DEVELOPMENT OF FILM-FORMING SOLUTIONS CONTAINING *KAEMPFERIA PARVIFLORA* EXTRACT FOR APHTHOUS ULCERS

NATTANICH WATTANAPHRAYA

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Cosmetic Sciences
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By Nattanich Wattanaphraya

has been approved by the Graduate School as partial fulfillment of the requirements

for the Master of Science in Cosmetic Sciences of Naresuan University

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ABSTRACT

This study aimed to develop a mucoadhesive film containing black ginger extract for the treatment of oral ulcers. Various formulations were prepared using different polymers and their characteristics were evaluated. PVP K90, HPMC E15 LV, and HPC SL were chosen as mucoadhesive polymers based on their solubility and solution characteristics. The addition of mucoadhesive polymers led to a significant improvement in the residence time of the film due to the increased number of hydrogen binding sites for mucin. Furthermore, the hydrophilicity of the polymers was found to be the main mechanism by which film adhesion was improved. The release profile of methoxyflavone from the film showed an initial slow release followed by a sharp release phase due to film erosion. Stability studies demonstrated that the film remained stable under different storage conditions, exhibiting no significant changes in film-forming time and residence time. However, a slight decrease in viscosity, pH, and drug content was observed, which can be addressed by optimizing the formulation process and storage conditions.

In conclusion, the developed mucoadhesive film containing black ginger extract has potential as an effective treatment for oral ulcers, providing extended residence time and controlled drug release. The study findings suggest that HPMC is the most effective mucoadhesive polymer for achieving longer residence time and increased viscosity. The addition of Eudragit® E100 can be used to extend the duration of action by reducing the effectiveness of dissolution over time. The results

of this study provide a basis for further investigation of the film's efficacy and safety in *in vivo* studies.

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Nattanich Wattanaphraya

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Chapter 1

Introduction

1.1. Statement of the study

Aphthous stomatitis, also known as aphthous ulcers, canker sores, or aphthae, is a condition characterized by painful, round or ovoid lesions on the oral mucosa with distinct erythematous margins (1, 2, 3). These ulcers cause considerable discomfort for patients, making it difficult to eat, speak, laugh, and maintain oral hygiene. Moreover, the pain associated with aphthous stomatitis can negatively impact emotional well-being and overall quality of life (4, 5). While the exact etiology of aphthous stomatitis remains elusive, potential causative factors include nutritional deficiencies, weakened immune system, and viral or bacterial infections (6). Consequently, the primary goals of treatment focus on pain relief, lesion duration reduction, and minimization of recurrence in cases of constant or frequent ulcers (3).

Various topical dosage forms are currently available for aphthous stomatitis treatment, including pastes, creams, ointments, gels, films, and solutions (7). Comparative analyses reveal that semi-solid and liquid forms disperse more easily across the oral mucosa but are prone to being rapidly washed away compared to solid forms like adhesive patches or films. Notably, films are flexible and offer protection to the wound surface (8). Given the advantages and limitations of different topical dosage forms, this study aims to investigate film-forming systems (FFS). FFS is a non-solid dosage form that generates a film in situ upon application to the skin or any surface, offering easy application and target area protection.

Corticosteroids serve as the first-line treatment for pain and inflammation; however, prolonged treatment and frequent exposure may result in fungal infections, drug resistance, and potentially life-threatening complications (7). Alternative therapies using natural herbal medicines have gained attention, such as allicin tablets (9), *Aloe vera* and myrrh oral gels (10), and tobacco leaves mouthwash (11). In Thailand, the rhizome of *Kaempferia parviflora* (kra-chai-dam; black ginger) is utilized for treating aphthous ulcers (12). The anti-inflammatory and antimicrobial properties of *Kaempferia parviflora* extract are attributed to its methoxyflavones content (13). Consequently, this study selected *Kaempferia parviflora* extract as the model active ingredient in the FFS.

Currently, a limited number of FFS products are available on the market for treating aphthous stomatitis, such as Urgo Filmogel® Aphtes and Zilactin®-B. These FFS products primarily aim to provide a protective layer over the ulcerated surface, preventing further irritation. Standard treatment recommendations include antibiotics and antiseptics, but herbal treatments have demonstrated potential as alternative medications due to their lower incidence of side effects compared to synthetic drugs. This project seeks to develop a film-forming system containing *Kaempferia parviflora*

extract as an active ingredient for the treatment of minor aphthous ulcers, with a focus on evaluating the physical properties, efficacy, and stability of the film-forming formulation.

1.2.Objectives

The objectives of this study were

1. To develop an FFS containing Kaempferia parviflora extract for aphthous ulcers, capable of forming a mucoadhesive film.
2. To investigate the film properties of variable formulation.

1.3. Hypotheses of the Study

1. The FFS containing *Kaempferia parviflora* extract can form the films in the oral cavity environment.
2. The hydrophilic additive polymers can improve the mucoadhesive property of films.

Chapter 2
Literature Reviews

2.1. Aphthous stomatitis

Aphthous stomatitis, also referred to as aphthous ulcers, canker sores, or aphthae, is the most prevalent inflammatory and ulcerative oral condition. The term "aphthous" is derived from the Greek word "aphtha," which means ulceration. Aphthous ulcer is a type of oral mucosal ulceration that typically progresses through several distinct stages. During the pre-ulcerative stage, patients may experience a burning sensation prior to the onset of anesthesia. This stage is characterized by the infiltration of mononuclear cells, primarily lymphocytes, into the affected area. Subsequently, edema and localized vasculitis ensue, leading to local swelling and ulceration in the ulcerative stage. The ulcer becomes infiltrated by plasma cells, neutrophils, and lymphocytes, as it progresses toward healing. The final stage of the healing process is characterized by epithelial mucosal regeneration (as shown in Figure 1). The cell-mediated immune response is implicated in the pathogenesis of aphthous stomatitis, with TNF-α being released as the main inflammatory cytokine, implicating T-cells and other leukocytes, including mast cells and macrophages. TNF-α has chemotactic activity on neutrophils, which cause inflammation and the release of Major Histocompatibility Complex (MHC), resulting in damage to epithelial cells via cytotoxic T cells (CD8+), Interleukin (IL) such as IL-2, IL-6, IL-10, and IL-1β. Gamma delta (γϴ) T lymphocytes are important in the release of antibodies mediated by cytotoxic reactions in the epithelium of the oral mucosa. The factors that affect the inflammatory processes during aphthous ulcer are described in Figure 1 (14). This disorder primarily affects young adults, with certain studies indicating a prevalence as high as 60% in students. The frequency and severity of the condition generally decrease after 50 years of age. The cause of aphthous stomatitis is unknown in most patients. However, in some cases, the disease may be a manifestation of an underlying infectious, inflammatory, immunologic, or nutritional disorder (3, 15).

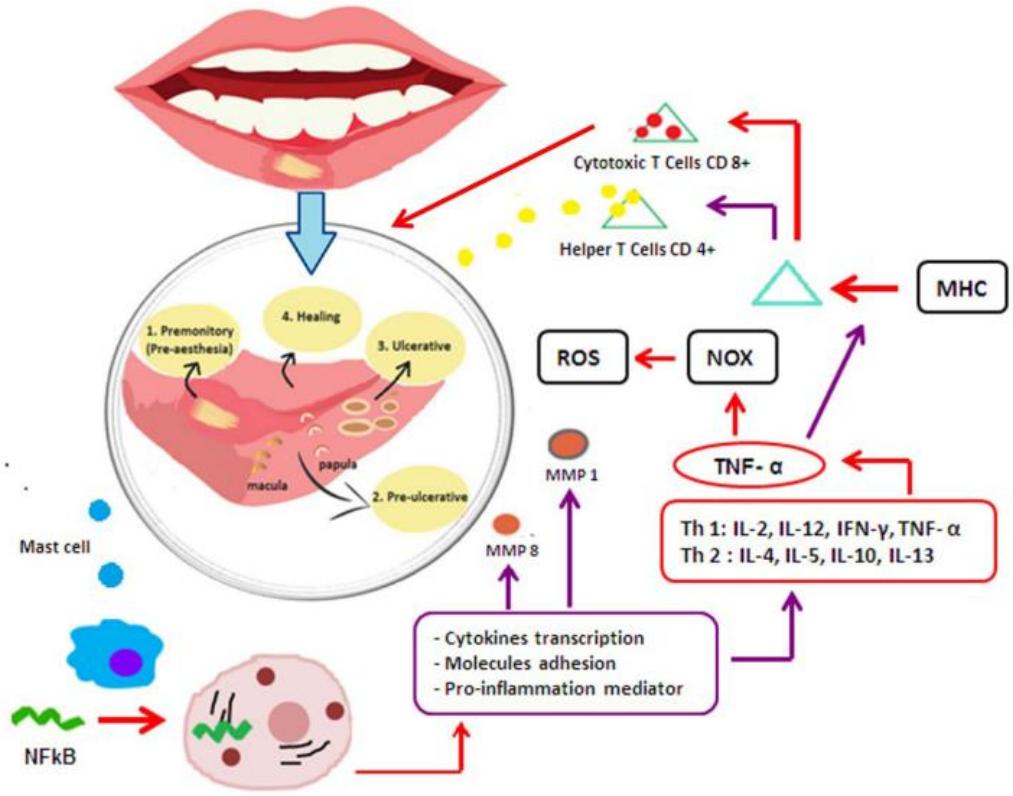


Figure 1 Inflammation process induced Aphthous Stomatitis.
Source: Suharyani I, Fouad Abdelwahab Mohammed A, Muchtaridi M, Wathoni N, Abdassah M. Drug Des Devel Ther. 2021; 15:4071-89.

2.1.1. Clinical Characteristics

A typical presentation of aphthous ulcers in the oral mucosa is characterized by painful, circular, shallow ulcerations that are covered by a yellowish-tan pseudomembrane, surrounded by an erythematous halo. The lesions are well-defined, without any tissue tags, and are self-limiting, lasting between 1 or 2 weeks. Common sites for these ulcers include the tongue, buccal mucosa, floor of the mouth, and lingual mucosa. In contrast, less frequently affected areas include heavily keratinized surfaces such as the palate and gingiva (2).

2.1.2. Classification

Aphthous ulcer has been classified based on the size and evolution of lesions into 3 forms: minor aphthae (Figure 2), major aphthae (Figure 3), and herpetiform ulcerations (Figure 4). Table 1 describes the general characteristics of the 3 types.(1, 15)

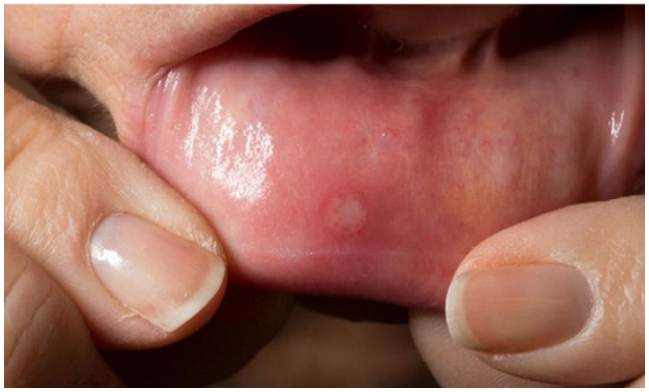


Figure 2 Minor aphthae on the anterior oral mucosa
Source: Cui RZ, Bruce AJ, Rogers RS. Clinics in Dermatology. 2016;34(4):475-81.

