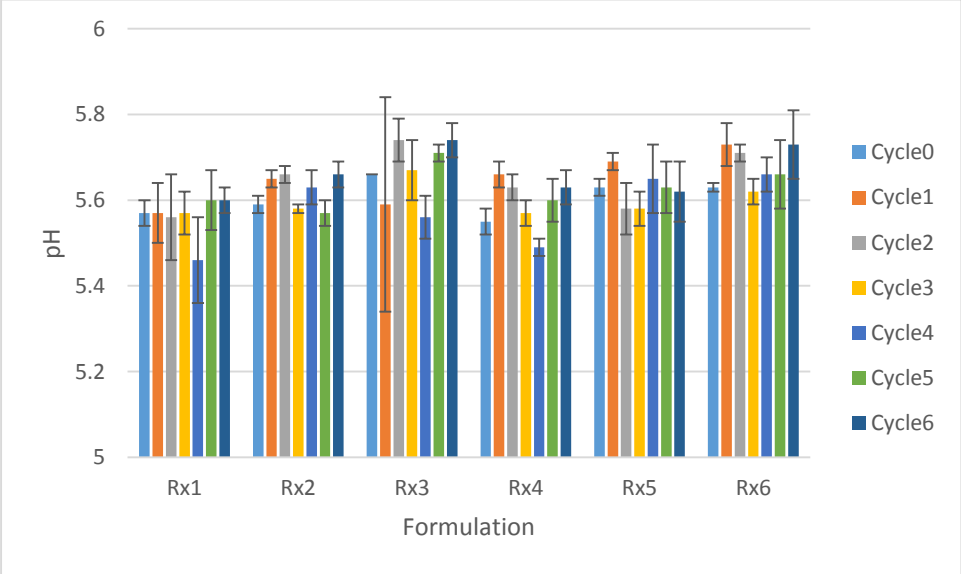


Figure 4.6 pH values of six formulations of artificial saliva before and after Heating-cooling cycle totally 6 cycles

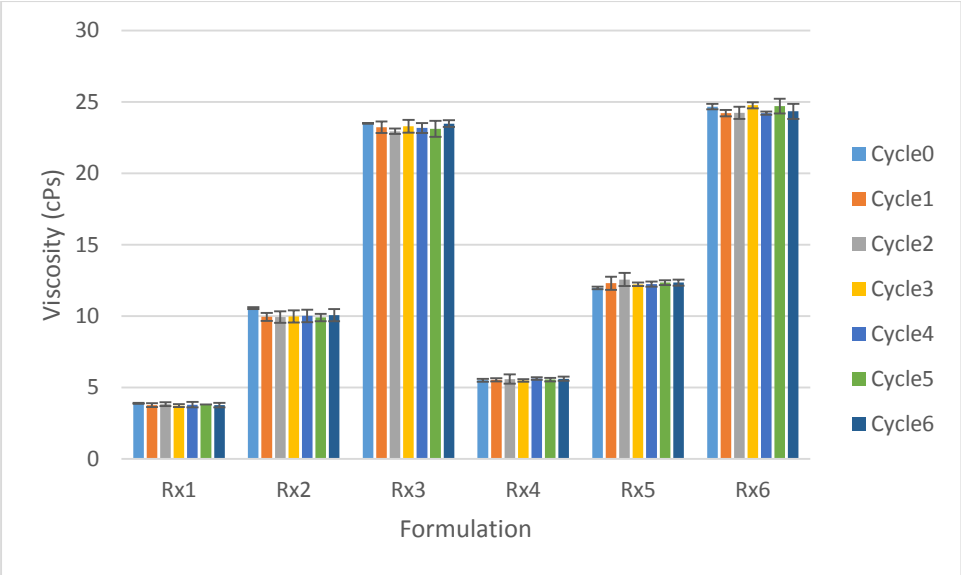


Figure 4.7 Viscosity of six formulations of artificial saliva before and after Heating-cooling cycle totally 6 cycles

4.4 Simulated gelation time

Table 4.3 shows the simulated gelation time of *in situ* artificial saliva gel. The viscosity of Rx1, Rx2 and Rx3 (0.1% w/v gellan gum) was significantly higher than the initial values within 45 to 60 minute. Rx5 and Rx6 (0.15% w/v gellan gum) had less simulated gelation time than Rx2 and Rx3, respectively. The result was consistent with the previous study of Meng, Hong and Jin (2013), who reported that gelling temperature and gelling rate increased with an increase of content of gellan gum (Meng et al., 2013). Rx5 and Rx6 showed shorter simulated gelation time than Rx4 with the same concentration of gellan gum, but a similar effect was not noticed in Rx 1, Rx 2, and Rx 3. Thus, this result implies that HEC also affected on the simulated gelation time at sufficient concentration of gellan gum. In this study, simulated gelation time was determined by the change of viscosity of *in situ* gel artificial saliva, so the viscosity-enhancing property of HEC leads to that the formulations containing HEC (Rx5 and Rx6) were more viscose than that without HEC (Rx4).

Table 4.3 Simulated gelation time of *in situ* artificial saliva gel

Formulation	Simulated gelation time
Rx1	+++
Rx2	++++
Rx3	++++
Rx4	+++
Rx5	+
Rx6	+

Gelation time: + within 0 – 15 min, ++ within 16 – 30 min,
+++ within 31 – 45 min, ++++ within 46 – 60 min

4.5 Mucoadhesive test

All formulations of *in situ* gel-forming artificial saliva was coated on porcine buccal mucosa around 0.1 gram per 0.8 cm^2. Figure 4.8 shows adhesive work of six formulation of artificial saliva with porcine buccal mucosa. Adhesive work of Rx6 with porcine buccal mucosa was the highest and significant different (p-value < 0.05) from Rx1, Rx2 and Rx3. This result shows that gellan gum and HEC with sufficient concentration enhanced mucoadhesive property due to HEC's binding property. Gellan gum and HEC formed hydrogen bonding to the mucosa and anionic property of gellan gum caused electron transfer between gellan gum and mucus surfaces (Salamat-Miller et al., 2005). The important factor of strong adhesive bond with the mucosa is the concentration of both polymer. The interaction between polymer and mucus is unstable, when the concentration is too low, due to the number of penetrating polymer chains per unit volume of the mucus is small (Salamat-Miller et al., 2005). Thus, Rx 6 was selected for satisfaction study.

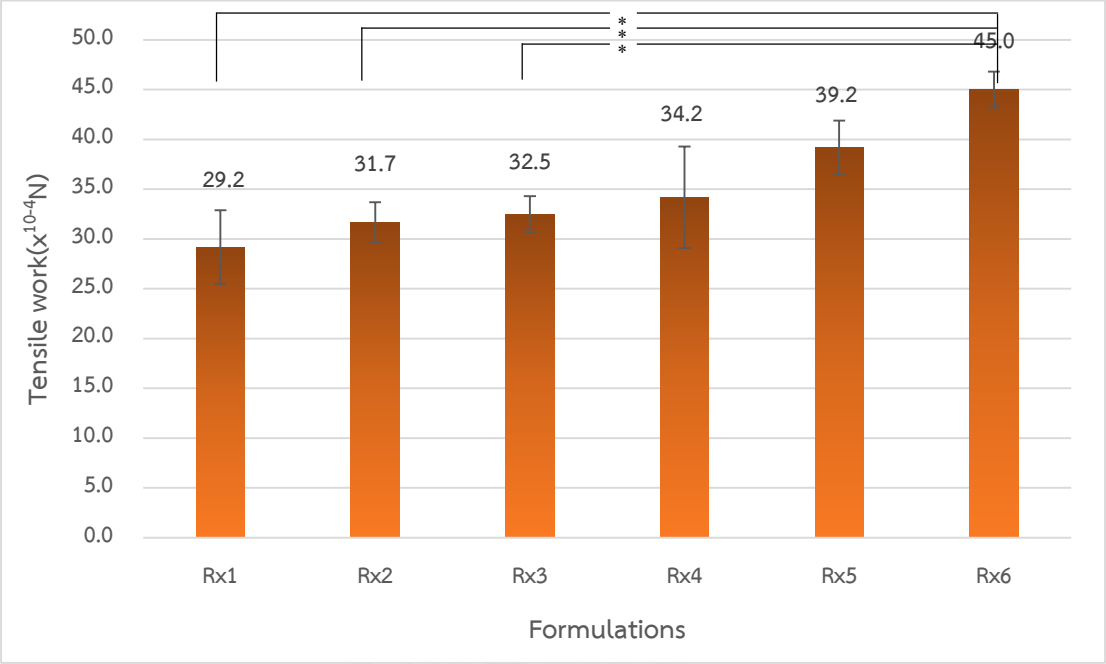


Figure 4.8 Mucoadhesion of *in situ* gel-forming artificial saliva

*significant difference (p-value < 0.05)

4.6 Satisfaction study

Fifteen volunteers with dry mouth were recruited into this study. This group consisted of 9 females and 6 males with a mean age of 40.5 years, range from 26-56 years Table 4.4 shows that *in situ* gel-forming artificial saliva could reduce the dryness of mouth, tongue and throat of volunteers with dry mouth and decrease difficulty in speaking and swallowing. However, it could not reduce the dryness of the lips, because the mechanism of this *in situ* gel-forming artificial saliva is to moisturize the oral cavity by forming gel when contacting with electrolyte in saliva was not available on lips. Previous study by Vadcharavivad and Boonroung found that CMC-containing artificial saliva also reduced the dryness of mouth and difficulty in speaking and swallowing, the change of mean VAS scores were 31.1, 23.4 and 23.2, respectively (Vadcharavivada & Boonroungb, 2013), while the change of means of VAS scores of the dryness of mouth and difficulty in speaking and swallowing from present study were 28.00, 28.78 and 35.74, respectively. Our *in situ* gel-forming artificial saliva demonstrated better efficacy in reducing the difficulty in speaking and swallowing due to soft gel forming and covering all areas in the mouth but showed worse efficacy in reducing the dryness

of mouth since the moisturizing effect of *in situ* gel formulation was less than CMC-base gel formulation.

Table 4.4 Means of VAS scores of xerostomia questionnaire before and after using *in situ* gel-forming artificial saliva

Parameter	VAS score (mm), mean(SD)		
	Before	After	Difference
Dryness of mouth	56.53(16.99)	28.53(14.61)*	28.00(18.24)
Dryness of throat	60.53(14.41)	33.27(15.75)*	27.27(21.57)
Dryness of lips	66.66(24.41)	54.07(25.49)	13.06(22.75)
Dryness of tongue	52.26(19.16)	31.27(18.39)*	20.99(22.62)
Quantity of saliva	48.14(21.93)	68.53(17.27)*	22.26(17.39)
Stickiness of saliva	59.98(19.65)	38.60(21.30)*	24.31(24.29)
Thirst	66.97(15.38)	38.73(20.50)*	28.24(27.10)
The need to use artificial saliva	35.56(28.63)	29.10(25.41)	27.00(24.24)
Dryness of mouth while speaking	68.30(15.05)	37.40(19.12)*	30.90(25.21)
Quantity of saliva while speaking	47.70(22.60)	72.20(15.41)*	27.30(22.34)
Stickiness of saliva while speaking	59.07(16.22)	39.53(22.36)*	21.93(21.04)
Thirst while speaking	71.90(20.32)	42.20(21.80)*	29.96(28.19)
The need to use artificial saliva while speaking	38.53(28.89)	32.84(25.05)	28.90(22.53)
Difficulty in speaking	45.91(26.44)	17.13(17.78)*	28.78(24.13)
Difficulty in swallowing	61.08(18.63)	25.33(18.42)*	35.74(25.58)

*significant difference (p-value < 0.05)

Figure 4.9 shows that volunteers were satisfied with our *in situ* gel-forming artificial saliva in term of ease of use, almost satisfied in term of spreadability, long lasting, taste, efficacy, overall of *in situ* gel-forming artificial saliva and did not detected salty from electrolyte compositions in formulation. Mom et al. found that the criteria that patients used to choose the artificial saliva products is not only its efficacy but also its taste and the convenience of use (Momm, Volegova-Neher, Schulte-Monting, & Guttenberger, 2005). In this study almost volunteers wanted to continue on using

the product due to its good taste and its easy handling. The volunteer with scanty saliva commented that this product had short-term duration compared with candies, because less intensity of gelation occurred in volunteer with less saliva and not enough electrolytes to interact with gellan gum. Nevertheless, candies are effective only in patients who still have residual salivary gland function. The study of Field et al. found that the prevalence of xerostomia depended on gender (Field et al., 2001), but, in the present study, the satisfaction did not depend on gender (significant difference at p-value < 0.05). No side effect of this product had been reported in any volunteers.

