DEVELOPMENT OF MUCOADHESIVE PATCHES CONTAINING ALPHA-MANGOSTIN FOR APHTHOUS ULCERS

JIRATCHAYA LERDSRIMONGKOL

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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ABSTRACT

This study aimed to develop alpha-mangostin (α-MN) loaded mucoadhesive patches for aphthous ulcers and investigates their physicochemical properties. Patches, prepared by solvent casting method, consisted of an ethylcellulose backing layer supporting a mucoadhesive gelatin layer loaded with α-MN. For mucoadhesive layer, the higher gelatin bloom strength correlated with increased puncture strength and elongation at break. Notably, the 300-bloom gelatin exhibited superior mechanical characteristics. However, the introduction of glycerin as a plasticizer resulted in decreased puncture strength without any significant increase in elongation at the break. Furthermore, an escalation in gelatin concentration from 1% to 7% increased patch thickness and puncture strength. Importantly, increasing gelatin concentration correlated with an extended in vitro residence time. Successful loading of α-MN into the patches was achieved in a range of 144.12 ± 27.10 to 441.05 ± 94.79 μg/cm^2 At optimal conditions, the prepared patch exhibited good mechanical strength and flexibility. It demonstrated an ability to adhere to mucous membranes for up to 8 hours, serving as a potential aid in concealing wounds while ensuring sustained release of α-MN. These findings demonstrate the successful development of mucoadhesive patches with promising properties for potential oral mucosal drug delivery applications. This research lays the foundation for further optimization and advancement in the development of mucoadhesive patches, offering potential solutions for various oral mucosal diseases

Thesis entitled "Development of mucoadhesive patches containing alpha-mangostin for aphthous ulcers"

By Jiratchaya Lerdsrimongkol

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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Jiratchaya Lerdsrimongkol

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CHAPTER I

INTRODUCTION

Statement of the problem

Aphthous ulcers, also known as canker sores, are a common oral mucosal disorder that affects approximately 20% of the population (1). The ulcers can cause pain, discomfort, and difficulty in speaking, eating, and swallowing, which can have a significant impact on the patient's quality of life. While there are various topical treatments available for Aphthous ulcers, such as antimicrobial mouthwashes, corticosteroids, or anesthetics, they may have limitations in terms of efficacy, convenience, and patient compliance. Corticosteroids are commonly used to treat aphthous ulcers because they are effective anti-inflammatory agents. However, prolonged use of corticosteroids can lead to a range of negative side effects, including the development of candidiasis (a fungal infection) and other systemic effects (2). To address these limitations, there has been a growing interest in developing alternative dosage forms, such as mucoadhesive patches, for the treatment of aphthous ulcers. Mucoadhesive patches offer several advantages over traditional topical treatments, including targeted and sustained drug delivery, improved drug bioavailability, and patient convenience (3). Gelatin is a biocompatible and biodegradable polymer that has been widely used in various drug delivery systems due to its excellent mucoadhesive properties and biocompatibility. Alpha-mangostin (α-MN) was used as an herbal active ingredient because of its anti-inflammatory and antibacterial properties (4). Mohan et al. found that α-MN at doses of 8 and 14 µg/ml effectively inhibited cytokines nitric oxide, PGE2, TNF-α, IL-4, and COX-2. COX-2 activity was reduced by 31.5 ± 4.2% at 8 µg/ml and 74.04 ± 5.8% at 14 µg/ml (5). These results suggest the potential of α-MN as a therapeutic agent for cytokine regulation. To improve the patch formulation, we studied how various formulation variables, including the type of gelatin, quantity of gelatin, and plasticizer, impacted the mucoadhesive and mechanical characteristics of the patches.

Purposes of the study

1. To develop mucoadhesive patches based on gelatin as a new potential dosage form for the treatment of aphthous ulcers.

2. To evaluate the patches' physicochemical properties; scanning electron microscopy (SEM), and puncture test, including *in vitro* residence time, wettability, and alpha-mangostin loading and release.

Hypotheses of the study

1. Gelatin-based patches will have a long residence time on a simulated oral mucosa.

2. Gelatin-based patches will have an appropriate α-MN loading.

CHAPTER II

LITERATURE REVIEW

Aphthous ulcers

1. General conditions in the oral cavity

The human oral cavity is composed of the lips, buccal mucosa, the palate, the tongue, and other parts. A mucous membrane of stratified squamous epithelium lines and protects the oral cavity. Major and minor salivary glands secrete saliva to moisten and lubricate the oral cavity (6). The oral mucosa has two layers: the oral epithelial layer and the connective tissue layer (7).