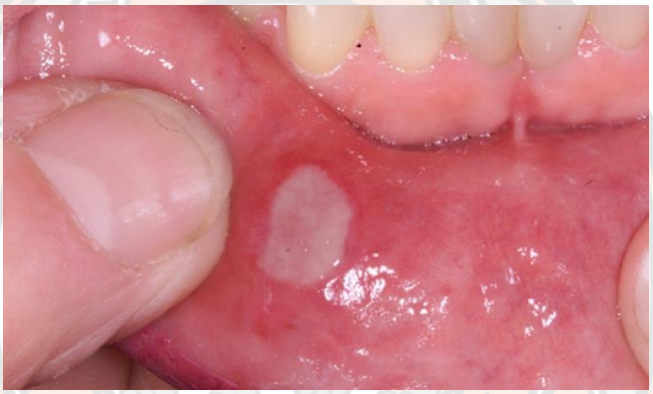


Figure 3 Major aphthae on the anterior oral mucosa
Source: Cui RZ, Bruce AJ, Rogers RS. Clinics in Dermatology. 2016;34(4):475-81.

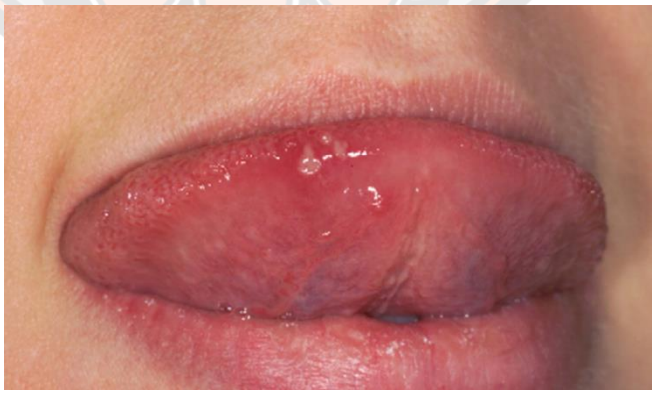


Figure 4 Herpetiform ulcer on the tip of the tongue
Source: Cui RZ, Bruce AJ, Rogers RS. Clinics in Dermatology. 2016;34(4):475-81.

Table 1 Classification of aphthous ulcer

General characteristics of aphthous ulcer	Types of aphthous ulcer		
	Minor	Major	Herpetiform
Frequency	75%	10–15%	5–10%
Gender predilection	M=F	M=F	F > M (usually)
Age of onset (years)	5–19	10–19	20–29
Number of ulcers	1–5	1–10	10–100
Size of ulcers (mm)	<10	>10	1–2 (Larger if coalesced)
Duration (days)	4–14	>30	<30
Systemic features	None	Fever, odynophagia	None
Location	Buccal/labial	Labial/soft palate or tongue/pharynx	Any location orally
Scarring	No	Yes	No

* M = Male, F = Female

2.1.3. Etiology

The underlying cause of aphthous stomatitis remains unknown, despite extensive research in this field. Several factors have been suggested as potential causative agents, including local, systemic, immunologic, genetic, allergic, nutritional, and microbial factors. Furthermore, some medications, such as immunosuppressive drugs like calcineurin and mTOR (Mammalian Target of Rapamycin) inhibitors, have been associated with severe aphthous-like stomatitis (Table 2) (1, 15).

Table 2 Predisposing factors of aphthous stomatitis.

Local factors	Trauma Smoking Dysregulated saliva composition
Microbial factors	Bacterial: Streptococci Viral: Varicella zoster, Cytomegalovirus
Systemic factors	Behçet's disease Mouth and genital ulcers with inflamed cartilage (MAGIC) syndrome Crohn's disease Ulcerative colitis HIV infection Periodic fever, aphthosis, pharyngitis, and adenitis (PFAPA) or Marshall's syndrome Cyclic neutropenia Stress; psychological imbalance, menstrual cycle
Nutritional factors	Gluten sensitive enteropathy Iron, folic acid, zinc deficiencies Vitamin B1, B2, B6 and B12 deficiencies
Genetic factors	Ethnicity HLA haplotypes
Allergic/Immunologic factors	Local T-lymphocyte cytotoxicity Abnormal CD4:CD8 ratio Dysregulated cytokine levels Microbe-induced hypersensitivity Sodium lauryl sulfate (SLS) sensitivity Food sensitivity
Others	Non-steroidal anti-inflammatory drugs (NSAIDS) Beta blockers Immunosuppressive drugs

2.1.4. Management of aphthous stomatitis

The etiology of aphthous stomatitis remains elusive despite extensive research efforts. the main objective of treating aphthous stomatitis is to alleviate the accompanying symptoms and minimize the size of the ulceration. In order to achieve this goal, a variety of drug delivery systems for aphthous stomatitis have been developed over time (16).

Drug delivery systems (DDS) for aphthous stomatitis can be classified into several forms based on their physical state. Liquid DDS options, such as mouth rinse or mouthwash solutions, oral liquid preparations, lotions, and inhalers, are frequently used for the treatment of aphthous stomatitis due to their ease of use and convenience.

On the other hand, semi-solid DDS formulations, such as gels, pastes, patches, creams, and ointments, are preferred in some cases because of their longer residence time at the oral mucosa. The use of polymers in semi-solid preparations increases their adhesion to the oral mucosa, which in turn, decreases the effect of "saliva wash out" and enhances their therapeutic efficacy.

Solid DDS options, including powders, granules, and tablets, are also commonly used for the management of aphthous stomatitis. Solid preparations can be applied directly to the affected area, providing localized delivery and promoting ulcer healing. The choice of DDS form will depend on a variety of factors, including the patient's preference, the severity and location of the ulcers, and the desired treatment outcome.

Topical therapy is the first choice of treatment for aphthous stomatitis. They are cheap, effective and safe. Treatment is directed toward symptomatic relief (of pain), prevention of recurrences, and accelerating healing. The American Academy of Oral Medicine has recommended topical treatments for aphthous stomatitis. Topical medications include anesthetics, antihistamines, antimicrobials, and anti-inflammatory agents. Evidence of successful use of these agents for aphthous ulcers is primarily anecdotal (17).

Some problem of topical therapy is obtaining the optimum effect of active ingredients because semisolid and liquid topical dosage forms applied to mucosal surfaces are inevitably rubbed or rinsed away. To ensure maximum effect, patients are instructed to dab the area of ulcer dry, apply a small amount of gel or cream after rinsing, and avoid eating or drinking for 30 min. This is repeated three or four times daily while ulcers persist (3). Previously study, Needleman and Frederick investigated 3 gel bioadhesion properties in human volunteers (18). The tissue culture coverslip plastic films with adherent gel were applied to the dried site and seated into position with gentle finger pressure for approximately 2 s. The subjects were then observed for retention of the film every 15 min during the first hour and hourly for 6 h. The results shown the mean adhesion times for the gels varied quite substantially, ranging from 42.6 min for the chitosan formulation, through 89.3 min for poly (ethylene oxide) and

153.5 min for the xanthan gum formulation. While Javed Ali and co-worker prepared buccoadhesive erodible disk for treatment of oro-dental infections by using SCMC PVP and HPMC K4M as film former. The results shown adhesion time of erodible disk in healthy human volunteers was longer than 5 hours (5.58 ± 0.33) and released the drug for a period of over 6.0 hours (19). From this study demonstrated that films have more adhesion time than semisolid and liquid forms. Moreover, oral films may be preferred over solid forms in terms of flexibility and comfort. Previously, Collins and Deasy were prepare bioadhesive Lozenge for cetylpyridinium chloride delivery (20). The device was evaluated *in vivo* by three human volunteers. The volunteers encountered with the device was that residual material remained attached to the buccal mucosa when the device was removed. Moreover, they need to wipe off excessive saliva before applying the device to the oral mucosa and the need to remove gently would need to be stressed. While Javed Ali and co-worker developed erodible buccoadhesive disks of cetylpyridinium chloride (19). The *in vivo* evaluation of the optimized disks on healthy human volunteers revealed that the disks eroded completely and none had to be removed due to irritation. The advantage of this disk was its erodible character as compared to the lozenges prepared earlier.

2.2. Film forming systems

Film forming systems is a novel approach which can be used as an alternative to conventional topical and transdermal formulations. It is defined as non-solid dosage form that produces a film *in situ*, i.e., after application on the skin or any other body surface (Figure 5). These systems contain the drug and film forming excipients in a vehicle which, upon contact with the skin, leaves behind a film of excipients along with the drug up on solvent evaporation (21).

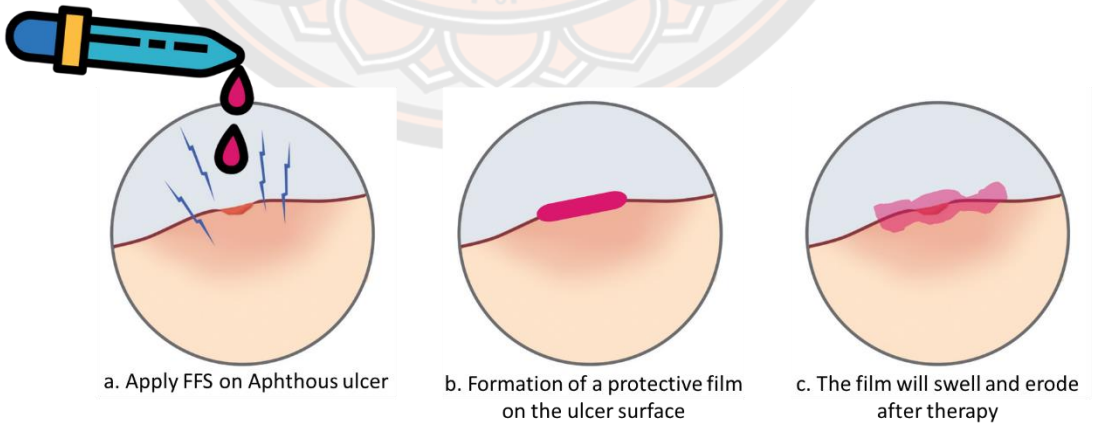


Figure 5 Application of film forming solution on the aphthous ulcer surface.