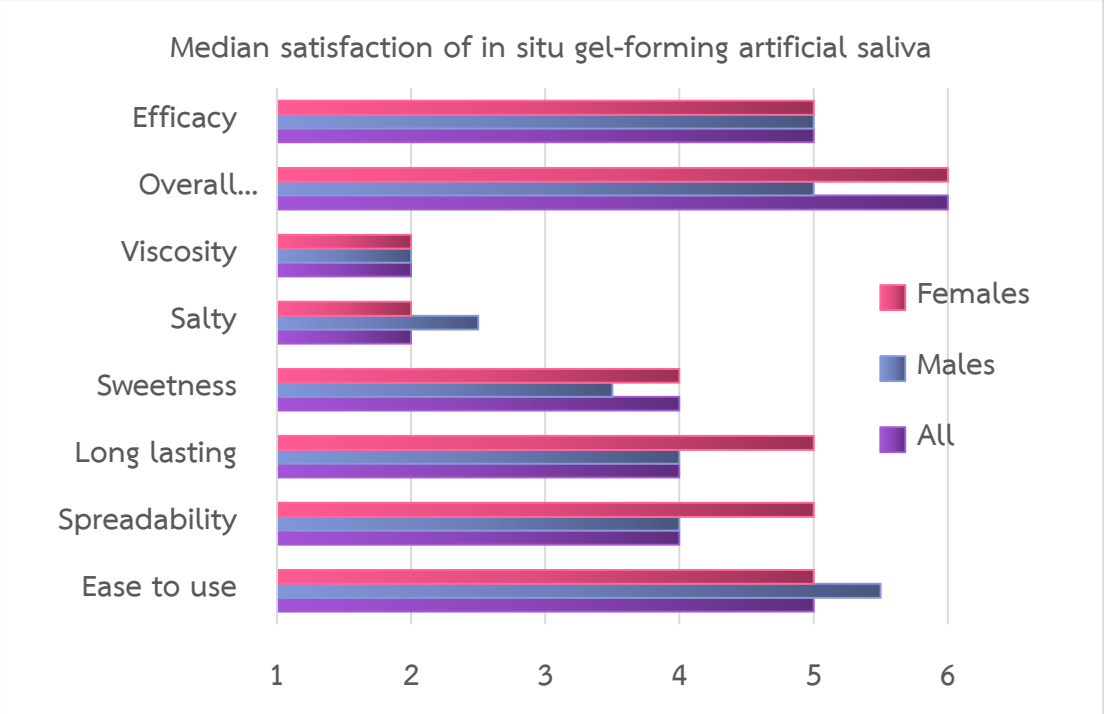


Figure 4.9 Median satisfaction of *in situ* gel-forming artificial saliva

Satisfaction rate: 1 least satisfied, 2 less satisfied, 3 little satisfied, 4 quite satisfied, 5 very satisfied, 6 most satisfied

CHAPTER V
CONCLUSION

The present study aimed to develop the *in situ* gel-forming artificial saliva containing gellan gum and HEC in sprayable form. The gelation time, mucoadhesive property and satisfaction of *in situ* gel-forming artificial saliva were evaluated for finding the most effective *in situ* gel-forming artificial saliva formulation.

The findings obtained in this study can be concluded as follows:

1. 0.15% w/v of gellan gum was suitable for prepared the *in situ* gel-forming artificial saliva in sprayable form.
2. The *in situ* gel-forming artificial saliva that contains 0.1% and 0.15%w/v of gellan gum and 0.15% and 0.3% of HEC was stable.
3. Increasing of gellan gum concentration decreases the gelation time of *in situ* gel-forming artificial saliva.
4. The *in situ* gel-forming artificial saliva, which contain 0.15% w/v of gellan gum and 0.3%w/v of HEC had the highest mucoadhesive property.
5. The *in situ* gel-forming artificial saliva can reduce dryness of mouth and almost volunteers are satisfaction with it.

From the results of the present study, the combination of gellan gum and HEC could be used for development of *in situ* gel-forming artificial saliva with suitable gelation time and mucoadhesion.

For future development of *in situ* gel-forming artificial saliva, other experiments that should be studied are following:

- Development of formulations with other mucoadhesive polymers to improve mucoadhesion property
- Addition of the moisturizing agents such as sodium hyaluronate for the better moisturizer effect

Efficacy of *in situ* gel-forming artificial saliva in patients with xerostomia

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APPENDICES

APPENDIX A

Effect of pH and Hydroxyethyl cellulose on gellan gum solution

Table A-1 Effect of pH and Hydroxyethyl cellulose on gellan gum solution

pH	Viscosity Before cPs.			Mean	SD	Viscosity After cPs.			Mean	SD
	N1	N2	N3			N1	N2	N3		
5.00	7.07	6.94	7.11	7.04	0.09	517.40	515.90	520.40	517.90	2.29
6.00	6.94	6.73	6.73	6.80	0.12	586.20	580.20	590.40	585.60	5.13
7.00	6.73	6.60	6.47	6.60	0.13	484.20	487.60	482.50	484.77	2.60

Table A-2 Effect of HEC and Hydroxyethyl cellulose on gellan gum solution

%HEC	Viscosity (cPs.) Before			Mean	SD	Viscosity (cPs.) After			Mean	SD
	N1	N2	N3			N1	N2	N3		
0%	6.94	7.28	7.07	7.10	0.17	602.40	606.70	603.30	604.13	2.27
0.15%	15.50	15.70	16.30	15.83	0.42	617.00	614.40	610.20	613.87	3.43
0.30%	37.90	37.50	37.70	37.70	0.20	674.40	676.10	671.00	673.83	2.60

Table A-3 One-way analysis of variance of effect of pH on gellan gum solution before and after gelation

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
before	Between Groups	.291	2	.146	11.058	.010
	Within Groups	.079	6	.013		
	Total	.370	8			
after	Between Groups	15848.469	2	7924.234	621.130	.000
	Within Groups	76.547	6	12.758		
	Total	15925.016	8			

Table A-4 Multiple comparison of pH on gellan gum solution before and after gelation

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) ph	(J) ph	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
before	5.00	6.00	.24000	.09369	.094	-.0475	.5275
		7.00	.44000*	.09369	.008	.1525	.7275
	6.00	5.00	-.24000	.09369	.094	-.5275	.0475
		7.00	.20000	.09369	.163	-.0875	.4875
	7.00	5.00	-.44000*	.09369	.008	-.7275	-.1525
		6.00	-.20000	.09369	.163	-.4875	.0875
after	5.00	6.00	-67.70000*	2.91637	.000	-76.6482	-58.7518
		7.00	33.13333*	2.91637	.000	24.1851	42.0815
	6.00	5.00	67.70000*	2.91637	.000	58.7518	76.6482
		7.00	100.83333*	2.91637	.000	91.8851	109.7815
	7.00	5.00	-33.13333*	2.91637	.000	-42.0815	-24.1851
		6.00	-100.83333*	2.91637	.000	-109.7815	-91.8851

*. The mean difference is significant at the 0.05 level.

Table A-5 One-way analysis of variance of effect of HEC on gellan gum solution before and after gelation

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
before	Between Groups	1491.044	2	745.522	9212.825	.000
	Within Groups	.486	6	.081		
	Total	1491.530	8			
after	Between Groups	8548.829	2	4274.414	541.980	.000
	Within Groups	47.320	6	7.887		
	Total	8596.149	8			

Table A-6 Multiple comparison of HEC on gellan gum solution before and after gelation

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) HEC	(J) HEC	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
before	.00	.15	-8.73667*	.23227	.000	-9.4493	-8.0240
		.30	-30.60333*	.23227	.000	-31.3160	-29.8907
	.15	.00	8.73667*	.23227	.000	8.0240	9.4493
		.30	-21.86667*	.23227	.000	-22.5793	-21.1540
	.30	.00	30.60333*	.23227	.000	29.8907	31.3160
		.15	21.86667*	.23227	.000	21.1540	22.5793
after	.00	.15	-9.73333*	2.29298	.013	-16.7688	-2.6978
		.30	-69.70000*	2.29298	.000	-76.7355	-62.6645
	.15	.00	9.73333*	2.29298	.013	2.6978	16.7688
		.30	-59.96667*	2.29298	.000	-67.0022	-52.9312
	.30	.00	69.70000*	2.29298	.000	62.6645	76.7355
		.15	59.96667*	2.29298	.000	52.9312	67.0022

*. The mean difference is significant at the 0.05 level.

APPENDIX B

Physical stability of *in situ* gel-forming artificial saliva

Table B-1 Physical properties of Rx 1

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.61	5.55	5.55	5.57	0.03	3.87	3.87	3.93	3.89	0.03
1	5.65	5.51	5.56	5.57	0.07	3.62	3.87	3.81	3.77	0.13
2	5.67	5.53	5.47	5.56	0.1	3.99	3.75	3.75	3.83	0.14
3	5.62	5.52	5.57	5.57	0.05	3.62	3.81	3.75	3.73	0.1
4	5.57	5.43	5.38	5.46	0.1	3.99	3.75	3.62	3.79	0.19
5	5.67	5.6	5.53	5.6	0.07	3.81	3.81	3.81	3.81	0
6	5.59	5.57	5.63	5.6	0.03	3.75	3.93	3.62	3.77	0.16

Table B-2 Physical properties of Rx 2

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.58	5.58	5.61	5.59	0.02	10.5	10.56	10.62	10.56	0.06
1	5.67	5.63	5.65	5.65	0.02	10.25	9.7	9.89	9.95	0.28
2	5.68	5.66	5.63	5.66	0.02	9.82	9.58	10.38	9.93	0.41
3	5.58	5.57	5.59	5.58	0.01	10.44	9.82	9.64	9.97	0.42
4	5.67	5.63	5.58	5.63	0.04	9.7	10.5	9.82	10.01	0.43
5	5.59	5.53	5.59	5.57	0.03	10.19	9.7	9.82	9.9	0.26
6	5.62	5.68	5.68	5.66	0.03	10.56	9.82	9.82	10.07	0.43

Table B-3 Physical properties of Rx 3

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.66	5.66	5.66	5.66	0	23.27	23.64	23.58	23.5	0.02
1	5.71	5.3	5.76	5.59	0.25	23.52	23.39	22.78	23.23	0.4
2	5.78	5.68	5.76	5.74	0.05	22.9	22.78	23.15	22.94	0.19
3	5.61	5.65	5.74	5.67	0.07	23.52	22.78	23.58	23.29	0.44
4	5.57	5.61	5.51	5.56	0.05	22.78	23.33	23.39	23.17	0.34
5	5.68	5.72	5.72	5.71	0.02	23.64	22.53	23.19	23.12	0.56
6	5.78	5.69	5.75	5.74	0.04	23.21	23.64	23.58	23.48	0.23

Table B-4 Physical properties of Rx 4

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.52	5.55	5.58	5.55	0.03	5.53	5.59	5.4	5.51	0.1
1	5.66	5.69	5.63	5.66	0.03	5.4	5.59	5.59	5.53	0.11
2	5.6	5.66	5.62	5.63	0.03	5.4	5.4	5.96	5.59	0.32
3	5.6	5.55	5.55	5.57	0.03	5.53	5.4	5.53	5.49	0.08
4	5.49	5.51	5.47	5.49	0.02	5.71	5.65	5.53	5.63	0.09
5	5.63	5.63	5.55	5.6	0.05	5.59	5.4	5.65	5.55	0.13
6	5.6	5.67	5.62	5.63	0.04	5.53	5.53	5.77	5.61	0.14

Table B-5 Physical properties of Rx 5

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.62	5.61	5.65	5.63	0.02	12.03	12.03	11.85	11.97	0.1
1	5.68	5.71	5.68	5.69	0.02	12.83	12.03	12.03	12.3	0.46
2	5.54	5.65	5.56	5.58	0.06	12.03	12.83	12.83	12.56	0.46
3	5.54	5.59	5.61	5.58	0.04	12.1	12.22	12.34	12.22	0.12
4	5.56	5.68	5.71	5.65	0.08	12.03	12.34	12.34	12.24	0.18
5	5.68	5.65	5.56	5.63	0.06	12.16	12.46	12.4	12.34	0.16
6	5.68	5.65	5.54	5.62	0.07	12.59	12.16	12.28	12.34	0.22

Table B-6 Physical properties of Rx 6

	pH			Mean	S.D.	Viscosity cPs.			Mean	S.D.
Cycle	N1	N2	N3			N1	N2	N3		
0	5.64	5.62	5.63	5.63	0.01	24.87	24.5	24.62	24.66	0.19
1	5.71	5.79	5.7	5.73	0.05	24.19	24.44	24.01	24.21	0.22
2	5.68	5.73	5.71	5.71	0.02	24.38	23.76	24.56	24.23	0.42
3	5.65	5.59	5.61	5.62	0.03	24.74	24.56	24.99	24.76	0.22
4	5.71	5.65	5.62	5.66	0.04	24.07	24.31	24.25	24.21	0.12
5	5.62	5.75	5.61	5.66	0.08	25.11	24.13	24.87	24.7	0.51
6	5.64	5.75	5.79	5.73	0.08	23.76	24.44	24.81	24.34	0.53

Table B-7 Paired t-test physical stability of Rx 1

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.5700	3	.03464	.02000
	pH1	5.5733	3	.07095	.04096
Pair 2	pH0	5.5700	3	.03464	.02000
	pH2	5.5567	3	.10263	.05925
Pair 3	pH0	5.5700	3	.03464	.02000
	pH3	5.5700	3	.05000	.02887
Pair 4	pH0	5.5700	3	.03464	.02000
	pH4	5.4600	3	.09849	.05686
Pair 5	pH0	5.5700	3	.03464	.02000
	pH5	5.6000	3	.07000	.04041
Pair 6	pH0	5.5700	3	.03464	.02000
	pH6	5.5967	3	.03055	.01764

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	pH0 - pH1	-.00333	.04041	.02333	-.10373	.09706	-.143	2	.899
Pair 2	pH0 - pH2	.01333	.07024	.04055	-.16115	.18781	.329	2	.774
Pair 3	pH0 - pH3	.00000	.02646	.01528	-.06572	.06572	.000	2	1.000
Pair 4	pH0 - pH4	.11000	.06557	.03786	-.05290	.27290	2.905	2	.101
Pair 5	pH0 - pH5	-.03000	.04359	.02517	-.13828	.07828	-1.192	2	.355
Pair 6	pH0 - pH6	-.02667	.05033	.02906	-.15170	.09837	-.918	2	.456

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	3.8900	3	.03464	.02000
	viscosity1	3.7667	3	.13051	.07535
Pair 2	viscosity0	3.8900	3	.03464	.02000
	viscosity2	3.8300	3	.13856	.08000
Pair 3	viscosity0	3.8900	3	.03464	.02000
	viscosity3	3.7267	3	.09713	.05608
Pair 4	viscosity0	3.8900	3	.03464	.02000
	viscosity4	3.7867	3	.18771	.10837
Pair 5	viscosity0	3.8900	3	.03464	.02000
	viscosity5	3.8100	3	.00000	.00000
Pair 6	viscosity0	3.8900	3	.03464	.02000
	viscosity6	3.7667	3	.15567	.08988

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	viscosity0 - viscosity1	.12333	.12503	.07219	-.18727	.43393	1.709	2	.230
Pair 2	viscosity0 - viscosity2	.06000	.15875	.09165	-.33434	.45434	.655	2	.580
Pair 3	viscosity0 - viscosity3	.16333	.09609	.05548	-.07537	.40203	2.944	2	.099
Pair 4	viscosity0 - viscosity4	.10333	.21548	.12441	-.43196	.63863	.831	2	.494
Pair 5	viscosity0 - viscosity5	.08000	.03464	.02000	-.00605	.16605	4.000	2	.057
Pair 6	viscosity0 - viscosity6	.12333	.18502	.10682	-.33629	.58295	1.155	2	.368

Table B-8 Paired t-test physical stability of Rx 2

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.5900	3	.01732	.01000
	pH1	5.6500	3	.02000	.01155
Pair 2	pH0	5.5900	3	.01732	.01000
	pH2	5.6567	3	.02517	.01453
Pair 3	pH0	5.5900	3	.01732	.01000
	pH3	5.5800	3	.01000	.00577
Pair 4	pH0	5.5900	3	.01732	.01000
	pH4	5.6267	3	.04509	.02603
Pair 5	pH0	5.5900	3	.01732	.01000
	pH5	5.5700	3	.03464	.02000
Pair 6	pH0	5.5900	3	.01732	.01000
	pH6	5.6600	3	.03464	.02000

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	pH0 - pH1	-.06000	.02646	.01528	-.12572	.00572	-3.928	2	.059
Pair 2	pH0 - pH2	-.06667	.04163	.02404	-.17009	.03676	-2.774	2	.109
Pair 3	pH0 - pH3	.01000	.01000	.00577	-.01484	.03484	1.732	2	.225
Pair 4	pH0 - pH4	-.03667	.06110	.03528	-.18845	.11512	-1.039	2	.408
Pair 5	pH0 - pH5	.02000	.03000	.01732	-.05452	.09452	1.155	2	.368
Pair 6	pH0 - pH6	-.07000	.03000	.01732	-.14452	.00452	-4.041	2	.056

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	10.5600	3	.06000	.03464
	viscosity1	9.9467	3	.27934	.16128
Pair 2	viscosity0	10.5600	3	.06000	.03464
	viscosity2	9.9267	3	.41053	.23702
Pair 3	viscosity0	10.5600	3	.06000	.03464
	viscosity3	9.9667	3	.41968	.24230
Pair 4	viscosity0	10.5600	3	.06000	.03464
	viscosity4	10.0067	3	.43143	.24909
Pair 5	viscosity0	10.5600	3	.06000	.03464
	viscosity5	9.9033	3	.25541	.14746
Pair 6	viscosity0	10.5600	3	.06000	.03464
	viscosity6	10.0667	3	.42724	.24667

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	viscosity0 - viscosity1	.61333	.32130	.18550	-.18482	1.41149	3.306	2	.081
Pair 2	viscosity0 - viscosity2	.63333	.37220	.21489	-.29126	1.55793	2.947	2	.098
Pair 3	viscosity0 - viscosity3	.59333	.47721	.27552	-.59213	1.77880	2.154	2	.164
Pair 4	viscosity0 - viscosity4	.55333	.42724	.24667	-.50799	1.61465	2.243	2	.154
Pair 5	viscosity0 - viscosity5	.65667	.30172	.17420	-.09284	1.40617	3.770	2	.064
Pair 6	viscosity0 - viscosity6	.49333	.48014	.27721	-.69940	1.68606	1.780	2	.217

Table B-9 Paired t-test physical stability of Rx 3

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.6600	3	.00000	.00000
	pH1	5.5900	3	.25239	.14572
Pair 2	pH0	5.6600	3	.00000	.00000
	pH2	5.7400	3	.05292	.03055
Pair 3	pH0	5.6600	3	.00000	.00000
	pH3	5.6667	3	.06658	.03844
Pair 4	pH0	5.6600	3	.00000	.00000
	pH4	5.5633	3	.05033	.02906
Pair 5	pH0	5.6600	3	.00000	.00000
	pH5	5.7067	3	.02309	.01333
Pair 6	pH0	5.6600	3	.00000	.00000
	pH6	5.7400	3	.04583	.02646

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	pH0 - pH1	.07000	.25239	.14572	-.55697	.69697	.480	2	.678
Pair 2	pH0 - pH2	-.08000	.05292	.03055	-.21145	.05145	-2.619	2	.120
Pair 3	pH0 - pH3	-.00667	.06658	.03844	-.17207	.15874	-.173	2	.878
Pair 4	pH0 - pH4	.09667	.05033	.02906	-.02837	.22170	3.327	2	.080
Pair 5	pH0 - pH5	-.04667	.02309	.01333	-.10404	.01070	-3.500	2	.073
Pair 6	pH0 - pH6	-.08000	.04583	.02646	-.19384	.03384	-3.024	2	.094

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	23.4967	3	.19858	.11465
	viscosity1	23.2300	3	.39509	.22811
Pair 2	viscosity0	23.4967	3	.19858	.11465
	viscosity2	22.9433	3	.18877	.10899
Pair 3	viscosity0	23.4967	3	.19858	.11465
	viscosity3	23.2933	3	.44557	.25725
Pair 4	viscosity0	23.4967	3	.19858	.11465
	viscosity4	23.1667	3	.33620	.19411
Pair 5	viscosity0	23.4967	3	.19858	.11465
	viscosity5	23.1200	3	.55830	.32234
Pair 6	viscosity0	23.4967	3	.19858	.11465
	viscosity6	23.4767	3	.23288	.13445

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	viscosity0 - viscosity1	.26667	.52520	.30322	-1.03800	1.57133	.879	2	.472
Pair 2	viscosity0 - viscosity2	.55333	.26727	.15431	-.11060	1.21727	3.586	2	.070
Pair 3	viscosity0 - viscosity3	.20333	.58227	.33617	-1.24309	1.64976	.605	2	.607
Pair 4	viscosity0 - viscosity4	.33000	.15100	.08718	-.04510	.70510	3.785	2	.063
Pair 5	viscosity0 - viscosity5	.37667	.74009	.42729	-1.46182	2.21515	.882	2	.471
Pair 6	viscosity0 - viscosity6	.02000	.03464	.02000	-.06605	.10605	1.000	2	.423

Table B-10 Paired t-test physical stability of Rx 4

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.5500	3	.03000	.01732
	pH1	5.6600	3	.03000	.01732
Pair 2	pH0	5.5500	3	.03000	.01732
	pH2	5.6267	3	.03055	.01764
Pair 3	pH0	5.5500	3	.03000	.01732
	pH3	5.5667	3	.02887	.01667
Pair 4	pH0	5.5500	3	.03000	.01732
	pH4	5.4900	3	.02000	.01155
Pair 5	pH0	5.5500	3	.03000	.01732
	pH5	5.6033	3	.04619	.02667
Pair 6	pH0	5.5500	3	.03000	.01732
	pH6	5.6300	3	.03606	.02082

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	pH0 - pH1	-.11000	.05196	.03000	-.23908	.01908	-3.667	2	.067
Pair 2	pH0 - pH2	-.07667	.03512	.02028	-.16391	.01057	-3.781	2	.063
Pair 3	pH0 - pH3	-.01667	.05686	.03283	-.15792	.12459	-.508	2	.662
Pair 4	pH0 - pH4	.06000	.04359	.02517	-.04828	.16828	2.384	2	.140
Pair 5	pH0 - pH5	-.05333	.07371	.04256	-.23644	.12978	-1.253	2	.337
Pair 6	pH0 - pH6	-.08000	.04000	.02309	-.17937	.01937	-3.464	2	.074

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	5.5067	3	.09713	.05608
	viscosity1	5.5267	3	.10970	.06333
Pair 2	viscosity0	5.5067	3	.09713	.05608
	viscosity2	5.5867	3	.32332	.18667
Pair 3	viscosity0	5.5067	3	.09713	.05608
	viscosity3	5.4867	3	.07506	.04333
Pair 4	viscosity0	5.5067	3	.09713	.05608
	viscosity4	5.6300	3	.09165	.05292
Pair 5	viscosity0	5.5067	3	.09713	.05608
	viscosity5	5.5467	3	.13051	.07535
Pair 6	viscosity0	5.5067	3	.09713	.05608
	viscosity6	5.6100	3	.13856	.08000

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	viscosity0 - viscosity1	-.02000	.16093	.09292	-.41978	.37978	-.215	2	.850
Pair 2	viscosity0 - viscosity2	-.08000	.41677	.24062	-1.11532	.95532	-.332	2	.771
Pair 3	viscosity0 - viscosity3	.02000	.16093	.09292	-.37978	.41978	.215	2	.850
Pair 4	viscosity0 - viscosity4	-.12333	.06028	.03480	-.27307	.02640	-3.544	2	.071
Pair 5	viscosity0 - viscosity5	-.04000	.22068	.12741	-.58820	.50820	-.314	2	.783
Pair 6	viscosity0 - viscosity6	-.10333	.23288	.13445	-.68184	.47517	-.769	2	.523

Table B-11 Paired t-test physical stability of Rx 5

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.6267	3	.02082	.01202
	pH1	5.6900	3	.01732	.01000
Pair 2	pH0	5.6267	3	.02082	.01202
	pH2	5.5833	3	.05859	.03383
Pair 3	pH0	5.6267	3	.02082	.01202
	pH3	5.5800	3	.03606	.02082
Pair 4	pH0	5.6267	3	.02082	.01202
	pH4	5.6500	3	.07937	.04583
Pair 5	pH0	5.6267	3	.02082	.01202
	pH5	5.6300	3	.06245	.03606
Pair 6	pH0	5.6267	3	.02082	.01202
	pH6	5.6233	3	.07371	.04256

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	pH0 - pH1	-.06333	.03512	.02028	-.15057	.02391	-3.124	2	.089
Pair 2	pH0 - pH2	.04333	.07234	.04177	-.13637	.22304	1.038	2	.408
Pair 3	pH0 - pH3	.04667	.03055	.01764	-.02922	.12256	2.646	2	.118
Pair 4	pH0 - pH4	-.02333	.07234	.04177	-.20304	.15637	-.559	2	.633
Pair 5	pH0 - pH5	-.00333	.08145	.04702	-.20565	.19899	-.071	2	.950
Pair 6	pH0 - pH6	.00333	.09292	.05364	-.22748	.23415	.062	2	.956

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	11.9700	3	.10392	.06000
	viscosity1	12.2967	3	.46188	.26667
Pair 2	viscosity0	11.9700	3	.10392	.06000
	viscosity2	12.5633	3	.46188	.26667
Pair 3	viscosity0	11.9700	3	.10392	.06000
	viscosity3	12.2200	3	.12000	.06928
Pair 4	viscosity0	11.9700	3	.10392	.06000
	viscosity4	12.2367	3	.17898	.10333
Pair 5	viscosity0	11.9700	3	.10392	.06000
	viscosity5	12.3400	3	.15875	.09165
Pair 6	viscosity0	11.9700	3	.10392	.06000
	viscosity6	12.3433	3	.22189	.12811

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	viscosity0 - viscosity1	-.32667	.41968	.24230	-1.36922	.71588	-1.348	2	.310
Pair 2	viscosity0 - viscosity2	-.59333	.52166	.30118	-1.88922	.70255	-1.970	2	.188
Pair 3	viscosity0 - viscosity3	-.25000	.21633	.12490	-.78740	.28740	-2.002	2	.183
Pair 4	viscosity0 - viscosity4	-.26667	.24786	.14310	-.88238	.34905	-1.863	2	.203
Pair 5	viscosity0 - viscosity5	-.37000	.21633	.12490	-.90740	.16740	-2.962	2	.098
Pair 6	viscosity0 - viscosity6	-.37333	.22053	.12732	-.92116	.17449	-2.932	2	.099