Film forming systems is used in oral ulcer and mucositis treatment as local anesthetic formulation. Hydroxypropyl cellulose (HPC) gel preparation that also contains the topical anesthetic agent benzocaine hydrochloride (15%). It forms a durable, mucoadhesive, protective film when applied to the mucosa (22). In this study, film forming polymer is HPC that has mucoadhesion property. Leung and Robinson (23) described mucoadhesion as the interaction between a mucin surface and a synthetic or natural polymer.

2.2.1. Mucoadhesion

Mucoadhesion refers to the binding between two materials, where one of the materials is a mucosal surface. In the context of drug delivery, bioadhesion refers to the attachment of a drug carrier system to a specific biological location, such as epithelial tissue or the mucous layer on the surface of a tissue. When the adhesion is to the mucous layer, it is called mucoadhesion. It is important to note that mucoadhesion is distinct from bioadhesion; while bioadhesion refers to the attachment of a polymer to a biological membrane, mucoadhesion specifically refers to adhesion to a mucous membrane. Mucoadhesion occurs in two stages depending on nature of dosage form and its delivery. Stage I (Contact Stage): wetting, spreading and swelling of the mucoadhesive surface creates close contact between a mucoadhesive and a mucosa. Sometimes additional forces like mechanical system in vaginal delivery, aero dynamics in nasal delivery and peristaltic motions in intestinal delivery of dosage form. Stage II (Consolidation Stage): moisture breaks molecules and interpenetration or dominant attractive interaction between two surfaces starts due to Van der Waals forces, electrostatic attractions, hydrogen bonding and hydrophobic interactions (24). Consolidation stage is explained by 6 theories (25) as shown in Figure 6.

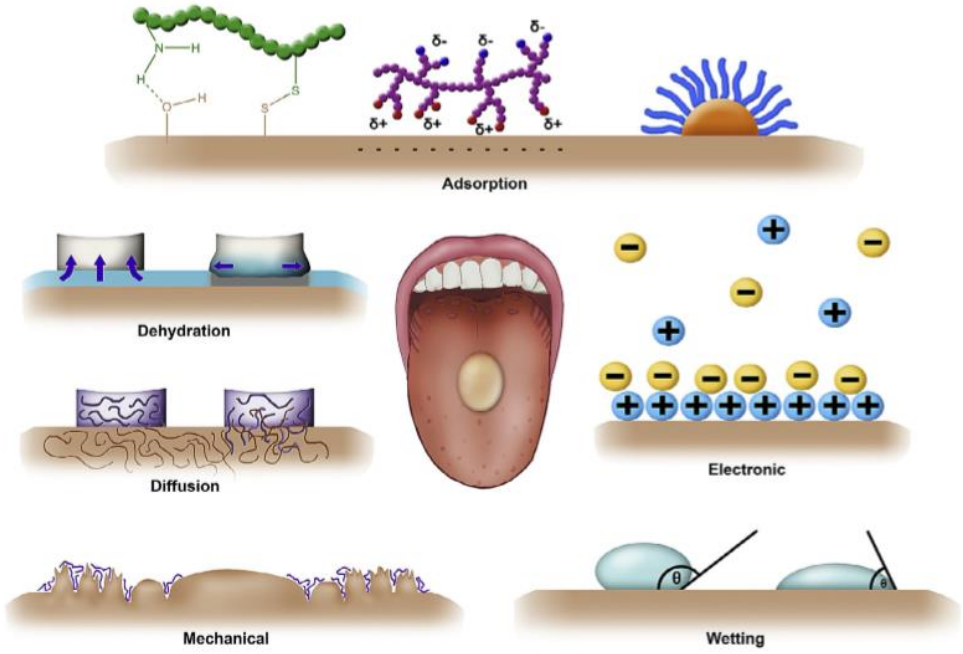


Figure 6 The six main mechanisms of mucoadhesion: adsorption, dehydration, diffusion, electronic, mechanical and wetting.
Source: Cook SL, Bull SP, Methven L, Parker JK, Khutoryanskiy VV. Food Hydrocolloids. 2017;72:281-96.

Wetting theory is concerned with polymer spread and ability to swell on the wet mucosal surface. A higher affinity to spread on the mucosa results in stronger mucoadhesion. Typically, the wetting phenomena are important for liquid mucoadhesives.

Dehydration theory describes the process where a material capable of gelling is brought into contact with a moist mucosal membrane. The movement of water from the mucous gel to the water-absorbing material reaches equilibrium and facilitates an adhesive joint. An example of this is the water uptake by a solid dosage form containing a hydrophilic polymer, such as poly (-acrylic acid), when placed on a moist surface. Once in contact with the wet mucosa, the dosage form will rapidly dehydrate the surface and adhesion will occur.

Diffusion theory considers the entanglement of polymer and mucin chains due to interpenetration, allowing for further primary and potentially secondary bonds to form, strengthening the adhesion.

Adsorption theory considers interactions between the mucosal surface and polymer; including Van der Waals forces, hydrogen bonds, and hydrophobic interactions. These non-covalent interactions are likely to form the majority of interactions; however, covalent bonding is possible depending on the chemical properties of the polymer. Thiolate polymers can form disulfide bonds with cysteine groups in mucins via thiol exchange reactions, or the oxidation of free thiol groups.

The protein backbone of some mucins contains large regions high in cysteine residues and low in oligosaccharides, which provide a potential area for strong chemical bonds to occur.

Electronic theory describes the transfer of electrons between the mucoadhesive and the mucous layer, resulting in the formation of a charged double layer at the interface of the mucin and polymer networks.

Mechanical theory describes the effect of contact area on the interaction between the polymer and mucosal surface. The effect of this will be particularly relevant in the oral cavity, which has a very thin layer of saliva in some areas; therefore, the mucoadhesive is more likely to contact the rough underlying tissue. Irregular surfaces and micro-cracks give a larger contact area and thus mucoadhesive strength. The papillae on the tongue provide a suitably rough surface and therefore greater surface area for penetration by mucoadhesives.

Mucoadhesive formulations use polymers as the adhesive component. These polymers also form viscous liquids when hydrated with water that increases their retention time over mucosal surfaces and may lead to adhesive interactions. Mucoadhesive polymers should possess certain physicochemical features including hydrophilicity, numerous hydrogen bond-forming groups, flexibility for interpenetration with mucous and epithelial tissue, and viscoelastic properties (26).

2.2.2. Film-forming system for aphthous ulcer

Film-forming system was used as a novel preparation form for aphthous ulcer, this system can be directly applied to the affected area, allowing for localized delivery of the medication. Additionally, the film-forming system has the ability to protect the ulcer surface, thereby promoting healing. Rodu et al. was evaluated the mucosal binding characteristics and the pain relief and protection properties of Zilactin, a hydroxypropyl cellulose (HPC) film former. They prepare HPC film forming gel in several formulas include HPC with tannic acid, HPC with salicylic acid, HPC with boric acid and HPC without the 3 acids. The results shown HPC with boric acid and HPC without the 3 acids can’t form films on oral mucosa while HPC with tannic acid, HPC with salicylic acid can form adherent white opaque films (Figure 7).

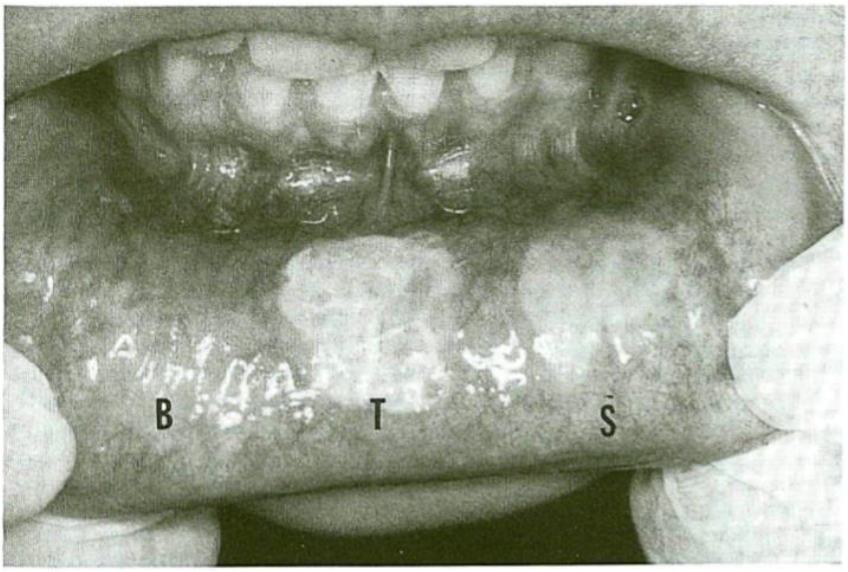


Figure 7 Film formation by HPC coupled with tannic (t) and salicylic (s) acids but not with boric (b) acid.
Source: Rodu B, Russell CM. Oral Surgery, Oral Medicine, Oral Pathology. 1988;65(6):699-703.

Tannic and salicylic acids are key elements responsible for the clinical properties demonstrated by their studies. The carboxyl groups of these acids provide esterification of the hydroxyl groups of HPC (Figure 8). This esterification decreases water solubility by blocking the water loving hydroxyl groups, causing the cloud point to decrease. As Zilactin is placed in contact with saliva upon application, the esterified polymers of both acids become opaque, insoluble (27).

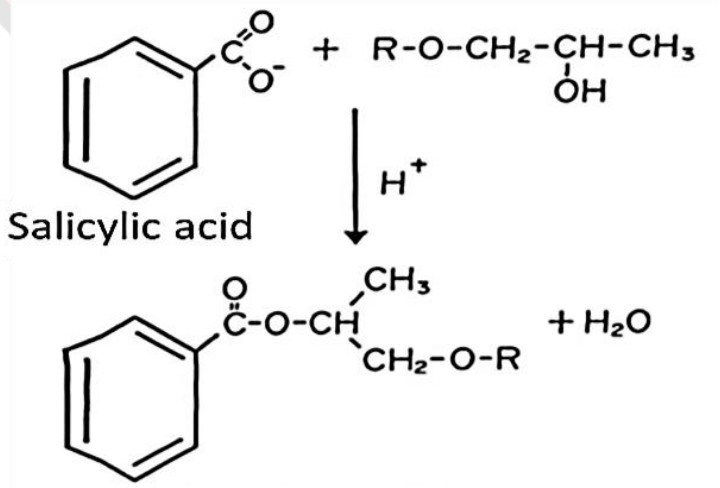


Figure 8 Esterification of the hydroxypropyl group on the polymer backbone (R-) by salicylic acid. The process is similar for tannic acid, a larger molecule.
Source: Rodu B, Russell CM. Oral Surgery, Oral Medicine, Oral Pathology. 1988;65(6):699-703.