Table B-12 Paired t-test physical stability of Rx 6

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	pH0	5.6300	3	.01000	.00577
	pH1	5.7333	3	.04933	.02848
Pair 2	pH0	5.6300	3	.01000	.00577
	pH2	5.7067	3	.02517	.01453
Pair 3	pH0	5.6300	3	.01000	.00577
	pH3	5.6167	3	.03055	.01764
Pair 4	pH0	5.6300	3	.01000	.00577
	pH4	5.6600	3	.04583	.02646
Pair 5	pH0	5.6300	3	.01000	.00577
	pH5	5.6600	3	.07810	.04509
Pair 6	pH0	5.6300	3	.01000	.00577
	pH6	5.7267	3	.07767	.04485

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	pH0 - pH1	-.10333	.05774	.03333	-.24676	.04009	-3.100	2	.090
Pair 2	pH0 - pH2	-.07667	.03512	.02028	-.16391	.01057	-3.781	2	.063
Pair 3	pH0 - pH3	.01333	.02082	.01202	-.03838	.06504	1.109	2	.383
Pair 4	pH0 - pH4	-.03000	.04000	.02309	-.12937	.06937	-1.299	2	.324
Pair 5	pH0 - pH5	-.03000	.08660	.05000	-.24513	.18513	-.600	2	.609
Pair 6	pH0 - pH6	-.09667	.08505	.04910	-.30794	.11461	-1.969	2	.188

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	viscosity0	24.6633	3	.18877	.10899
	viscosity1	24.2133	3	.21595	.12468
Pair 2	viscosity0	24.6633	3	.18877	.10899
	viscosity2	24.2333	3	.41968	.24230
Pair 3	viscosity0	24.6633	3	.18877	.10899
	viscosity3	24.7633	3	.21595	.12468
Pair 4	viscosity0	24.6633	3	.18877	.10899
	viscosity4	24.2100	3	.12490	.07211
Pair 5	viscosity0	24.6633	3	.18877	.10899
	viscosity5	24.7033	3	.51082	.29492
Pair 6	viscosity0	24.6633	3	.18877	.10899
	viscosity6	24.3367	3	.53257	.30748

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	viscosity0 - viscosity1	.45000	.33956	.19604	-.39351	1.29351	2.295	2	.149
Pair 2	viscosity0 - viscosity2	.43000	.34395	.19858	-.42441	1.28441	2.165	2	.163
Pair 3	viscosity0 - viscosity3	-.10000	.25239	.14572	-.72697	.52697	-.686	2	.563
Pair 4	viscosity0 - viscosity4	.45333	.31342	.18095	-.32525	1.23192	2.505	2	.129
Pair 5	viscosity0 - viscosity5	-.04000	.35511	.20502	-.92213	.84213	-.195	2	.863
Pair 6	viscosity0 - viscosity6	.32667	.68981	.39826	-1.38691	2.04024	.820	2	.498

APPENDIX C

Simulated Gelation time data

Table C-1 Viscosity of *in situ* gel-forming artificial saliva after gelation

	Before gelation	15 min	30 min	45 min	60 min
Rx1	9.06	7.74	8.78	9.81	10.6
	9.05	7.77	8.81	9.83	10.4
	8.98	7.73	8.74	9.86	10.6
Rx2	24.3	19.6	21.7	23.6	25.4
	24.1	19.4	22.9	25.1	26.9
	24.2	19.1	22.9	25.7	27.3
Rx3	49.8	40.0	45.7	49.5	51.0
	49.4	40.2	45.4	49.7	51.2
	50.0	40.6	45.3	48.9	50.8
Rx4	17.1	10.8	13.5	20.5	30.3
	17.8	11.3	13.6	20.4	29.8
	17.7	10.5	13.4	21.0	30.0
Rx5	30.0	37.3	53.4	67.0	76.1
	29.5	37.4	53.2	67.1	76.3
	30.3	37.1	53.2	67.0	76.1
Rx6	56.9	73.1	87.7	102.0	117.0
	56.2	73.5	87.3	102.0	117.0
	56.5	73.4	87.4	102.0	117.0

Table C-2 One-way analysis of variance of simulated gelation time of *in situ* gel-forming artificial saliva

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Rx1	Between Groups	13.494	4	3.373	962.003	.000
	Within Groups	.035	10	.004		
	Total	13.529	14			
Rx2	Between Groups	88.391	4	22.098	40.521	.000
	Within Groups	5.453	10	.545		
	Total	93.844	14			
Rx3	Between Groups	229.867	4	57.467	648.120	.000
	Within Groups	.887	10	.089		
	Total	230.753	14			
Rx4	Between Groups	665.311	4	166.328	1720.631	.000
	Within Groups	.967	10	.097		
	Total	666.277	14			
Rx5	Between Groups	4538.860	4	1134.715	26185.731	.000
	Within Groups	.433	10	.043		
	Total	4539.293	14			
Rx6	Between Groups	6719.573	4	1679.893	39997.460	.000
	Within Groups	.420	10	.042		
	Total	6719.993	14			

Table C-3 Multiple comparison of Viscosity of *in situ* gel-forming artificial saliva

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Rx1	0	15	1.28333*	.04835	.000	1.1242	1.4425
		30	.25333*	.04835	.003	.0942	.4125
		45	-.80333*	.04835	.000	-.9625	-.6442
		60	-1.50333*	.04835	.000	-1.6625	-1.3442
	15	0	-1.28333*	.04835	.000	-1.4425	-1.1242
		30	-1.03000*	.04835	.000	-1.1891	-.8709
		45	-2.08667*	.04835	.000	-2.2458	-1.9275
		60	-2.78667*	.04835	.000	-2.9458	-2.6275
	30	0	-.25333*	.04835	.003	-.4125	-.0942
		15	1.03000*	.04835	.000	.8709	1.1891
		45	-1.05667*	.04835	.000	-1.2158	-.8975
		60	-1.75667*	.04835	.000	-1.9158	-1.5975
	45	0	.80333*	.04835	.000	.6442	.9625
		15	2.08667*	.04835	.000	1.9275	2.2458
		30	1.05667*	.04835	.000	.8975	1.2158
		60	-.70000*	.04835	.000	-.8591	-.5409
	60	0	1.50333*	.04835	.000	1.3442	1.6625
		15	2.78667*	.04835	.000	2.6275	2.9458
		30	1.75667*	.04835	.000	1.5975	1.9158
		45	.70000*	.04835	.000	.5409	.8591
Rx2	0	15	4.83333*	.60296	.000	2.8490	6.8177
		30	1.70000	.60296	.103	-.2844	3.6844
		45	-.60000	.60296	.852	-2.5844	1.3844
		60	-2.33333*	.60296	.020	-4.3177	-.3490
	15	0	-4.83333*	.60296	.000	-6.8177	-2.8490
		30	-3.13333*	.60296	.003	-5.1177	-1.1490
		45	-5.43333*	.60296	.000	-7.4177	-3.4490

		60	-7.16667*	.60296	.000	-9.1510	-5.1823
	30	0	-1.70000	.60296	.103	-3.6844	.2844
		15	3.13333*	.60296	.003	1.1490	5.1177
		45	-2.30000*	.60296	.022	-4.2844	-.3156
		60	-4.03333*	.60296	.000	-6.0177	-2.0490
	45	0	.60000	.60296	.852	-1.3844	2.5844
		15	5.43333*	.60296	.000	3.4490	7.4177
		30	2.30000*	.60296	.022	.3156	4.2844
		60	-1.73333	.60296	.095	-3.7177	.2510
	60	0	2.33333*	.60296	.020	.3490	4.3177
		15	7.16667*	.60296	.000	5.1823	9.1510
		30	4.03333*	.60296	.000	2.0490	6.0177
		45	1.73333	.60296	.095	-.2510	3.7177
Rx3	0	15	9.46667*	.24313	.000	8.6665	10.2668
		30	4.26667*	.24313	.000	3.4665	5.0668
		45	.36667	.24313	.580	-.4335	1.1668
		60	-1.26667*	.24313	.003	-2.0668	-.4665
	15	0	-9.46667*	.24313	.000	-10.2668	-8.6665
		30	-5.20000*	.24313	.000	-6.0002	-4.3998
		45	-9.10000*	.24313	.000	-9.9002	-8.2998
		60	-10.73333*	.24313	.000	-11.5335	-9.9332
	30	0	-4.26667*	.24313	.000	-5.0668	-3.4665
		15	5.20000*	.24313	.000	4.3998	6.0002
		45	-3.90000*	.24313	.000	-4.7002	-3.0998
		60	-5.53333*	.24313	.000	-6.3335	-4.7332
	45	0	-.36667	.24313	.580	-1.1668	.4335
		15	9.10000*	.24313	.000	8.2998	9.9002
		30	3.90000*	.24313	.000	3.0998	4.7002
		60	-1.63333*	.24313	.000	-2.4335	-.8332
	60	0	1.26667*	.24313	.003	.4665	2.0668
		15	10.73333*	.24313	.000	9.9332	11.5335
		30	5.53333*	.24313	.000	4.7332	6.3335
		45	1.63333*	.24313	.000	.8332	2.4335

Rx4	0	15	6.66667*	.25386	.000	5.8312	7.5021
		30	4.03333*	.25386	.000	3.1979	4.8688
		45	-3.10000*	.25386	.000	-3.9355	-2.2645
		60	-12.50000*	.25386	.000	-13.3355	-11.6645
	15	0	-6.66667*	.25386	.000	-7.5021	-5.8312
		30	-2.63333*	.25386	.000	-3.4688	-1.7979
		45	-9.76667*	.25386	.000	-10.6021	-8.9312
		60	-19.16667*	.25386	.000	-20.0021	-18.3312
	30	0	-4.03333*	.25386	.000	-4.8688	-3.1979
		15	2.63333*	.25386	.000	1.7979	3.4688
		45	-7.13333*	.25386	.000	-7.9688	-6.2979
		60	-16.53333*	.25386	.000	-17.3688	-15.6979
	45	0	3.10000*	.25386	.000	2.2645	3.9355
		15	9.76667*	.25386	.000	8.9312	10.6021
		30	7.13333*	.25386	.000	6.2979	7.9688
		60	-9.40000*	.25386	.000	-10.2355	-8.5645
	60	0	12.50000*	.25386	.000	11.6645	13.3355
		15	19.16667*	.25386	.000	18.3312	20.0021
		30	16.53333*	.25386	.000	15.6979	17.3688
		45	9.40000*	.25386	.000	8.5645	10.2355
Rx5	0	15	-7.33333*	.16997	.000	-7.8927	-6.7740
		30	-23.33333*	.16997	.000	-23.8927	-22.7740
		45	-37.10000*	.16997	.000	-37.6594	-36.5406
		60	-46.23333*	.16997	.000	-46.7927	-45.6740
	15	0	7.33333*	.16997	.000	6.7740	7.8927
		30	-16.00000*	.16997	.000	-16.5594	-15.4406
		45	-29.76667*	.16997	.000	-30.3260	-29.2073
		60	-38.90000*	.16997	.000	-39.4594	-38.3406
	30	0	23.33333*	.16997	.000	22.7740	23.8927
		15	16.00000*	.16997	.000	15.4406	16.5594
		45	-13.76667*	.16997	.000	-14.3260	-13.2073
		60	-22.90000*	.16997	.000	-23.4594	-22.3406
	45	0	37.10000*	.16997	.000	36.5406	37.6594

		15	29.76667*	.16997	.000	29.2073	30.3260
		30	13.76667*	.16997	.000	13.2073	14.3260
		60	-9.13333*	.16997	.000	-9.6927	-8.5740
	60	0	46.23333*	.16997	.000	45.6740	46.7927
		15	38.90000*	.16997	.000	38.3406	39.4594
		30	22.90000*	.16997	.000	22.3406	23.4594
		45	9.13333*	.16997	.000	8.5740	9.6927
Rx6	0	15	-16.80000*	.16733	.000	-17.3507	-16.2493
		30	-30.93333*	.16733	.000	-31.4840	-30.3826
		45	-45.46667*	.16733	.000	-46.0174	-44.9160
		60	-60.46667*	.16733	.000	-61.0174	-59.9160
	15	0	16.80000*	.16733	.000	16.2493	17.3507
		30	-14.13333*	.16733	.000	-14.6840	-13.5826
		45	-28.66667*	.16733	.000	-29.2174	-28.1160
		60	-43.66667*	.16733	.000	-44.2174	-43.1160
	30	0	30.93333*	.16733	.000	30.3826	31.4840
		15	14.13333*	.16733	.000	13.5826	14.6840
		45	-14.53333*	.16733	.000	-15.0840	-13.9826
		60	-29.53333*	.16733	.000	-30.0840	-28.9826
	45	0	45.46667*	.16733	.000	44.9160	46.0174
		15	28.66667*	.16733	.000	28.1160	29.2174
		30	14.53333*	.16733	.000	13.9826	15.0840
		60	-15.00000*	.16733	.000	-15.5507	-14.4493
	60	0	60.46667*	.16733	.000	59.9160	61.0174
		15	43.66667*	.16733	.000	43.1160	44.2174
		30	29.53333*	.16733	.000	28.9826	30.0840
		45	15.00000*	.16733	.000	14.4493	15.5507

*. The mean difference is significant at the 0.05 level.

APPENDIX D

Mucoadhesive test

Table D-1 Tensile work of with porcine buccal mucosa

	Tensile work (N)				
Formulation	N1	N2	N3	Mean	SD
Rx1	0.00275	0.00250	0.00350	0.00292	0.00037
Rx2	0.00300	0.00300	0.00350	0.00317	0.00020
Rx3	0.00350	0.00325	0.00300	0.00325	0.00018
Rx4	0.00300	0.00300	0.00425	0.00342	0.00051
Rx5	0.00400	0.00350	0.00425	0.00392	0.00027
Rx6	0.00425	0.00450	0.00475	0.00450	0.00018

Table D-2 One-way analysis of variance of tensile work of with porcine buccal mucosa

ANOVA

Mucoadhesive

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0	5	0	5.309	0.008
Within Groups	0	12	0		
Total	0	17			

Table D-3 Multiple comparison of tensile work of tensile work of with porcine buccal mucosa

Multiple Comparisons

Mucoadhesive
Tukey HSD

(I) formulation	(J) formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-0.00025	0.0003568	0.98	-0.001449	0.0009485
	3	-0.00033333	0.0003568	0.93	-0.001532	0.0008652
	4	-0.0005	0.0003568	0.73	-0.001699	0.0006985
	5	-0.001	0.0003568	0.12	-0.002199	0.0001985
	6	-.00158333*	0.0003568	0.01	-0.002782	-0.000385
2	1	0.00025	0.0003568	0.98	-0.000949	0.0014485
	3	-0.00008333	0.0003568	1	-0.001282	0.0011152
	4	-0.00025	0.0003568	0.98	-0.001449	0.0009485
	5	-0.00075	0.0003568	0.35	-0.001949	0.0004485
	6	-.00133333*	0.0003568	0.03	-0.002532	-0.000135
3	1	0.00033333	0.0003568	0.93	-0.000865	0.0015318
	2	0.00008333	0.0003568	1	-0.001115	0.0012818
	4	-0.00016667	0.0003568	1	-0.001365	0.0010318
	5	-0.00066667	0.0003568	0.46	-0.001865	0.0005318
	6	-.00125000*	0.0003568	0.04	-0.002449	-5.15E-05
4	1	0.0005	0.0003568	0.73	-0.000699	0.0016985
	2	0.00025	0.0003568	0.98	-0.000949	0.0014485
	3	0.00016667	0.0003568	1	-0.001032	0.0013652
	5	-0.0005	0.0003568	0.73	-0.001699	0.0006985
	6	-0.00108333	0.0003568	0.09	-0.002282	0.0001152
5	1	0.001	0.0003568	0.12	-0.000199	0.0021985
	2	0.00075	0.0003568	0.35	-0.000449	0.0019485
	3	0.00066667	0.0003568	0.46	-0.000532	0.0018652
	4	0.0005	0.0003568	0.73	-0.000699	0.0016985
	6	-0.00058333	0.0003568	0.59	-0.001782	0.0006152
6	1	.00158333*	0.0003568	0.01	0.0003848	0.0027818
	2	.00133333*	0.0003568	0.03	0.0001348	0.0025318
	3	.00125000*	0.0003568	0.04	0.0000515	0.0024485
	4	0.00108333	0.0003568	0.09	-0.000115	0.0022818
	5	0.00058333	0.0003568	0.59	-0.000615	0.0017818

*. The mean difference is significant at the 0.05 level.

APPENDIX E

Ethics for research involving Human Research Participants

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
โทรศัพท์/โทรสาร: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 114/2561

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 083.1/61 : การพัฒนาเจลก่อตัวเองเพื่อใช้เป็นน้ำลายเทียมและการศึกษาความพึงพอใจ

ผู้วิจัยหลัก : นางสาววิ ติษยาธิคม

หน่วยงาน : คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม.....
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)
ประธาน

ลงนาม.....
(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
กรรมการและเลขานุการ

วันที่รับรอง : 16 พฤษภาคม 2561 **วันหมดอายุ** : 15 พฤษภาคม 2562

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย
4) แบบสอบถาม
5) ใบประชาสัมพันธ์

เลขที่โครงการวิจัย 083.1/61
วันที่รับรอง 16 พ.ค. 2561
วันหมดอายุ 15 พ.ค. 2562

เงื่อนไข

1. *ข้าพเจ้ารับทราบว่าเป็นการผิดจริยธรรม หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ*
2. *หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องหยุด เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย*
3. *ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด*
4. *ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารเชิญเข้าร่วมวิจัย (ถ้ามี) **เฉพาะที่ประทับตราคณะกรรมการเท่านั้น***
5. *หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ*
6. *หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ*
7. *โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น*

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 114/2018

Certificate of Approval

Study Title No. 083.1/61 : DEVELOPMENT OF *IN SITU* GEL FOR ARTIFICIAL SALIVA AND SATISFACTION STUDY

Principal Investigator : MISS RAWI TISHYADHIGAMA

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: Prida Tasanapradit ..... Signature: Nuntaree Chaichanawongsaroj

(Associate Professor Prida Tasanapradit, M.D.) Chairman

(Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.) Secretary

Date of Approval : 16 May 2018 Approval Expire date : 15 May 2019

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire
5) Advertising leaflet

Protocol No. 083.1/61
Date of Approval 16 MAY 2018
Approval Expire Date 15 MAY 2019

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

AF 04-07

ข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย

ชื่อโครงการวิจัย การพัฒนาเจลก่อตัวเองเพื่อใช้เป็นน้ำลายเทียม และการศึกษาความพึงพอใจ

ชื่อผู้วิจัยหลัก นางสาวรวิ ดิษยาธิคม ตำแหน่ง นิสิตระดับปริญญาโท

สถานที่ติดต่อผู้วิจัย (ที่ทำงาน) ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

(ที่บ้าน) 406/559 คอนโดยูดีไลท์ แอท อ่อนนุชสเตชั่น แขวงสวนหลวง เขตสวนหลวง กรุงเทพมหานคร 10250

โทรศัพท์ (ที่ทำงาน) 08-5199-3694 โทรศัพท์ที่บ้าน -

โทรศัพท์มือถือ 08-5199-3694 E-mail : rawitish@gmail.com

ชื่อผู้วิจัยร่วม อาจารย์ เภสัชกรหญิง ดร.จุฑารัตน์ กิจส่งเสริมธน ตำแหน่ง อาจารย์ประจำ

สถานที่ติดต่อผู้วิจัย (ที่ทำงาน) ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ (ที่ทำงาน) 0-2218-8402 โทรศัพท์ที่บ้าน -

โทรศัพท์มือถือ - E-mail : jutarat.kit@gmail.com

1. ขอเรียนเชิญท่านเข้าร่วมในการวิจัยก่อนที่ท่านจะตัดสินใจเข้าร่วมในการวิจัย มีความจำเป็นที่ท่านควรทำความเข้าใจว่างานวิจัยนี้ทำเพราะเหตุใด และเกี่ยวข้องกับอะไร กรุณาใช้เวลาในการอ่านข้อมูลต่อไปนี้อย่างละเอียดรอบคอบ และสอบถามข้อมูลเพิ่มเติมหรือข้อมูลที่ไม่ชัดเจนได้ตลอดเวลา
2. วัตถุประสงค์ของการวิจัย

   เพื่อศึกษาความพึงพอใจต่อน้ำลายเทียมแบบเจลก่อตัวเอง ในผู้ป่วยที่มีอาการภาวะปากแห้ง
3. รายละเอียดของกลุ่มอาสาสมัคร
   - ลักษณะของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย

     คุณสมบัติของอาสาสมัครที่เข้าร่วมโครงการวิจัย
     - *ผู้ใช้บริการ*ในช่วงอายุ 18-70 ปี
     - *ผู้ใช้บริการ*ที่มีภาวะปากแห้ง หรือมีการใช้น้ำลายเทียมอยู่แล้ว
     - *ผู้ใช้บริการ*ที่ไม่มีประวัติแพ้ยาทุกชนิด

     การคัดเลือกอาสาสมัครออกจากโครงการวิจัย
     - *ผู้ใช้บริการ*ที่มีประวัติการแพ้ส่วนประกอบในตำรับ
     - *ผู้ใช้บริการ*ที่มีความผิดปกติของระบบภูมิคุ้มกัน เช่น โรคเบาหวาน โรคติดเชื้อ HIV หรือได้รับยากดภูมิคุ้มกัน

     การถอนตัวของอาสาสมัครออกจากโครงการวิจัย
     - *ผู้ใช้บริการ*ไม่สามารถปฏิบัติตามเงื่อนไขที่ระบุไว้ในการวิจัย
     - *ผู้ใช้บริการ*เกิดอาการแพ้ส่วนประกอบของตำรับระหว่างการวิจัย
     - *ผู้ใช้บริการ*ไม่ประสงค์จะเข้าร่วมโครงการวิจัยอีกต่อไป
   - มีจำนวนทั้งหมด 20 คน

- วิธีการได้มาซึ่งกลุ่มอาสาสมัคร คือ การติดประกาศหาอาสาสมัครที่สนใจเข้าร่วมวิจัย ณ ห้องจ่ายยาโรงพยาบาลคณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย จากนั้นทำการนัดหมายอาสาสมัครเพื่อทดลองใช้น้ำลายเทียม พร้อมตอบแบบสอบถามเกี่ยวกับอาการปากแห้ง
ณ ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

4. กระบวนการการวิจัยที่กระทำต่อกลุ่มอาสาสมัคร คือ

- กระบวนการการวิจัยที่กระทำต่อกลุ่มอาสาสมัคร คือ
    1) *อาสาสมัครจะได้รับการสัมภาษณ์ โดยคำถามตามแบบคัดกรอง เมื่อผ่านเกณฑ์การคัดเข้าอาสาสมัครจะได้รับเอกสารข้อมูลการวิจัย*
    2) *อาสาสมัครจะต้องลงชื่อในใบยินยอม หากสมัครใจในการเข้าร่วมงานวิจัย*
    3) อาสาสมัครแต่ละคนจะได้รับน้ำลายเทียม พร้อมแบบสอบถามเกี่ยวกับอาการปากแห้ง และอธิบายวิธีใช้ และรายละเอียดแบบสอบถามโดยผู้วิจัย ใช้เวลา 10 นาที
    4) อาสาสมัครตอบแบบสอบถามเกี่ยวกับอาการปากแห้งของตนเองก่อนใช้น้ำลายเทียม ใช้เวลา 10 – 15 นาที
    5) อาสาสมัครทดลองใช้น้ำลายเทียม และตอบแบบสอบถามเกี่ยวกับอาการปากแห้งอีกครั้งครั้งจากทดลองใช้ 15 นาที โดยกระบวนการทั้งหมดใช้เวลาประมาณ 30-60 นาที

5. กระบวนการให้ข้อมูลแก่กลุ่มอาสาสมัคร จะทำด้วยวิธีแจกเอกสารข้อมูล คำอธิบาย/คำชี้แจง กระบวนการวิจัยให้อาสาสมัครอ่าน โดยมีผู้วิจัยเป็นผู้ชี้แจง และเปิดโอกาสให้ผู้เข้าร่วมวิจัยสอบถามข้อสงสัย

6. เงื่อนไขการปฏิบัติตัว

อาสาสมัครจะต้องปฏิบัติตามคำอธิบาย/คำชี้แจ้งของผู้วิจัยอย่างเคร่งครัด หากเกิดข้อสงสัยสามารถสอบถามผู้วิจัยได้ตลอดเวลา และตอบคำถาม/แบบสอบถามด้วยความจริง

7. **อันตรายหรือความเสี่ยง**ที่อาจเกิดขึ้นแก่กลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย

งานวิจัยนี้มีความเสี่ยงจากการใช้น้ำลายเทียมแบบเจลก่อตัวเอง คือ การแพ้ส่วนประกอบในน้ำลายเทียม ซึ่งผู้วิจัยได้มีการสอบถามอาสาสมัครถึงข้อมูลการแพ้ยา สารเคมีหรืออาหารที่อาจจะเกี่ยวข้องกับการแพ้ตัวยาสำคัญหรือส่วนประกอบในน้ำลายเทียมและกำหนดเป็นเกณฑ์ในการคัดอาสาสมัครออกเพื่อป้องกันไม่ให้ผู้ที่อาจแพ้ตัวยาสำคัญหรือส่วนประกอบในน้ำลายเทียมได้รับผลกระทบจากการใช้น้ำลายเทียม และผู้วิจัยได้เลือกใช้ส่วนประกอบของน้ำลายเทียมที่เป็นเกรดสำหรับเตรียมยาและอาหาร และมีข้อมูลด้านความปลอดภัยครบถ้วน เพื่อลดความเสี่ยงในการแพ้สารปนเปื้อนอีกทางหนึ่งด้วย ดังนั้นจึงอาจสรุปได้ว่าน้ำลายเทียมแบบเจลก่อตัวเองมีความปลอดภัยในการใช้และมีโอกาสเกิดผลข้างเคียงดังกล่าวต่ำมาก นอกจากนี้ยังมีความเสี่ยงเล็กน้อยที่ไม่มากกว่าความเสี่ยงในชีวิตประจำวัน เช่น การเสียเวลาในการเดินทางและการพบแพทย์ที่นานขึ้น ความไม่สะดวกในการใช้น้ำลายเทียมและบันทึกข้อมูล การสูญเสียรายได้เนื่องจากมาพบแพทย์ เป็นต้น