From this previous study shown the stimuli responsive polymers are interested for development of a novel FFS. Eudragit® L, S, FS, RL, RS and E polymers are a pH-sensitive polymers trademark of Evonik Industries in Germany, first marketed in 1950s. Eudragit prepared by the polymerization of acrylic and methacrylic acids or their esters, e.g., butyl ester or dimethylaminoethyl ester**.** Eudragit provided good adhesive strength and produced transparent, elastic, and self-adhesive films (21, 28). For example, aminoalkyl methacrylate copolymer (Eudragit® E) is a Food and Drug Administration (FDA)-approved cationic polymer having high solubility below pH 5.0 and swellable above pH 5.0 (29). Eudragit® E 100 is widely used in topical formulations and transdermal drug delivery system as a film-forming agent because of nontoxic, nonirritant and safe in humans. Sritharadol et al. prepared mupirocin spray for antibacterial and wound-healing applications using Eudragit® E 100 as a film-forming agent, glycerol as a humectant and plasticizer and PEG400 as a plasticizer. The result shown the optimized mupirocin spray formed the film immediately after sprays droplets reached the skin with a drying time less than 3 min. The stickiness of the film was low. The film was flexible and uniform without any crack. The optimized mupirocin spray was intact on the skin and resistant against washing with water after 15s of rinsing. This is an advantage of hydrophobic property of Eudragit® E 100 (30). From pH-sensitive, hydrophobic and bioadhesive properties of Eudragit® E 100, we hypothesized a film-forming solution using Eudragit® E 100 can provide a thin film in oral cavity and protect the wound surface of aphthous ulcer.

2.2.3. *Kaempferia parviflora* for aphthous ulcer

Kaempferia parviflora Wall. ex Baker or kra-chai-dam, a plant in a family of *Zingiberaceae* and sometimes referred as Thai ginseng, is very popular for health promotion in Thailand. This plant is a perennial herb that grows to 90 cm height with dark purple to black rhizomes and these colors lead to the name kra-chai-dam. Extracts and purified compounds of *Kaempferia parviflora* are used for the treatment of gout, aphthous ulcer, abscesses, allergy and gastrointestinal disorders, as well as an aphrodisiac.

Since the etiology and pathogenesis of aphthous ulcer remains unclear, there is currently no consensus regarding a definitive curative therapy. The commonly accepted treatment strategy is to lessen the pain and duration of lesions. For the treatment of aphthous ulcer normally, they use the standard topical agent include corticosteroid to reduce inflammatory of the ulcer so the anti-inflammatory effect of *Kaempferia parviflora* is focused.

Previously, Tewtrakul and Subhadhirasakul (2008) reported the effects of 5-hydroxy-3,7,3,4-tetramethoxyflavone from *Kaempferia parviflora* on nitric oxide (NO), PGE2 and tumor necrosis factor-alpha (TNF-) productions in RAW264.7 macrophage cells. It was found that compound 5 exhibited potent anti-inflammatory effect against LPS-induced NO and PGE2 release in RAW264.7 cells with IC50

values of 16.1 and 16.3 M, respectively (12). Therefore, Sae-wong and co-worker (2009) investigate the mechanism in transcriptional level of this plant on the suppression of iNOS and COX-2 genes as well as the anti-inflammatory effect in animal model (carrageenan-induced rat paw edema) and its acute toxicity in mice. In summary, the present study on both *in vitro* (macrophage cell line) and *in vivo* (carrageenan-induced rat paw edema) supports the traditional use of *Kaempferia parviflora* for treatment of inflammation. The anti-inflammatory properties of *Kaempferia parviflora* and its compound Retusin (also known as compound 5) were investigated. The study focused on their effects on RAW 264.7 macrophages, a type of immune cell. It was found that the anti-inflammatory mechanism involved mainly the downregulation of inducible nitric oxide synthase (iNOS) mRNA expression, which is responsible for the production of the pro-inflammatory molecule nitric oxide (NO). Additionally, there was a partial downregulation of cyclooxygenase-2 (COX-2) mRNA expression (31), another enzyme involved in inflammation. Three active components of Kaempferia parviflora, namely 5,7-dimethoxyflavone, trimethylapigenin, and tetramethylluteolin, were identified. These components significantly inhibited the production of NO and moderately inhibited the production of tumor necrosis factor-alpha (TNF-α), another inflammatory molecule. They also strongly inhibited the expression of iNOS mRNA and iNOS protein. However, they did not affect certain proteins involved in cellular signaling pathways related to inflammation. Trimethylapigenin (compound 4) exhibited the most potent anti-inflammatory activity and was found to inhibit the enzyme activity of spleen tyrosine kinase (SYK), suggesting its involvement in suppressing inflammatory signaling in macrophages. Overall, the study demonstrated that the anti-inflammatory activity of *Kaempferia parviflora* and its compounds was mainly attributed to the downregulation of iNOS mRNA expression, which reduces the production of NO. Additionally, the inhibition of SYK by trimethylapigenin may play a role in suppressing inflammatory signaling. These findings suggest that *Kaempferia parviflora* extract and its methoxyflavonoids have the potential to reduce inflammation, making them potentially beneficial for conditions such as aphthous ulcers.

Moreover, Tewtrakul and Subhadhirasakul (2008) reported the antimicrobial activity of the ethanol extract of *Kaempferia parviflora* against human pathogens, including bacteria, yeast and dermatophyte fungi (32). The ethanol extract and seven compounds of K. parviflora were further studied using agar dilution method against dermatophytes. Only 3, 5, 7-trimethoxyflavone present in this extract showed appreciable anti-fungal activity with MIC values of 250 µg/mL. According to the obtained results, 3, 5, 7-trimethoxyflavone could be responsible only in part of the antifungal effect of the *Kaempferia parviflora* extract.

Chapter 3

Materials and methods

3.1. Materials

1. *Kaempferia parviflora* (black ginger) extract (TISTR, Thailand)
2. Eudragit® E 100 (Evonik, Germany)
3. Hydroxypropyl cellulose (HPC) SL (Nippon Soda Co., Ltd, Japan)
4. Hydroxypropyl methylcellulose (HPMC) E15 LV (Colorcon Asia Pacific Pte., Ltd, Singapore)
5. Polyvinylpyrrolidone (PVP) K90 (Fluka, Switzerland)
6. Potassium dihydrogen phosphate (Ajax Finechem, Australia)
7. Sodium hydroxide (Ajax Finechem, Australia)

3.2. Equipment

1. High Performance Liquid Chromatography (Shimadzu, Japan)
2. Viscometer (Brookfield DV III ultra V6.0 RV, Brookfield Engineering Laboratories, Middleboro, MA)
3. Shear rheometer (Anton Paar Rheoplus, Austria)
4. Incubator shaker (GFL 1086 shaking water bath, Burgwedel, Germany)
5. Peristaltic pump (Cole-Parmer, Wertheim, Germany)
6. Drop shape analyzer (DSA100, KRÜSS, Germany)
7. Hot air oven (Model 600, Memmert, Schwabach, Germany)
8. Hot plate stirrer (C-MAG HS7, IKA, Selangor, Wilmington, North Carolina, United States)
9. Sonication bath (Transsonic TP690, Elma, Singen, Germany)
10. Water bath (WNE22, Memmert, Schwabach, Germany)

3.3. Preparation of Film Forming Solutions

A film-forming solution (FFS) was prepared by dissolving Eudragit® E100 in 70% w/w ethanol and stirred continuously using a magnetic stirrer at room temperature until a clear solution was obtained. Subsequently, *Kaempferia parviflora* extract was incorporated into the Eudragit® E100 solution various from 5-15% w/w, followed by the addition of an additive polymer (PVP K90, HPMC E15 LV, and HPC SL). The mixture was stirred continuously at room temperature until a clear solution was obtained.

3.4. Physicochemical characterization

3.4.1. Viscosity

The viscosity of the film-forming solutions (FFS) (0.5 g) was determined using a Brookfield DV III ultra V6.0 RV cone and plate Rheometer (Brookfield Engineering Laboratories, Middleboro, MA) using spindle # CP40, 51 and 52 at 25°C ± 0.3°C.

3.4.2. pH measurement

The pH of the FFS was measured by pH-indicator paper (Merck, Germany) to check whether each FFS causes irritation to the buccal mucosa. The mean of three observations was calculated.

3.4.3. Wettability

Films were cast on glass slides using the solvent evaporation method. Water contact angles were measured using a contact angle goniometer (Drop Shape Analyzer – DSA100, KRÜSS, Germany) by depositing 15.0 µL droplets of purified water onto the film surfaces using a microsyringe. The angle between the tangent line and the film surface from the goniometric scale was measured 10 s after depositing each droplet onto the surface, at both the right and left sides of the drop. Ten droplets were measured for each film sample, and the average water contact angle values were calculated.

3.4.4. Rheological analysis

The preliminary rheological analysis of the various FFS with and without *Kaempferia parviflora* extract, was conducted using a shear rheometer (Anton Paar Rheoplus, Austria) equipped with a temperature control plate. The rheological property was expressed in terms of the loss tangent (tangent phase angle) and the moduli, indicating the formation of a viscoelastic gel. This experiment was performed in two conditions: in the absence and presence of prewarmed PBS at 37 °C to simulate saliva (pH 6.8). A gap of 0.8 mm was set between the lower temperature control plate and the upper parallel plate (25 mm in diameter; PP25). The tangent phase angle (tan δ) and the moduli (G' and G") were measured by depositing 0.3 mL of the solution onto the hot plate, previously set to 37°C. The relationship between the tan δ and moduli was defined in Eq. (1)(33). To study the effect of PBS pH 6.8, 0.3 mL prewarmed PBS was deposited onto and around the sample. The rheological behavior was investigated at 1 Hz. The experimental setup is illustrated in Figure 9.

$$tan\ \delta = \frac{G''}{G'} \quad (1)$$

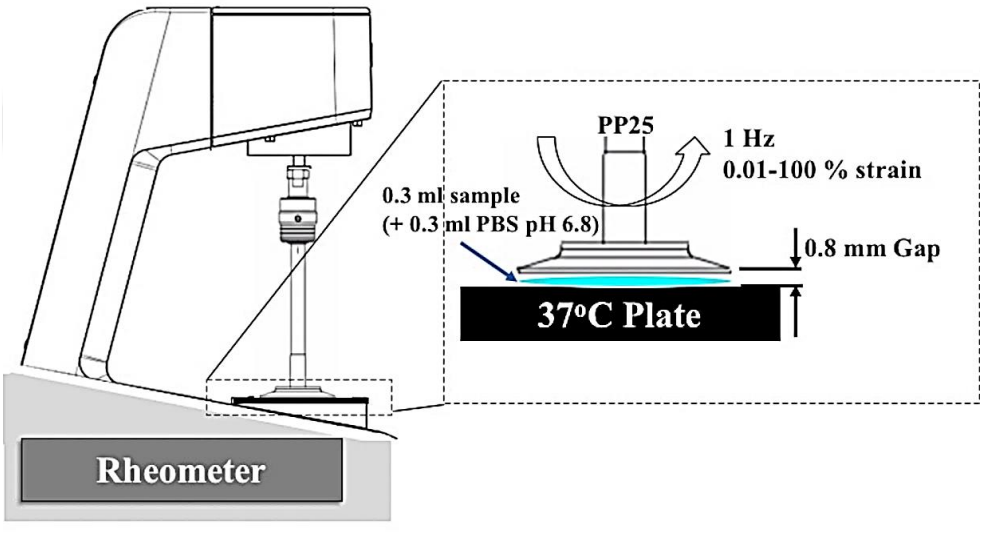


Figure 9 Experimental set-up of the dynamic rheological analysis. The analyses were performed under ambient conditions (25±3° C, 60±5 %relative humidity) on the warm plate controlled at 37 ° C