8. ***คำแนะนำหากเกิดอาการแพ้ อาการไม่พึงประสงค์***
หากอาสาสมัครมีอาการดังต่อไปนี้
    - *คัน*
    - *มีผื่นขึ้น*
    - *เวียนศีรษะ*
    - *คลื่นไส้*

ให้หยุดใช้ผลิตภัณฑ์ทันที และทำการบ้วนปากด้วยน้ำสะอาด หากหลังจากหยุดใช้ผลิตภัณฑ์แล้วอาการยังไม่บรรเทา ควรรีบพบแพทย์ พร้อมแจ้งอาการที่เกิดทันที

9. **ประโยชน์**ในการเข้าร่วมวิจัย

ท่านจะไม่ได้รับประโยชน์โดยตรง ในการร่วมการวิจัยครั้งนี้ แต่ผลจากการวิจัยที่ได้จะทำให้ได้ผลิตภัณฑ์น้ำลายเทียมที่มีประสิทธิภาพเป็นที่น่าพอใจแก่ผู้ป่วยต่อไป

10. การเข้าร่วมในการวิจัยของท่านเป็นโดย**สมัครใจ** และสามารถ**ปฏิเสธ**ที่จะเข้าร่วมหรือ**ถอนตัว**จากการวิจัยได้ทุกขณะ โดยไม่ต้องให้เหตุผลและไม่สูญเสียประโยชน์ที่พึงได้รับ

11. ข้อมูลที่เกี่ยวข้องกับท่านจะเก็บเป็น**ความลับ** หากมีการเสนอผลการวิจัยจะเสนอเป็นภาพรวม ข้อมูลใดที่สามารถระบุถึงตัวท่านได้จะไม่ปรากฏในรายงาน

12. อาสาสมัครจะได้รับค่าเสียเวลาในการเข้าร่วมงานวิจัยครั้งนี้ จำนวน 100 บาท ตลอดระยะเวลาในการเข้าร่วมงานวิจัย

13. "หากท่านไม่ได้รับการปฏิบัติตามข้อมูลดังกล่าวสามารถร้องเรียนได้ที่ คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2218-3202 E-mail: eccu@chula.ac.th

เอกสารรับรองโครงการวิจัย 083.1/61
วันที่รับรอง 16 พ.ค. 2561
วันหมดอายุ 15 พ.ค. 2562

AF05-07

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ทำที่.....

วันที่.....เดือน.....พ.ศ. .....

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย การพัฒนาเจลก่อตัวเองเพื่อใช้เป็นน้ำลายเทียม และการศึกษาความพึงพอใจ

ชื่อผู้วิจัย นางสาววริ ติษยาธิคม

ที่อยู่ที่ติดต่อ ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

โทรศัพท์ 08-5199-3694

ข้าพเจ้า **ได้รับทราบ**รายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด และ**ได้รับคำอธิบาย**จากผู้วิจัย **จนเข้าใจเป็นอย่างดี**แล้ว

ข้าพเจ้าจึง**สมัครใจ**เข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอม ตอบแบบสอบถามเกี่ยวกับอาการปากแห้ง ก่อนทดลองใช้น้ำลายเทียม *เวลา 10 – 15 นาที* และทดลองใช้น้ำลายเทียม และตอบแบบสอบถามหลังการทดลองใช้ *เวลา 15 นาที* รายละเอียดตามที่ระบุในเอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย (AF 04-07)

ข้าพเจ้ามี**สิทธิถอนตัว**ออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ **โดยไม่ต้องแจ้งเหตุผล** ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะ**เก็บรักษาเป็นความลับ** โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2218-3202

E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....

(นางสาววริ ติษยาธิคม)

ผู้วิจัยหลัก

ลงชื่อ.....

(.....)

ผู้มีส่วนร่วมในการวิจัย

ลงชื่อ.....

(ผู้ช่วยศาสตราจารย์ เภสัชกรหญิง ดร.จุฑารัตน์ กิจส่งเสริมชน)

พยาน

ลขที่โครงการวิจัย 083.1/61

วันที่รับรอง 16 พ.ค. 2561

วันหมดอายุ 15 พ.ค. 2562

V.2.4/2558

แบบคัดกรองอาสาสมัครตามเกณฑ์การคัดเข้า-คัดออก

เรื่อง การพัฒนาเจลก่อตัวเองเพื่อใช้เป็นน้ำลายเทียม และการศึกษาความพึงพอใจ

สำหรับเจ้าหน้าที่กรอก		
หมายเลขที่.....		
พิจารณาจากการสัมภาษณ์อาสาสมัคร		
เกณฑ์การคัดเข้า	ผ่าน	ไม่ผ่าน
1. อายุ 18 – 70 ปี	☐	☐
2. มีภาวะปากแห้ง หรือมีการใช้น้ำลายเทียมอยู่	☐	☐
3. ไม่มีประวัติแพ้ยาทุกชนิด	☐	☐
4. อาสาสมัครต้องให้ความยินยอมเป็นลายลักษณ์อักษร	☐	☐
5. มีความยินดีที่จะเข้าร่วมงานวิจัย	☐	☐
เกณฑ์การคัดออก	ผ่าน	ไม่ผ่าน
1. มีประวัติการแพ้ส่วนประกอบในตำรับ	☐	☐
2. มีความผิดปกติของระบบภูมิคุ้มกัน	☐	☐
ผลการคัดกรอง		
ผู้วิจัย พิจารณาแล้วว่า อาสาสมัคร		
☐ ผ่าน ☐ ไม่ผ่าน		
การคัดกรองอาสาสมัครตามเกณฑ์การคัดเข้า-คัดออก		

เลขที่โครงการวิจัย 083.1/61

วันที่รับรอง 16 พ.ค. 2561

วันหมดอายุ 15 พ.ค. 2562

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ผู้คัดกรอง

แบบสอบถามความพึงพอใจของผู้ป่วยในการใช้ผลิตภัณฑ์น้ำลายเทียม

รหัสผู้ป่วย : _____ วันที่ _____

เพศ ❒ หญิง ❒ ชาย อายุ _____

เคี้ยวหมาก ❒ เคี้ยว ❒ ไม่เคี้ยว สูบบุหรี่ ❒ สูบ ❒ ไม่สูบ

คำชี้แจง คำถามต่อไปนี้เป็นคำถามเกี่ยวกับสภาวะช่องปาก ประสิทธิภาพและความพึงพอใจในการใช้ผลิตภัณฑ์น้ำลายเทียมของท่าน ขอให้ท่านทำเครื่องหมาย | ลงบนเส้นที่กำหนดตามระดับความคิดเห็นของท่าน โปรดตอบคำถามตามความเป็นจริง

1. ให้คะแนนระดับความแห้งในช่องปากของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

หลังใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

2. ให้คะแนนระดับความแห้งในช่องคอของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

หลังใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

3. ให้คะแนนระดับความแห้งของริมฝีปากของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

หลังใช้ _____

0 ไม่แห้งเลย — 10 แห้งมาก

4. ให้คะแนนระดับความแห้งของลิ้นของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ ——————————————————

0 ไม่แห้งเลย — 10 แห้งมาก

หลังใช้ ——————————————————

0 ไม่แห้งเลย — 10 แห้งมาก

5. ให้คะแนนระดับความชุ่มของน้ำลายในช่องปากของคุณขณะอยู่เฉยๆจากมากไปน้อย

ก่อนใช้ ——————————————————

0 ไม่ชุ่มชื้นเลย — 10 ชุ่มชื้นมาก

หลังใช้ ——————————————————

0 ไม่ชุ่มชื้นเลย — 10 ชุ่มชื้นมาก

6. ให้คะแนนระดับความหนืดของน้ำลายในช่องปากของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ ——————————————————

0 ไม่หนืดเลย — 10 หนืดมาก

หลังใช้ ——————————————————

0 ไม่หนืดเลย — 10 หนืดมาก

7. ให้คะแนนระดับความรู้สึกกระหายน้ำของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้ ——————————————————

0 ไม่กระหายเลย — 10 กระหายมาก

หลังใช้ ——————————————————

0 ไม่กระหายเลย — 10 กระหายมาก

8. ให้คะแนนระดับความต้องการใช้น้ำลายเทียมของคุณขณะอยู่เฉยๆจากน้อยไปมาก

ก่อนใช้

0 ไม่ต้องการเลย — 10 ต้องการมาก

หลังใช้

0 ไม่ต้องการเลย — 10 ต้องการมาก

9. ให้คะแนนระดับความแห้งในช่องปากของคุณขณะพูดจากน้อยไปมาก

ก่อนใช้

0 ไม่แห้งเลย — 10 แห้งมาก

หลังใช้

0 ไม่แห้งเลย — 10 แห้งมาก

10. ให้คะแนนระดับความชุ่มของน้ำลายในช่องปากของคุณขณะพูดคุยจากมากไปน้อย

ก่อนใช้

0 ไม่ชุ่มชื้นเลย — 10 ชุ่มชื้นมาก

หลังใช้

0 ไม่ชุ่มชื้นเลย — 10 ชุ่มชื้นมาก

11. ให้คะแนนระดับความหนืดของน้ำลายในช่องปากของคุณขณะพูดคุยจากน้อยไปมาก

ก่อนใช้

0 ไม่หนืดเลย — 10 หนืดมาก

หลังใช้

0 ไม่หนืดเลย — 10 หนืดมาก

12. ให้คะแนนระดับความรู้สึกกระหายน้ำของคุณขณะพูดคุยจากน้อยไปมาก

ก่อนใช้ ———————————————

0 ไม่กระหายเลย — 10 กระหายมาก

หลังใช้ ———————————————

0 ไม่กระหายเลย — 10 กระหายมาก

13. ให้คะแนนระดับความต้องการใช้น้ำลายเทียมของคุณขณะพูดคุยจากน้อยไปมาก

ก่อนใช้ ———————————————

0 ไม่ต้องการเลย — 10 ต้องการมาก

หลังใช้ ———————————————

0 ไม่ต้องการเลย — 10 ต้องการมาก

14. ให้คะแนนระดับความยากลำบากในการพูดเนื่องจากภาวะช่องปากแห้ง

ก่อนใช้ ———————————————

0 ไม่ยากเลย — 10 ยากมาก

หลังใช้ ———————————————

0 ไม่ยากเลย — 10 ยากมาก

15. ให้คะแนนระดับความยากลำบากในการกลืนน้ำลายเนื่องจากภาวะช่องปากแห้ง

ก่อนใช้ ———————————————

0 ไม่ยากเลย — 10 ยากมาก

หลังใช้ ———————————————

0 ไม่ยากเลย — 10 ยากมาก

รายการประเมิน	ระดับความคิดเห็น					
	มากที่สุด	มาก	ค่อนข้างมาก	ค่อนข้างน้อย	น้อย	น้อยที่สุด
ความพึงพอใจต่อผลิตภัณฑ์						
ผลิตภัณฑ์สามารถใช้ได้ง่าย						
ผลิตภัณฑ์สามารถกระจายได้ทั่วช่องปาก						
ผลิตภัณฑ์สามารถคงอยู่ในช่องปากได้นาน						
ระดับความหวานของผลิตภัณฑ์						
ระดับความเค็มของผลิตภัณฑ์						
ระดับความหนืดของผลิตภัณฑ์						
ผลิตภัณฑ์มีรสชาติที่ยอมรับได้ มีความหนืดเหมาะสม สามารถใช้ได้ง่าย (ความพึงพอใจต่อผลิตภัณฑ์โดยรวม)						
ผลิตภัณฑ์สามารถช่วยให้สภาวะปากแห้งดีขึ้น (ประสิทธิภาพของผลิตภัณฑ์โดยรวม)						

ท่านเคยใช้ผลิตภัณฑ์น้ำลายเทียมมาก่อนหรือไม่ หากเคยใช้ ท่านรู้สึกพึงพอใจกับผลิตภัณฑ์ในการทดลองอย่างไร (อธิบาย)

❒ เคย ❒ ไม่เคย

_____

_____

ความถี่ในการใช้น้ำลายเทียม ช่วงกลางวัน: _____ครั้งต่อวัน

ความถี่ในการใช้น้ำลายเทียม ช่วงกลางคืน: _____ครั้งต่อวัน

ท่านรู้สึกอยากใช้ผลิตภัณฑ์ต่อไป หรือไม่ เพราะอะไร

_____

_____

_____

ท่านพบผลข้างเคียง หรืออาการไม่พึงประสงค์ระหว่างใช้ผลิตภัณฑ์นี้หรือไม่ อย่างไร

_____

_____

_____

ขอบพระคุณที่ให้ความร่วมมือ

ลงชื่อโครงการวิจัย 083.1/61

วันที่รับรอง 16 พ.ค. 2561

วันหมดอายุ 15 พ.ค. 2562

เปิดรับอาสาสมัครทดสอบผลิตภัณฑ์น้ำลายเทียม
จำนวน 20 คน

คุณสมบัติผู้สมัคร

- เพศหญิงหรือชาย สัญชาติไทย เชื้อชาติไทย
- อายุระหว่าง 18 – 70 ปี
- มีภาวะปากแห้ง หรือมีการใช้น้ำลายเทียมอยู่แล้ว
- ไม่มีประวัติแพ้ยา

ข้อมูลการทดสอบเบื้องต้น

- ตอบแบบสอบถามเกี่ยวกับอาการปากแห้งก่อน และหลังทดลองใช้ผลิตภัณฑ์
- ทดลองใช้ผลิตภัณฑ์น้ำลายเทียมเป็นเวลา 15 นาที
- มีค่าเสียเวลาจำนวน 100 บาทให้อาสาสมัครที่เข้าร่วม

ผู้สนใจสามารถสอบถามและลงชื่อพร้อมช่องทางติดต่อกลับ ได้ที่เภสัชกร และเจ้าหน้าที่ ณ ห้องจ่ายยา

สนใจติดต่อได้ที่

นางสาวรวิ ติษยาธิคม
ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม (ชั้น 4)
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
เบอร์โทรศัพท์: 085-199-3694
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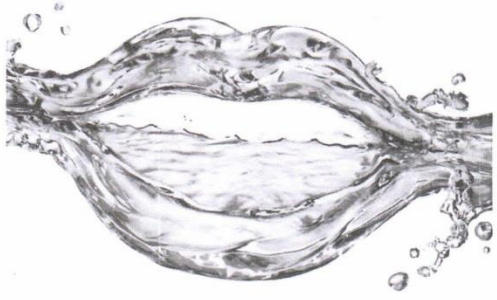

A dry mouth questionnaire used for screening volunteers (Stewart et al., 1998)

1. Does your mouth feel dry at night or on awakening?
2. Does your mouth feel dry at other times of the day?
3. Do you keep a glass of water by your bed?
4. Do you sip liquids to aid in swallowing dry foods?
5. Does your mouth feel dry when eating a meal?
6. Do you have difficulties swallowing any foods?