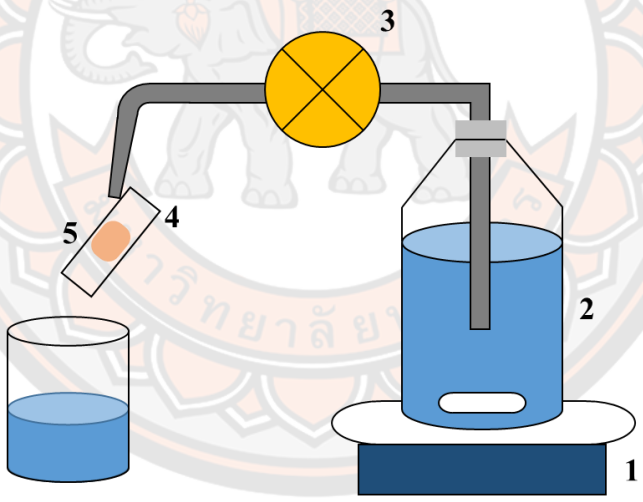
3.5. Film-forming time

The film-forming time was determined under two conditions: in the absence and presence of PBS pH 6.8. The film-forming formulations were applied to a Petri dish at a temperature of 37 ± 0.5°C with the help of a plastic positioning device and a dropper. The applied weight was 200 mg. After 5 s, a glass slide was placed on the film without pressure. If there were no visible remains of liquid on the glass slide after removal, the film was considered dry. If remains of visible liquid were present on the

glass slide, the experiment was repeated every 5 s until the film completely dried. The time to form a film was noted with the help of a stopwatch (34, 35).

3.6. *In vitro* residence time

This experiment was adapted from Gajdošová M, et al. (36), Srichaivatana K, et al. (37) and Semalty M, et al.(38). Buccal mucosa was simulated using a cellophane membrane covered with a 5% mucin dispersion (w/w) in phosphate buffer of pH 6.8 (10 mL per 1 cm^2). A cellophane membrane was mounted on a glass slide, and then 30 mg of the formulations were spread on the cellophane membrane (1 cm^2) and allowed to equilibrate for 1 min. The *in vitro* residence time was measured using the wash-off method. The membrane was exposed to a continuous flow of phosphate buffer at 37±2 °C at a 45° angle. A flow rate of 10 mL/min was maintained using a peristaltic pump (Cole-Parmer, Wertheim, Germany), and the phosphate buffer was dropped from 10 cm above the membrane. The time required for complete detachment of the film from the membrane surface was recorded, and the process was repeated three times.



1. Hot plate
2. Rising solution (Phosphate buffer pH 6.8)
3. Peristaltic pump
4. Glass slide
5. Cellophane membrane cover by a 5% mucin dispersion (w/w)

Figure 10 Schematic representation of wash-off model for measurement of in vitro residence time of FFS

3.7. *In vitro* drug release study

The film-forming solution (100 mg) was applied to a petri dish in area 1 cm^2 and immersed in 10 mL of PBS pH 6.8. The system was maintained at 37±0.5 °C

under shaking at 50 rpm and covered to prevent water evaporation. At predetermined time intervals (60, 120, 180 and 240 min), 5.0 mL of each sample was withdrawn and an equal volume of medium was added. The concentration of black ginger extract in each sample was measured by HPLC with UV-VIS detector at 254 nm**.** The analytical column was an ACE® C18 column (4.6 mm × 150 mm, 5 µm) controlled at 40°C. The mobile phase was water and methanol using the following gradient: 0-35 min: isocratic at 35% water/65% methanol at a flow rate 0.6 mL/min; and 35-60 min isocratic at 20% water/80% methanol at a flow rate 0.6 mL/min. The injection volume was 5 µL.

3.8. Stability test

Stability testing was performed under accelerated conditions. FFS was stored at 45°C for a 4-week period in an airtight container. Viscosity, pH, film-forming time, residence time, and drug content were used as parameters to compare the physical properties of FFS before and after storage at 45°C.

3.9. Statistical analysis

Data was presented as mean ± standard deviation and were analyzed using analysis of variance (ANOVA) testing. Statistical significance was set at *p*-value < 0.05.

Chapter 4
Results and discussion

4.1. Preparation and characterization of Film Forming Solutions

The Film Forming Solutions (FFS) containing black ginger extract were prepared according to Table 3. The FFS appeared as brownish-green clear viscous solutions with a characteristic odor. The pH value of the FFS was measured to be 7.00. The addition of mucoadhesive polymer and black ginger extract did not significantly alter the pH value of the FFS.

Table 3 Compositions of the tested film-forming solutions.

Component (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F7PVP	F7HPMC	F7HPC
Eudragit® E100	10	20	30	40	50	30	30	30	30	30	30
Black ginger extract	-	-	-	-	-	5	10	15	10	10	10
PVP K90	-	-	-	-	-	-	-	-	5	-	-
HPMC E15 LV	-	-	-	-	-	-	-	-	-	5	-
HPC SL	-	-	-	-	-	-	-	-	-	-	5

Upon application to the aphthous ulcer (Figure 11a), the FFS rapidly formed a thin film by a solvent exchange mechanism, covering the ulcer surface and protecting it from mechanical stress (Figure 11b). The black ginger extract was gradually released from the film as an anti-inflammatory agent, providing therapeutic benefits to the ulcer (Figure 11c).

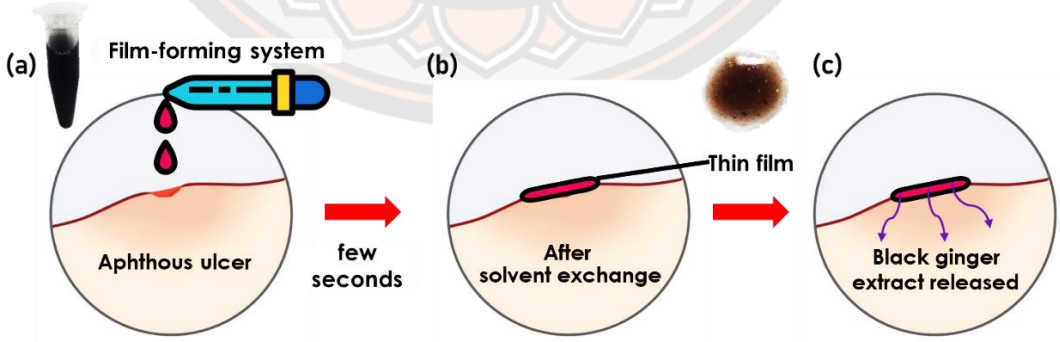


Figure 11 Mucoadhesive film-forming systems (FFSs) containing black ginger extract as a treatment for Aphthous ulcers.

4.2. Effect of amount of Eudragit® E100

4.2.1. Film-forming time

The film-forming time of the FFS was determined under both wet and dry conditions. The wet condition involved the use of a phase separation mechanism, while the dry condition involved solvent evaporation. Figure 12a shows that the film-forming time was very rapid in the wet state, taking only a few seconds. This was due to the rapid changes in solubility of Eudragit® E100 in response to a change in solvent composition. The increase in Eudragit® E100 concentration from 30% to 50% w/w did not result in a significant change in film-forming time, as the polymer molecules may have become tightly packed at -high concentrations (39). In contrast, the film formed more slowly in dry conditions due to the rate of solvent evaporation, as shown in Figure 12b. However, increasing the relative loading of Eudragit® E100 led to a decrease in film-forming time because of the increased polymer content and reduced solvent volume, resulting in faster solvent evaporation. Nevertheless, the results in Figure 12b showed that 10% w/w of Eudragit® E100 dried faster than 20% w/w of Eudragit® E100 due to the larger evaporation area (40). The viscosity of 10% w/w of Eudragit® E100 was found to be quite low (Figure 13), leading to more spreadability and faster film formation.

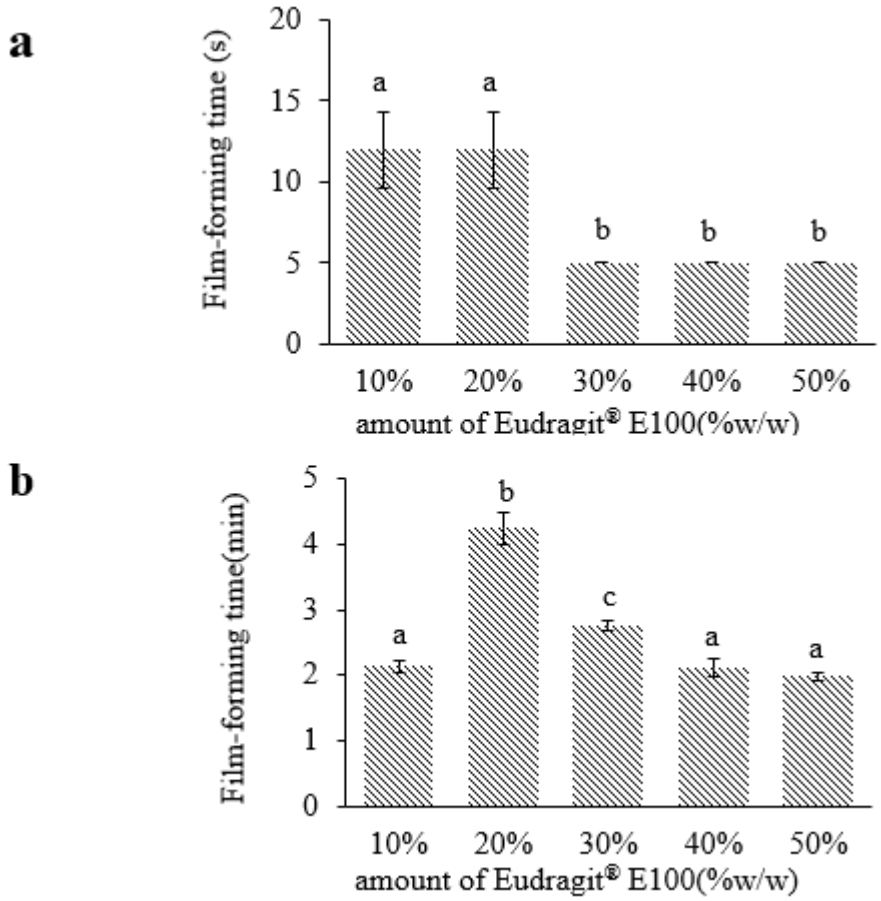


Figure 12 Effect of the amount of Eudragit® E100 on the film-forming time of FFS as follows (a) the film-forming time in wet state, (b) the film-forming time in dry state (mean ± SD, n=3), different letters indicate statistically significant differences ($p < 0.05$)

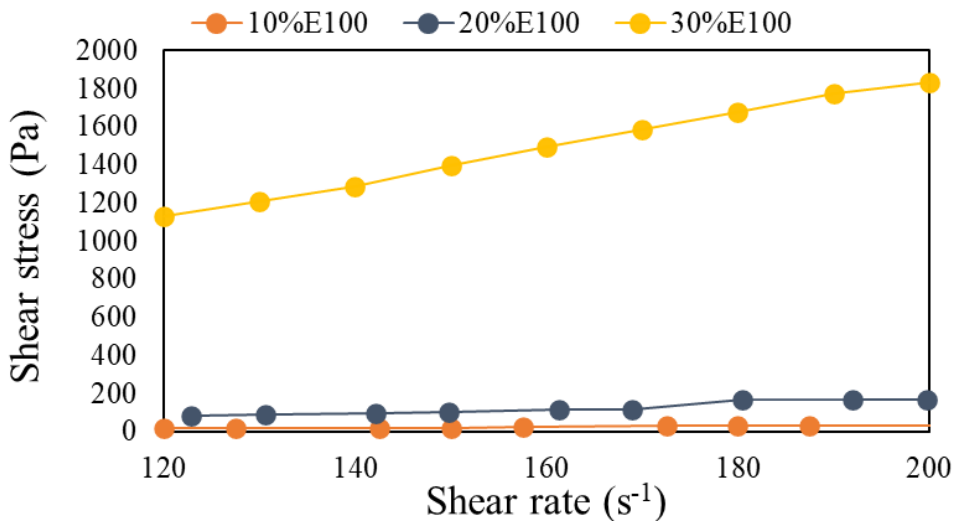


Figure 13 Flow rheograms of formulations containing 10-30% w/w Eudragit® E100

4.2.2. *In vitro* residence time

To analyze the adhesive force between the formulation and mucin, the residence time was measured by the wash-off method. The time taken for the film to detach completely from the surface of mucin-coated cellophane was recorded as the residence time, which indicated the mucoadhesion of the film-forming system. Eudragit® E100 is a cationic methacrylate polymer that can interact with the negatively charged groups, such as carboxyl or sulfate, on the mucin through electrostatic interactions (41). Figure 14 displays the residence time of the FFS prepared from various concentrations of Eudragit® E100 (10-50% w/w). The results indicated that an increase in of the amount of Eudragit® E100 decreased the resident time of the FFS, The FFS containing 10% w/w Eudragit® E100 exhibited the highest residence time at approximate 6 minutes, followed by 20%, 30%, 40%, and 50% w/w Eudragit® E100 respectively.

In the present study, the residence time of Eudragit® E100 was measured in a wet state. The results showed that a change in solvent composition caused the Eudragit® E100 to become tightly packed, resulting in the formation of a hard solid film at high concentrations. This led to a decrease in the interaction with mucin, which in turn resulted in a lower residence time as the concentration of Eudragit® E100 was increased.

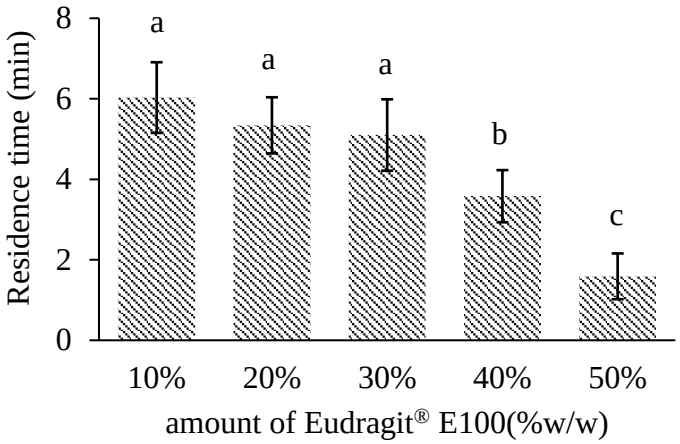


Figure 14 Effect of the amount of Eudragit® E100 on the residence time of FFS (mean ± SD, n=3), different letters indicate statistically significant differences ($p < 0.05$)

4.2.3. Rheological analysis

The rheological properties of various polymeric formulations were investigated in the present study. The influence of Eudragit® E100 concentration on the viscoelastic characteristics of formulations containing 10-50% w/w Eudragit® E100 was evaluated and represented graphically in Figures 15 and 16. The study used

the loss tangent (tangent phase angle) and moduli to evaluate the rheological property, which indicates the formation of a viscoelastic gel. As shown in Figure 15a, all the formulations displayed a decrease in the value of tan δ over time, indicating an increase in viscosity. The samples demonstrated solid-like behavior (tan $\delta < 1$), rather than the typical liquid solution, potentially due to the partial evaporation of the ethanol-based solvent during the 10-minute measurement period per sample. The sol-gel transition point was determined by the time at which tan δ reached 1. This point represents the critical gel point at which the storage and loss moduli are equivalent (33). In the absence of PBS buffer pH 6.8 (Figure 15), it was found that 10% and 20% w/w Eudragit® E100 had similar gel points at approximately 350 s, while highly concentrated 30-50% w/w Eudragit® E100 samples displayed faster gelation (Figure 15a). Figures 15b and 15c display solid-like elastic modulus (G') and liquid-like viscous modulus (G") as a function of strain. The augmentation of Eudragit® E100 concentration was observed to significantly increase the storage and loss moduli and decrease the critical gel point of each formulation, possibly attributed to the increased entanglement of adjacent polymer chains.

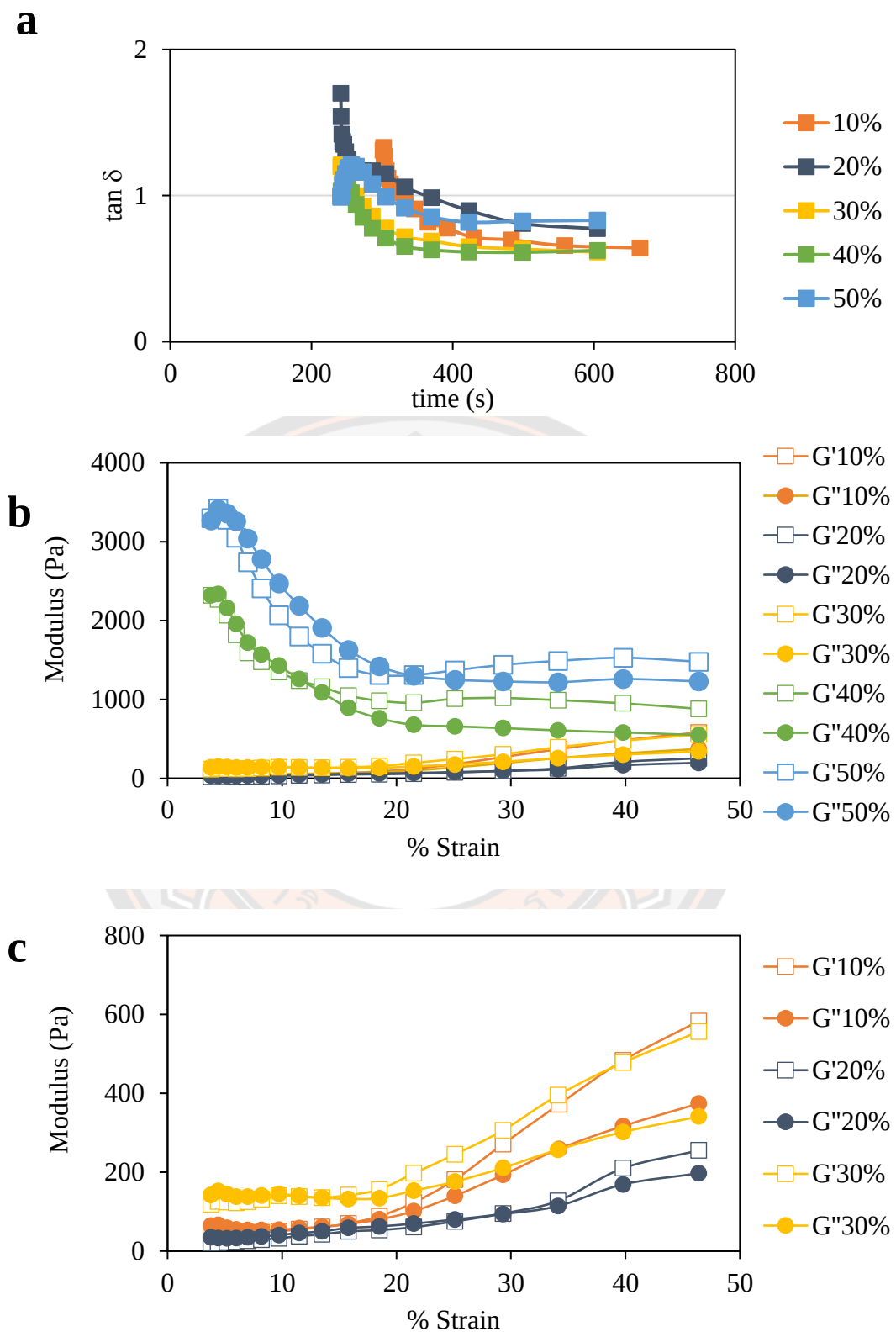


Figure 15 Loss tangent (tan δ) versus time (a) and strain-dependent changes of storage and loss moduli (G' and G") (b) of the 5 formulations (10-50% w/w Eudragit® E100) (c) specifically for the 10% to 30% w/w Eudragit® E100 formulations. The dash lines guide the critical gel point at tan δ = 1. For the moduli, open and filled symbols indicate G' and G", respectively

The sol-gel developed in this study exhibited a response to the simulated saliva (PBS pH 6.8). As shown in Figure 16, the gel formed quickly within a few seconds for concentrations ranging from 10-50% w/w. However, samples with high concentrations (40 and 50% w/w) of Eudragit® E100 displayed an increased tan δ due to phase separation between PBS and the viscous gels. This suggests that G" values might predominantly indicate PBS-dominated G' of the gel. Across the entire strain range and for all samples except for the 40-50% w/w Eudragit® E100, G' ≈ G". The gel strength of 10% w/w Eudragit® E100 was relatively low, measuring less than 100 Pa, while those of 20-40% w/w Eudragit® E100 were in a similar range of 300-700 Pa, depending on the applied strain. The moduli tended to decrease when the applied strain was increased, which may be attributed to the degree of swelling in the altered pH environment (42). Overall, these results suggest that the developed sol-gel has the potential to be used as a mucoadhesive film-forming system for the treatment of aphthous ulcers.