APPENDIX F

VAS scores of xerostomia questionnaire before and after using *in situ* gel-forming artificial saliva

Table F-1 VAS scores of dryness of mouth before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of dryness of mouth (mm)		
	Before	After	Difference
1	25	17	8
2	83	60	23
3	78	16	62
4	54	9	45
5	76	23	53
6	64	58	6
7	43	21	22
8	55	42	13
9	49	25	24
10	47	23	24
11	49	23	26
12	78	20	58
13	48	29	19
14	35	27	8
15	64	35	29
Mean	56.53	28.53	28.00
SD	16.99	14.61	18.24

Table F-2 VAS scores of dryness of throst before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of dryness of throat (mm)		
	Before	After	Difference
1	33	24	9
2	61	59	2
3	79	12	67
4	67	10	57
5	73	16	57
6	65	64	1
7	45	30	15
8	60	47	13
9	70	25	45
10	71	41	30
11	52	39	13
12	72	41	31
13	49	33	16
14	36	25	11
15	75	33	42
Mean	60.53	33.27	27.27
SD	14.41	15.75	21.57

Table F-3 VAS scores of dryness of lips before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of dryness of lips (mm)		
	Before	After	Difference
1	67	67	0
2	84	81	3
3	91	21	70
4	91	33	58
5	10	10	0
6	78	74	4
7	67	59	8
8	73	35	38
9	24	23	1
10	93	89	4
11	51	45	6
12	88	89	1
13	66	68	2
14	49	48	1
15	69	69	0
Mean	66.73	54.07	13.07
SD	24.49	25.49	22.83

Table F-4 VAS scores of dryness of tonge before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of dryness of tonge (mm)		
	Before	After	Difference
1	29	21	8
2	83	76	7
3	76	5	71
4	68	16	52
5	77	17	60
6	69	65	4
7	20	20	0
8	45	36	9
9	58	21	37
10	48	32	16
11	48	38	10
12	50	34	16
13	29	24	5
14	38	31	7
15	46	33	13
Mean	52.27	31.27	21.00
SD	19.16	18.39	22.62

Table F-5 VAS scores of quantity of saliva before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of quantity of saliva (mm)		
	Before	After	Difference
1	76	84	8
2	2	68	66
3	79	99	20
4	63	94	31
5	23	77	54
6	72	68	4
7	61	86	25
8	49	39	10
9	57	73	16
10	26	55	29
11	54	68	14
12	31	50	19
13	53	59	6
14	29	49	20
15	47	59	12
Mean	48.13	68.53	22.27
SD	21.93	17.27	17.39

Table F-6 VAS scores of stickiness of saliva before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of stickiness of saliva (mm)		
	Before	After	Difference
1	47	53	6
2	92	75	17
3	83	2	81
4	79	13	66
5	78	23	55
6	72	69	3
7	50	66	16
8	48	37	11
9	65	33	32
10	74	42	32
11	48	39	9
12	64	53	11
13	27	19	8
14	31	24	7
15	42	31	11
Mean	60.00	38.60	24.33
SD	19.69	21.30	24.28

Table F-7 VAS scores of thirst before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of thirst (mm)		
	Before	After	Difference
1	69	50	19
2	80	67	13
3	95	2	93
4	84	8	76
5	79	23	56
6	74	71	3
7	61	50	11
8	65	51	14
9	48	33	15
10	65	61	4
11	56	46	10
12	80	40	40
13	45	31	14
14	41	21	20
15	63	27	36
Mean	67.00	38.73	28.27
SD	15.38	20.50	27.08

Table F-8 VAS scores of the need to use artificial saliva before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of the need to use artificial saliva (mm)		
	Before	After	Difference
1	15	0	15
2	86	61	25
3	91	4	87
4	60	21	39
5	0	75	75
6	33	48	15
7	40	60	20
8	55	48	7
9	48	33	15
10	0	31	31
11	1	2	1
12	29	7	22
13	32	0	32
14	27	39	12
15	16	7	9
Mean	35.53	29.07	27.00
SD	28.58	25.37	24.24

Table F-9 VAS scores of dryness of mouth while speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of dryness of mouth while speaking (mm)		
	Before	After	Difference
1	75	58	17
2	85	67	18
3	97	3	94
4	81	9	72
5	80	22	58
6	69	64	5
7	64	41	23
8	58	49	9
9	58	34	24
10	51	26	25
11	61	51	10
12	84	42	42
13	60	33	27
14	41	19	22
15	60	43	17
Mean	68.27	37.40	30.87
SD	15.02	19.12	25.22

Table F-10 VAS scores of quantity of saliva while speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of quantity of saliva while speaking (mm)		
	Before	After	Difference
1	76	55	21
2	14	46	32
3	7	98	91
4	48	89	41
5	77	92	15
6	70	79	9
7	25	76	51
8	60	69	9
9	50	60	10
10	69	86	17
11	65	71	6
12	36	73	37
13	34	71	37
14	29	48	19
15	56	70	14
Mean	47.73	72.20	27.27
SD	22.55	15.41	22.33

Table F-11 VAS scores of stickiness of saliva while speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of stickiness of saliva while speaking (mm)		
	Before	After	Difference
1	36	43	7
2	85	66	19
3	84	4	80
4	67	9	58
5	78	89	11
6	53	48	5
7	47	27	20
8	63	52	11
9	61	29	32
10	65	45	20
11	55	51	4
12	53	48	5
13	33	18	15
14	39	19	20
15	67	45	22
Mean	59.07	39.53	21.93
SD	16.22	22.36	21.04

Table F-12 VAS scores of thirst while speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of thirst while speaking (mm)		
	Before	After	Difference
1	89	72	17
2	82	63	19
3	93	5	88
4	72	12	60
5	95	18	77
6	68	59	9
7	73	36	37
8	68	51	17
9	49	49	0
10	86	47	39
11	54	54	0
12	93	39	54
13	54	56	2
14	22	8	14
15	80	64	16
Mean	71.87	42.20	29.93
SD	20.30	21.80	28.18

Table F-13 VAS scores of the need to use artificial saliva while speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of the need to use artificial saliva while speaking (mm)		
	Before	After	Difference
1	26	17	9
2	85	64	21
3	89	17	72
4	57	14	43
5	6	84	78
6	24	50	26
7	51	16	35
8	69	69	0
9	54	34	20
10	0	39	39
11	1	2	1
12	49	24	25
13	23	1	22
14	15	45	30
15	29	17	12
Mean	38.53	32.87	28.87
SD	28.89	25.09	22.54

Table F-14 VAS scores of difficulty in speaking before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of difficulty in speaking (mm)		
	Before	After	Difference
1	30	0	30
2	72	54	18
3	91	10	81
4	54	1	53
5	79	21	58
6	51	45	6
7	23	7	16
8	48	43	5
9	67	31	36
10	16	3	13
11	3	3	0
12	70	9	61
13	27	9	18
14	43	17	26
15	15	4	11
Mean	45.93	17.13	28.80
SD	26.41	17.78	24.11

Table F-15 VAS scores of difficulty in swallowing before and after using in situ gel-forming artificial saliva

Volunteers code	VAS scores of difficulty in swallowing (mm)		
	Before	After	Difference
1	48	0	48
2	73	55	18
3	92	13	79
4	86	4	82
5	79	17	62
6	52	48	4
7	27	9	18
8	50	45	5
9	75	23	52
10	68	29	39
11	61	56	5
12	55	22	33
13	68	33	35
14	33	18	15
15	49	8	41
Mean	61.07	25.33	35.73
SD	18.59	18.42	25.52

Table F-16 Paired t-test VAS of xerostomia questionnaire before and after using in situ gel-forming artificial saliva

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	DMBefore	56.5333	15	16.98683	4.38598
	DMAfter	28.5333	15	14.61343	3.77317
Pair 2	DThBefore	60.5333	15	14.40668	3.71979
	DThAfter	33.2667	15	15.75013	4.06667
Pair 3	DLBefore	66.7333	15	24.49043	6.32340
	DLAfter	54.0667	15	25.49360	6.58242
Pair 4	DToBefore	52.2667	15	19.16271	4.94779
	DToAfter	31.2667	15	18.39047	4.74840
Pair 5	QSBefore	48.1333	15	21.93128	5.66263
	QSAfter	68.5333	15	17.27040	4.45920
Pair 6	SSBefore	60.0000	15	19.69409	5.08499
	SSAfter	38.6000	15	21.30325	5.50048
Pair 7	TBefore	67.0000	15	15.37623	3.97013
	TAfter	38.7333	15	20.49553	5.29192
Pair 8	NABefore	35.5333	15	28.57538	7.37813
	NAAfter	29.0667	15	25.37002	6.55051
Pair 9	DMsBefore	68.2667	15	15.02125	3.87847
	DMsAfter	37.4000	15	19.12291	4.93751
Pair 10	QSsBefore	47.7333	15	22.54667	5.82153
	QSsAfter	72.2000	15	15.41428	3.97995
Pair 11	SSsBefore	59.0667	15	16.21933	4.18781
	SSsAfter	39.5333	15	22.35706	5.77257
Pair 12	TsBefor	71.8667	15	20.30083	5.24165
	TsAfter	42.2000	15	21.80170	5.62918
Pair 13	NAsBefore	38.5333	15	28.89109	7.45965
	NAsAfter	32.8667	15	25.08804	6.47770
Pair 14	DifSpBefore	45.9333	15	26.41014	6.81907
	DifSpAfter	17.1333	15	17.77585	4.58971
Pair 15	DifSwBefore	61.0667	15	18.59134	4.80026
	DifSwAfter	25.3333	15	18.41842	4.75561

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	DMBefore - DMAfter	28.00000	18.24046	4.70967	17.89877	38.10123	5.945	14	.000
Pair 2	DThBefore - DThAfter	27.26667	21.56540	5.56816	15.32414	39.20919	4.897	14	.000
Pair 3	DLBefore - DLAfter	12.66667	23.06719	5.95592	-.10752	25.44085	2.127	14	.052
Pair 4	DToBefore - DToAfter	21.00000	22.62110	5.84074	8.47285	33.52715	3.595	14	.003
Pair 5	QSBefore - QSAfter	-20.40000	19.69336	5.08480	-31.30582	-9.49418	-4.012	14	.001
Pair 6	SSBefore - SSAfter	21.40000	27.08136	6.99238	6.40284	36.39716	3.060	14	.008
Pair 7	TBefore - TAfter	28.26667	27.07784	6.99147	13.27146	43.26188	4.043	14	.001
Pair 8	NABefore - NAAfter	6.46667	36.38262	9.39395	-13.68136	26.61469	.688	14	.502
Pair 9	DMsBefore - DMsAfter	30.86667	25.22433	6.51289	16.89790	44.83543	4.739	14	.000
Pair 10	QSsBefore - QSsAfter	-24.46667	25.57305	6.60293	-38.62855	-10.30478	-3.705	14	.002
Pair 11	SSsBefore - SSsAfter	19.53333	23.43949	6.05205	6.55298	32.51369	3.228	14	.006
Pair 12	TsBefor - TsAfter	29.66667	28.47723	7.35279	13.89650	45.43683	4.035	14	.001
Pair 13	NAsBefore - NAsAfter	5.66667	36.96846	9.54521	-14.80578	26.13911	.594	14	.562
Pair 14	DifSpBefore - DifSpAfter	28.80000	24.11342	6.22606	15.44643	42.15357	4.626	14	.000
Pair 15	DifSwBefore - DifSwAfter	35.73333	25.52161	6.58965	21.59994	49.86673	5.423	14	.000

APPENDIX G

Satisfaction of *in situ* gel-forming artificial saliva

Table G-1 satisfaction of *in situ* gel-forming artificial saliva

Volunteers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Ease of use	5	5	5	5	5	6	5	5	6	6	5	6	6	5	6
Spreadability	4	5	4	5	5	6	4	4	4	5	4	4	4	4	5
Long lasting	6	3	5	4	5	6	4	3	4	5	5	5	4	2	4
Sweetness	4	3	4	6	4	5	4	3	3	4	3	4	4	2	3
Salty	1	1	2	1	2	3	3	1	3	2	2	2	2	1	3
Viscosity	2	2	2	2	3	3	3	1	2	3	1	2	3	2	3
Overall	6	6	5	6	6	5	4	4	4	6	6	6	5	5	6
Efficacy	6	4	5	5	5	5	5	4	5	6	6	5	5	4	6
Sex	F	F	M	F	F	F	M	F	M	F	F	M	F	M	M

Satisfaction rate: 1 least satisfied, 2 less satisfied, 3 little satisfied, 4 quite satisfied, 5 very satisfied, 6 most satisfied

Sex: F=Female, M=Male

Table G-2 Chi-square test of satisfaction of *in situ* gel-forming artificial saliva

Test Statistics

	Ease of use	spreadability	longlasting	sweetness	salty	viscosity	Overall satisfaction	efficacy
Chi-Square	4.500[a]	1.750[b]	1.000[c]	3.000[c]	1.000[b]	1.750[b]	1.000[b]	3.250[b]
df	1	2	3	3	2	2	2	2
Asymp. Sig.	.034	.417	.801	.392	.607	.417	.607	.197

a. 2 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 4.0.

b. 3 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 2.7.

c. 4 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 2.0.