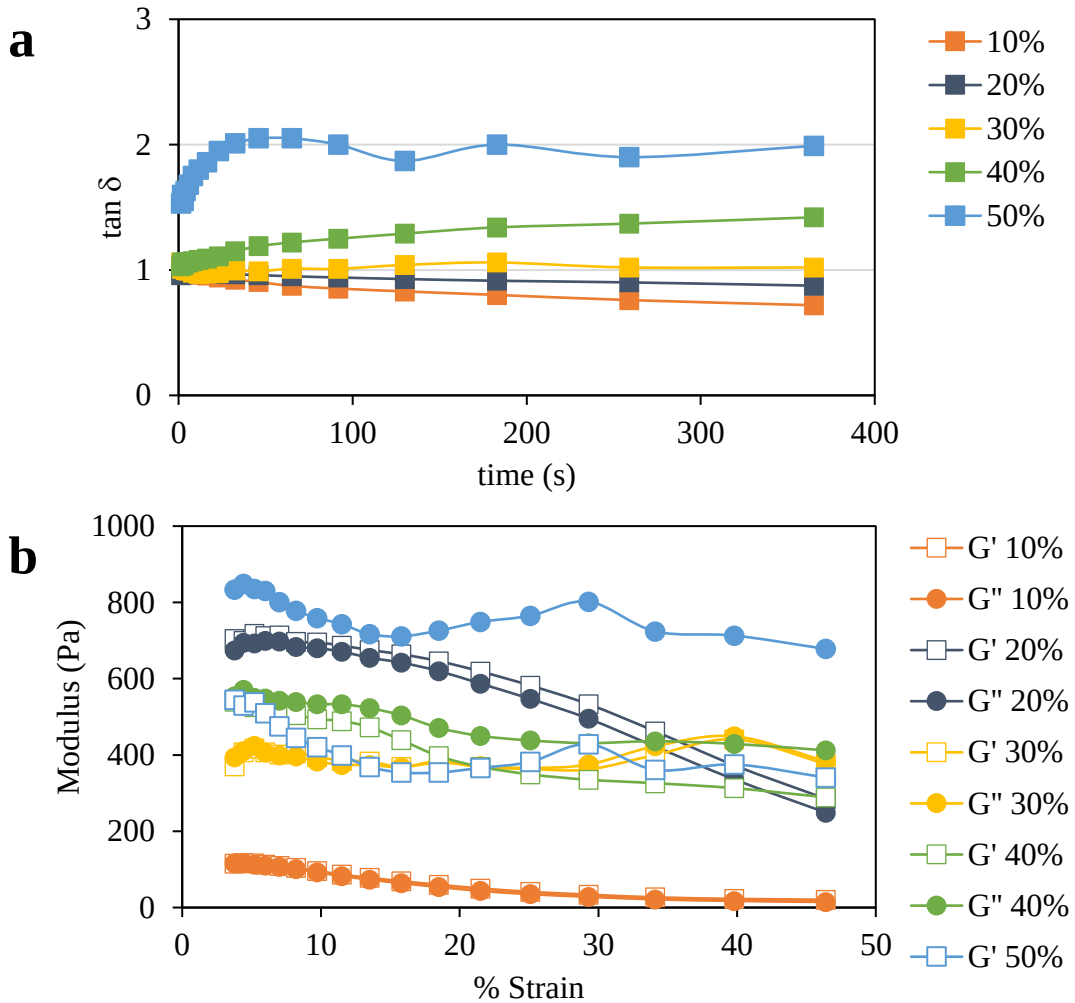


Figure 16 Loss tangent (tan δ) versus time (a) and strain-dependent changes of storage and loss moduli (G' and G") (b) of the 5 formulations (10-50% w/w Eudragit® E100) in the presence of prewarm PBS pH 6.8.

4.3. Effect of black ginger extract

4.3.1. Film-forming time

After adding black ginger extract, the film-forming time increased in the dry state, as shown in Figure 17. However, in the wet state, the FFS still formed a film within a few seconds (data not shown). The increased film-forming time in the dry state was due to factors such as the presence of large molecular weight compounds in the black ginger extract or the formation of aggregates or complexes between the extract and other components in the film-forming solution. The higher viscosity caused by these factors makes the solution more resistant to flow, which in turn makes it more difficult for the solvent to evaporate and for the film to solidify.

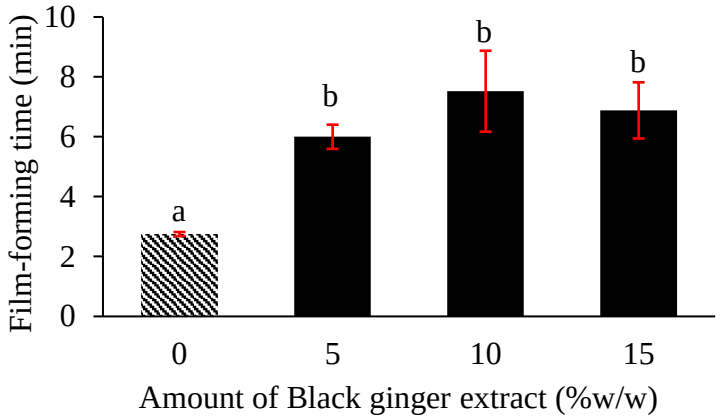


Figure 17 Effect of the amount of Black ginger extract on the film-forming time of FFS in dry state (mean ± SD, n=3), different letters indicate statistically significant differences ($p < 0.05$)

4.3.2. *In vitro* residence time

The residence times were longer when the FFS were incorporated with 5% and 10% black ginger extract, as shown in Figure 18. The FFS consisting of only Eudragit® E100 had lower residence time and viscosity (as shown in Table 4). The addition of black ginger extract to the film-forming solution can impact the residence time and viscosity. The increased residence time of the film can be attributed to the presence of certain components in the extract that interact with the mucin, allowing for a longer-lasting film. It is also possible that the increased viscosity of the film-forming solution containing black ginger extract contributes to a longer residence time by slowing down the diffusion of the active ingredients and slowing the release of the extract.

However, the solution containing 15% (F8) black ginger extract showed the lowest residence time. This may be due to the fact that too much black ginger extract can cause an increase in viscosity beyond a certain point, making it difficult for the solution to form a uniform and stable film.

Furthermore, the residence times were longer when the FFS were incorporated with 5% (F6) and 10% (F7) black ginger extract. Although the viscosity of F7 was higher than F6, it was easier to apply on the target site, and F7 formed a uniform and stable film compared to F8. Thus, F7 was the most suitable formulation for further development in increasing mucoadhesive activity.

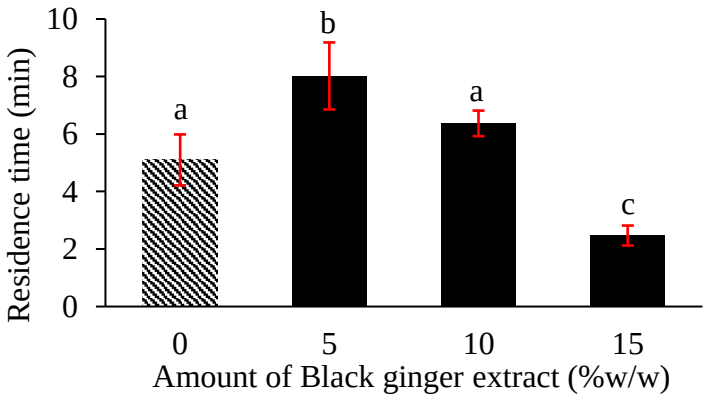


Figure 18 Effect of the amount of Black ginger extract on the Residence time of FFS (mean ± SD, n=3), different letters indicate statistically significant differences ($p < 0.05$)

Table 4 Effect of black ginger extract on physicochemical properties of FFS (mean ± SD)

Sample	Water contact angle (°) (n=6)	Viscosity (cP) (n=3)
F3	66.1±1.6	64.9±4.1
F6	69.6±2.0	92.4±8.7
F7	68.4±2.4	1293.3±35.4
F8	63.7±2.8*	1740.0±118.6

* represents $p < 0.05$

4.4. Effect of mucoadhesive polymers

4.4.1. Film-forming time

In this study, PVP K90, HPMC E15 LV, and HPC SL were chosen as mucoadhesive polymers based on their solubility and characteristics of the formed solutions. These polymers were found to be soluble in mixtures of water and ethanol and formed homogeneous solutions with slightly changed film-formation times in the wet stage (data not shown). Rapid solvent evaporation during the film-forming process created Marangoni instabilities, which roughened the air surface of the film. As the solvent evaporated, the viscosities of the films increased to a point at which the instabilities dissipated (43). In contrast, in the dry state (Figure 19), the film-forming

time was increased, which could be due to the hydrophilicity of the mucoadhesive polymers that are able to trap water clusters or create strong interactions of water with polar groups of the polymer. These interactions may result from direct binding to a chain (hydrogen bonding) or via another water molecule (44), leading to a slower evaporation rate.

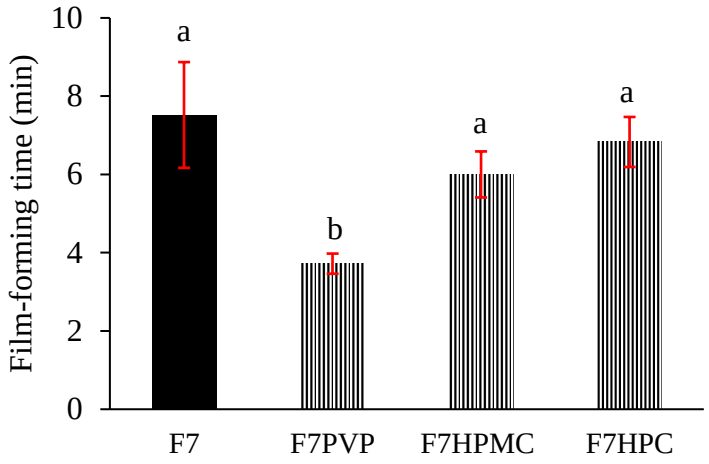


Figure 19 Effect of type of mucoadhesive polymers on the film-forming time of FFS in dry state (mean ± SD, n= 3), different letters indicate statistically significant differences (p < 0.05)

4.4.2. *In vitro* residence time

The incorporation of mucoadhesive polymers resulted in a significant improvement ($p<0.05$) in the residence time of F7 (Figure 20) due to an increased number of hydrogen binding sites for mucin (45). This enhanced interaction was confirmed by the lower water contact angles observed for F7HPMC and F7HPC compared to F7 alone, indicating that the film's wettability had been increased (Table 5). Therefore, it can be concluded that the primary mechanism by which film adhesion was improved was through the hydrophilicity of the polymers (25). The addition of mucoadhesive polymers led to a notable increase ($p<0.05$) in both viscosity and residence time. However, their effectiveness may diminish with time due to dissolution. To counteract this effect, water-insoluble Eudragit® E100 could be used to extend the duration of action.

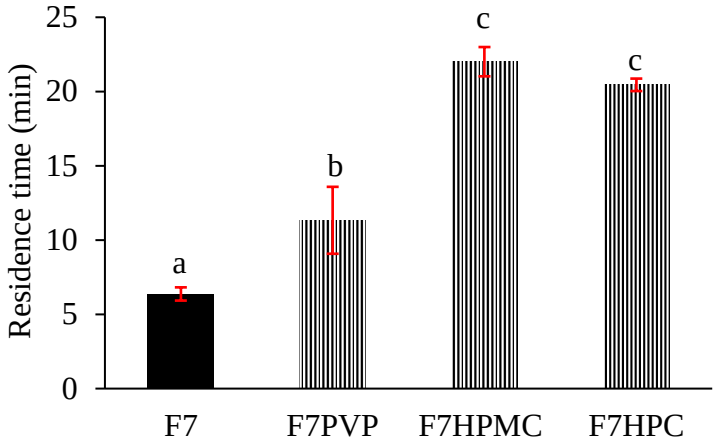


Figure 20 Effect of type of mucoadhesive polymers on the residence time of FFS (mean ± SD, n= 3), different letters indicate statistically significant differences ($p < 0.05$)

The relationship between viscosity and residence time for the FFS is summarized in Table 5. The results indicated that the addition of mucoadhesive polymers increased the viscosity of the FFS and promoted longer residence time. However, the FFS consisting of only Eudragit® E100 exhibited high viscosity but a shorter residence time. Based on these findings, the FFS containing HPMC had the highest viscosity and residence time, making it a promising formulation to further develop for increasing mucoadhesive activity.

Table 5 Effect of mucoadhesive polymers on physicochemical properties of FFS (mean ± SD)

Sample	Water contacts angle (°) (n=6)	Viscosity (cP)(n=3)
F7	68.4±2.4	1293.3±35.4
F7PVP	61.9±2.9	1387.3±70.5
F7HPMC	50.6±1.9*	1738.3±97.2
F7HPC	48.8±1.5*	1333.3±57.8

* represents $p < 0.05$

4.5. *In vitro* release studies

The cumulative release profiles of methoxyflavone in 100 mg of F7HPMC was displayed in Figure 21. The initial slow release of methoxyflavone in the first hour could be attributed to the extract being bound with the mucoadhesive polymer in the film. Subsequently, a sharp release phase was observed, which can be attributed to the erosion of the film, allowing methoxyflavone to gradually dissolve from the film into the ulcer and be washed away by saliva.

In a previous study, three compounds, namely 3,5,7,3,4-pentamethoxyflavone (PMF), 5,7-dimethoxyflavone (DMF), and 5,7,4-trimethoxyflavone (TMF), were found to inhibit the production of nitric oxide. Their respective IC50 values were determined as 5.1, 4.6, and 8.7 µg/ml (46). Building upon this research, our study focused on investigating the anti-inflammatory effect of black ginger extract as a potential active ingredient for treating aphthous ulcers. Our findings revealed that the cumulative release profiles of methoxyflavones indicated that our formulated delivery system provided a methoxyflavone content that exceeded the IC50 value within the first hour.

While the *in vitro* residence time measurement was adapted from Gajdošová M, et al.(36), Srichaivatana K, et al.(37) and Semalty M, et.al.(38) and the simulated saliva flow rate was 10 mL/min while the authentic saliva flow rate is about 0.3-0.4 mL/min (47). The residence time of F7HPMC was approximately 22 minutes (Figure 20). These results provide valuable data to develop an effective film-forming solution for optimal mucoadhesive efficacy. However, to obtain results that accurately reflect real-life conditions, further investigations using *in vivo* methods are warranted.

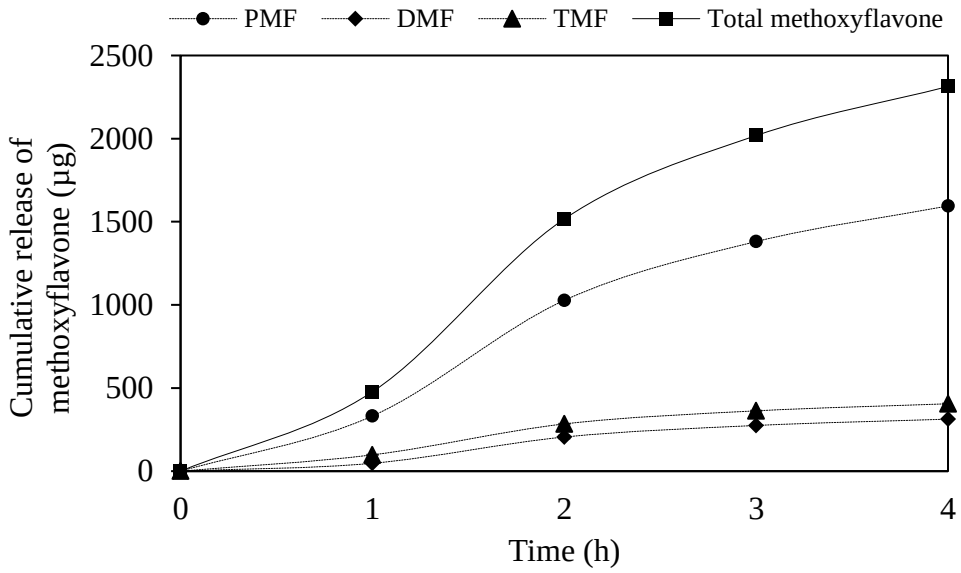


Figure 21 In vitro release profiles of 3,5,7,3',4'-pentamethoxyflavone (PMF) (●), 5,7-dimethoxyflavone (DMF) (♦), and 5,7,4'-trimethoxyflavone (TMF) (▲) in the formulation (F7HPMC) (n = 3)

4.6. Stability studies

The stability of the formulation containing 30% w/w Eudragit® E100, 10% w/w black ginger extract, and 5% w/w HPMC was evaluated by physical inspection on day 0 and day 30 under different storage conditions. The results showed (Figure 22) that the formulation remained stable over the storage period, as there were no significant changes in film-forming time and residence time, although a slight decrease in viscosity, pH, and drug content was observed. The preservation of film-forming time and residence time, even under elevated temperatures, is a crucial indicator of the stability and efficacy of the formulation. These findings suggest that the combination of Eudragit® E100, black ginger extract, and HPMC can serve as a stable and robust delivery system for methoxyflavone. The slight decrease in viscosity, pH, and drug content can potentially be addressed by optimizing the formulation process and storage conditions.

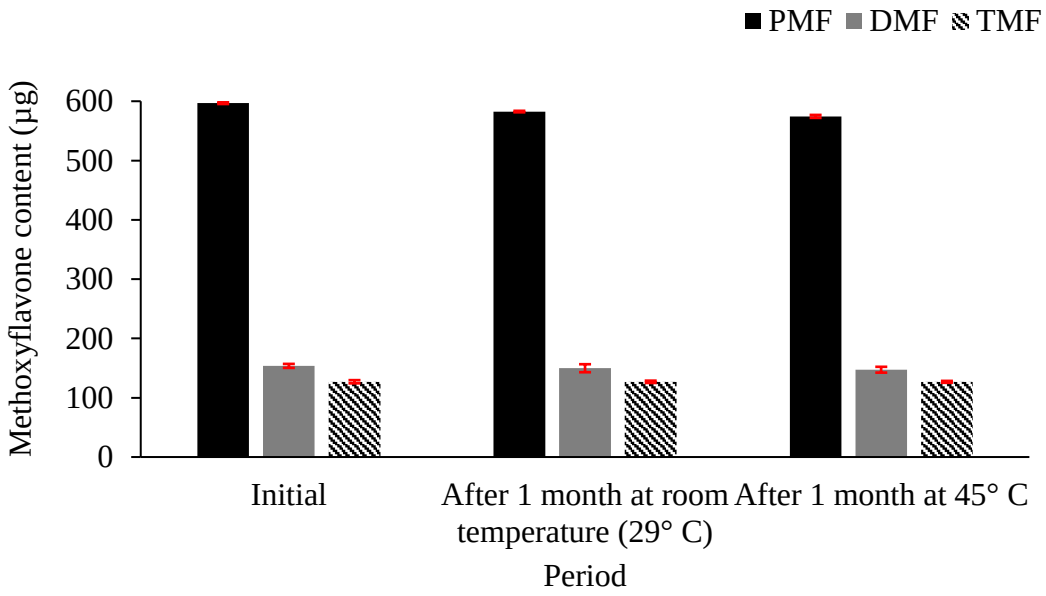


Figure 22 Methoxyflavone content in FFS over 30 days of stability studies.

Chapter 5

Conclusion

In conclusion, the aim of this study was to develop a mucoadhesive film containing black ginger extract as a delivery system for methoxyflavone for the treatment of oral ulcers. The results indicate that the mucoadhesive film containing black ginger extract can be used as an effective and stable delivery system for methoxyflavone.

The formulation containing 30% w/w Eudragit® E100, 10% w/w black ginger extract, and 5% w/w HPMC was found to be the most effective in terms of film-forming time, residence time, and drug release. The inclusion of mucoadhesive polymers, such as PVP K90, HPMC E15 LV, and HPC SL, significantly improved the residence time of the mucoadhesive film due to the increased number of hydrogen binding sites for mucin. The enhanced wettability of the film, as shown by the lower water contact angles of F7HPMC and F7HPC, was consistent with the results of the residence time, and can be attributed to the hydrophilicity of the polymers.

The *in vitro* release studies demonstrated an initial slow release of methoxyflavone, followed by a sharp release phase as the film started to erode, allowing methoxyflavone to gradually dissolve from the film into the ulcer and wash off by saliva. The stability studies indicated that the formulation remained stable under different storage conditions, with no significant changes in film-forming time and residence time, despite a slight decrease in viscosity, pH, and drug content. The preservation of film-forming time and residence time, even under elevated temperatures, is a crucial indicator of the stability and efficacy of the formulation.

Overall, this study demonstrates the potential of mucoadhesive films as a promising drug delivery system for the treatment of oral ulcers. The combination of mucoadhesive polymers, black ginger extract, and Eudragit® E100 can serve as a stable and robust delivery system for methoxyflavone. Further investigations are needed to evaluate the efficacy and safety of this delivery system *in vivo*. Additionally, optimization of the formulation process and storage conditions can potentially address the slight decrease in viscosity, pH, and drug content observed during stability studies.

In summary, this study provides a foundation for the development of an effective and stable delivery system for the treatment of oral ulcers. The use of natural compounds, such as black ginger extract, in combination with mucoadhesive polymers and Eudragit® E100 can offer a safe and effective alternative to current treatment options.

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