Table G-3 Chi-square test of ease of use satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
Ease of use	most	1	0	1
	much	5	2	7
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.381[a]	1	.537		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.622	1	.430		
Fisher's Exact Test				1.000	.750
Linear-by-Linear Association	.333	1	.564		
N of Valid Cases	8				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .25.

b. Computed only for a 2x2 table

Table G-4 Chi-square test of spreadability satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
spreadability	most	1	0	1
	much	3	0	3
	rather	2	2	4
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.667[a]	2	.264
Likelihood Ratio	3.452	2	.178
Linear-by-Linear Association	1.882	1	.170
N of Valid Cases	8		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is .25.

Table G-5 Chi-square test of long-lasting satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
Long-lasting	most	2	0	2
	much	2	1	3
	rather	1	1	2
	a bit	1	0	1
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.778[a]	3	.620
Likelihood Ratio	2.406	3	.493
Linear-by-Linear Association	.156	1	.693
N of Valid Cases	8		

a. 8 cells (100.0%) have expected count less than 5. The minimum expected count is .25.

Table G-6 Chi-square test of sweetness satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
sweetness	most	1	0	1
	much	2	0	2
	rather	2	2	4
	a bit	1	0	1
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.667[a]	3	.446
Likelihood Ratio	3.452	3	.327
Linear-by-Linear Association	.447	1	.504
N of Valid Cases	8		

a. 8 cells (100.0%) have expected count less than 5. The minimum expected count is .25.

Table G-7 Chi-square test of salty satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
salty	most	4	0	4
	much	1	1	2
	rather	1	1	2
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.667[a]	2	.264
Likelihood Ratio	3.452	2	.178
Linear-by-Linear Association	1.909	1	.167
N of Valid Cases	8		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is .50.

Table G-8 Chi-square test of viscosity satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
viscosity	most	1	0	1
	much	3	1	4
	rather	2	1	3
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.444[a]	2	.801
Likelihood Ratio	.680	2	.712
Linear-by-Linear Association	.333	1	.564
N of Valid Cases	8		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is .25.

Table G-9 Chi-square test of overall satisfaction of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
Overall satisfaction	most	4	0	4
	much	1	1	2
	rather	1	1	2
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.667[a]	2	.264
Likelihood Ratio	3.452	2	.178
Linear-by-Linear Association	1.909	1	.167
N of Valid Cases	8		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is .50.

Table G-10 Chi-square test of efficacy of *in situ* gel-forming artificial saliva versus sex

Crosstab

Count

		SEX		
		Female	Male	Total
efficacy	most	1	0	1
	much	3	2	5
	rather	2	0	2
Total		6	2	8

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.600[a]	2	.449
Likelihood Ratio	2.267	2	.322
Linear-by-Linear Association	.101	1	.750
N of Valid Cases	8		

a. 6 cells (100.0%) have expected count less than 5. The minimum expected count is .25.

APPENDIX H

Chemical substance Information

PRODUCT DATA SHEET

CPKelco
A HUBER COMPANY

KELCOGEL® GELLAN GUM

Document No.: 300-X
Effective Date: 18 May 2010

Description

KELCOGEL gellan gum is a multifunctional gelling agent for use in foods and personal care applications. **KELCOGEL** gellan gum is ideal for a variety of gelling, texturizing, stabilizing and film forming applications.

Features

- excellent stability
- high gel strength
- heat stable
- sparkling clarity
- outstanding flavor release
- easily combined with other hydrocolloids
- fluid gel suspension
- high compatibility with protein

Typical Applications

- aspics
- bakery fillings
- beverages / fluid gels
- confections
- dairy products
- dessert gels
- non-standard jams and jellies
- personal care
- fruit preparations

Typical Use Level

KELCOGEL gellan gum forms gels at extremely low gum use levels - as low as 0.05%. Gel strength can be increased by manipulating both gum and ion concentration.

Dispersion/Hydration

Model gels are produced by adding **KELCOGEL** gellan gum to tap water under shear, heating to 90°C, adding ions and cooling to set. Both monovalent and divalent ions can be used: K+, Na+, Ca++ and Mg++. Sequestrants such as sodium citrate or phosphates may be required for hydration in hard water.

Standard Packaging

Packed in 25-kg Leverpak drums (or their equivalent) with polyethylene liners (21 CFR §177.1520). All packaging materials comply with relevant UK, EU, and United States food contact legislation.

Ingredient/Labeling

KELCOGEL gellan gum
Food grade gellan gum, CAS: 71010-52-1; E418
For use as a stabilizer and thickener
Kosher approved; Halal approved

Regulatory Information

Gellan gum complies with requirements contained in the following regulations and standards: *Food Chemicals Codex,* 21 CFR § 172.665 (USA), *Canadian Food and Drug Law* (Item G.2, Table IV), JECFA, the purity criteria in the current EC Directive, 1829/2003/EC, and *Japan's Specifications and Standards for Food Additives*

Storage Conditions/ Shelf Life

Store in a roofed and well-ventilated area in the unopened original package. Functional properties of the product are guaranteed to conform with the stated sales specifications for **730 days** from the date of manufacture when stored under these conditions. Product quality should be re-evaluated prior to use if this "Best Before" date has been exceeded.

Quality System

Manufactured according to a Quality System registered to ISO 9001:2008.

KELCOGEL® GELLAN GUM

Document No.: 300-X
Effective Date: 18 May 2010

Specifications

Property	Requirement	Test Method
Particle Size	Tyler Standard Screen Scale, Ro-Tap	KTM146
- 28 mesh (600 µm)	Not less than 99% through	
- 42 mesh (355 µm)	Not less than 98% through	
Loss on Drying	Not more than 14%	KTM003
Powder Color	Not less than 72	KTM006
Solution pH		KTM005
- 1% gum in DI water	4.5 – 6.5	
Transmittance		KTM087
- 0.5% gum in 6 mM $CaCl_2$	Not less than 74%	
Isopropyl Alcohol	Not more than 750 mg/kg (ppm)	KTM520
Bacteria*	Not more than 10,000 cfu/g	KTM800
Fungal (Yeast & Mold) Count	Not more than 400 cfu/g	KTM803
Coliform	Negative by Most Probable Number (MPN)	KTM801
Escherichia coli	Absent in 25 g	KTM802
Salmonella spp.	Absent in 25 g	KTM804

* Total viable mesophilic aerobic count, 48 hr incubation

Specifications – Guaranteed to Comply

Testing to the following specifications is conducted on a skip-lot basis and may not be reported on the Certificate of Analysis. Product is guaranteed by CP Kelco to comply with compendial requirements applicable for each property.

Property	Requirement	Test Method
Identification	Pass	KTM519
Total Nitrogen	Not more than 3.0%	KTM516
Assay	3.3 – 6.8% CO_2	KTM503
Ash	4.0 – 14.0%	KTM007
Heavy Metals	Not more than 20.0 mg/kg (ppm)	KTM514
Lead	Not more than 2.0 mg/kg (ppm)	KTM514
Arsenic	Not more than 2.0 mg/kg (ppm)	KTM514
Mercury	Not more than 1.0 mg/kg (ppm)	KTM514
Cadmium	Not more than 1.0 mg/kg (ppm)	KTM514
Staphylococcus aureus	Absent in 1.0 g	KTM806
Pseudomonas aeruginosa	Absent in 1.0 g	KTM807

METHODS OF TESTING (For test methods not listed, follow the applicable compendium. Full details of test methods are available upon request)

Particle Size (KTM146)
Shake 50 g product on 28 and 42 mesh (600 and 355 µm) Tyler Standard Screens for 20 minutes using a Ro-Tap sieve shaker.

Loss on Drying (KTM003)
Spread 3-5 g product evenly on a tared weighing pan and weigh accurately. Dry in an oven at 105°C for 2½ hours. Cool in a desiccator and reweigh.

Powder Color (KTM006)
Test method is available upon request.

Solution pH (KTM005)
Slowly add 3 g product to 297 mL deionized water in a 400-mL beaker while stirring at 800 rpm using a low-pitched, propeller-type stirrer. After stirring for 30 min, measure the pH of this solution using a pH meter.

Transmittance (KTM087)
Slowly add 1.50 g product to 250 g deionized water in a tared 400-mL beaker while stirring at 600-800 rpm. Heat to 70-75°C (158-168°F) and hold at this temperature for 15 minutes with continued stirring. Pipet 4.8 mL of a calcium chloride solution (prepared by dissolving 37.755 g $CaCl_2 \cdot 2H_2O$ in 1 L of deionized water) into the heated solution and continue mixing for 1 to 2 minutes. Using deionized water at 80°C (176°F), adjust the weight of the solution to 301 g and mix for 30 seconds. Measure the transmittance of this solution using a Bausch and Lomb Spectronic 215, or other suitable spectrometer, at 490 nm. Use deionized water as the 100% transmittance standard. **Note:** After adding the solution to the cuvette, allow to cool to room temperature (approximately 1 hour) before measuring the transmittance.

NOTE: CP Kelco reserves the right to use company test methodology.

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Certificate of Analysis

1.05832.5000 Magnesium chloride hexahydrate cryst. EMPROVE® Ph Eur,BP,USP,JPC, FCC,E 511
Batch A1106632

	Spec. Values		Batch Values	
Assay (complexometric)	99.0 - 101.0	%	100.3	%
Identity	passes test		passes test	
Appearance of solution	passes test		passes test	
Insoluble matter	≤ 0.005	%	≤ 0.005	%
Acidity or alkalinity	passes test		passes test	
pH-value (5 %; water)	5.0 - 7.0		5.6	
Bromide (Br)	≤ 0.05	%	≤ 0.05	%
Sulphate (SO_4)	≤ 0.005	%	< 0.005	%
Heavy metals (as Pb)	≤ 0.001	%	≤ 0.001	%
Al (Aluminium)	≤ 0.0001	%	≤ 0.0001	%
As (Arsenic)	≤ 0.0002	%	≤ 0.0002	%
Ba (Barium)	passes test		passes test	
Ca (Calcium)	≤ 0.01	%	< 0.01	%
Fe (Iron)	≤ 0.0005	%	≤ 0.0005	%
Hg (Mercury)	≤ 0.0001	%	≤ 0.0001	%
K (Potassium)	≤ 0.0500	%	≤ 0.0500	%
NH_4 (Ammonium)	≤ 0.005	%	≤ 0.005	%
Pb (Lead)	≤ 0.0002	%	≤ 0.0002	%
Residual solvents (Ph.Eur./USP/ICH)	excluded by manufacturing process		excluded by manufacturing process	
Water	51.0 - 55.0	%	53.2	%
Endotoxins	≤ 3.0	I.U./g	≤ 3.0	I.U./g
Total aerobic microbial count	≤ 100	CFU/g	≤ 100	CFU/g
Total yeast and mould count	≤ 100	CFU/g	≤ 100	CFU/g

Residues of metal catalysts or metal reagents acc. to EMEA/CHMP/SWP/4446/2000 are not likely to be present.
Conforms to the purity criteria on food additives according to the current European Commission Regulation.
Corresponds to Ph Eur, BP, USP, JPC, FCC, E511

Date of examination (DD.MM.YYYY) 06.01.2017
Minimum shelf life (DD.MM.YYYY) 31.12.2018

Dr. Andreas Lang
Responsible laboratory manager quality control

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VITA

Miss Rawi Tishyadhigama was born on December 23th, 1990, in Nonthaburi, Thailand. In 2013, she received her Bachelor's degree of Food Sciences and Technology with first class honors from the Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand. After graduation, she worked at Multimax Co., Ltd. for two years. In 2015, she was entering the Master's degree program in Cosmetic Sciences at Chulalongkorn University.