1.1 Oral epithelial layer

The oral epithelial layer has a stratified squamous structure consisting of either keratinized or nonkeratinized oral mucosa, depending on its location in the mouth. Figure 1 shows that the oral epithelial layer has four sublayers: the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. The stratum basale adheres to the lamina basale in the connective tissue layer by hemidesmosomes. The stratum corneum is made of keratinized oral mucosa. Keratinization is the process of changing viable keratinocytes in the stratum granulosum to dead cells in the stratum corneum (8).

1.2 Connective tissue layer

The connective tissue layer mainly consists of lamina basale, which contains collagen fibers, fibroblasts, macrophages, plasma cells, mast cells and lymphocytes. Moreover, this layer also contains vascular components, nerves, and the ducts of salivary glands (Figure 2) (8).

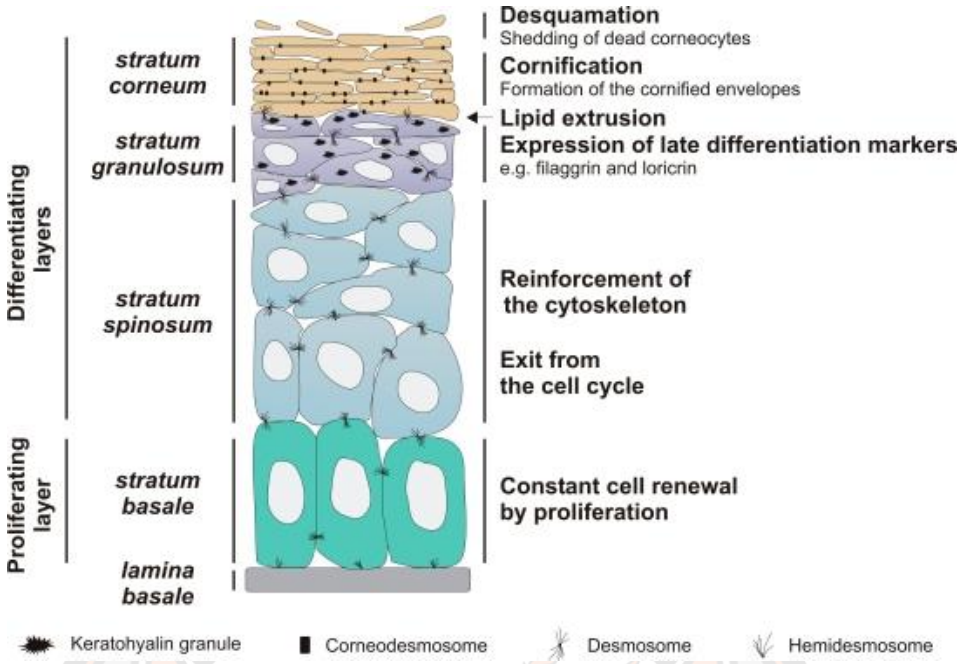

Figure 1 Oral epithelial layer (9)

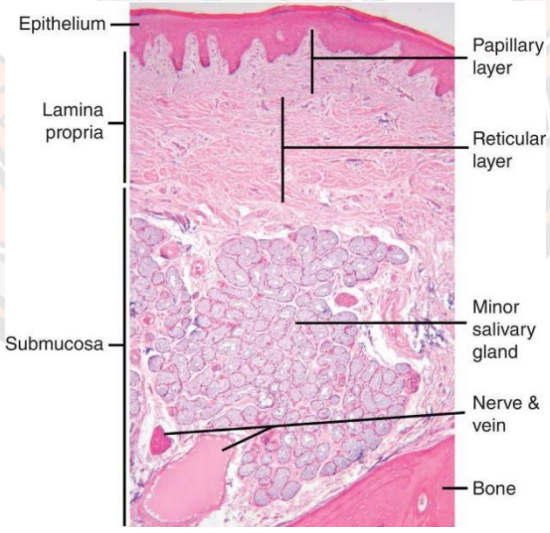

Figure 2 Overall layers with connective tissue layer of oral mucosa (10)

2. General characteristics of aphthous ulcers

Aphthous ulcers (AUs), also known as canker sores or recurrent aphthous stomatitis (RAS), are a common mouth disease in the oral mucosa. AUs are open ulcers that generally occur on nonkeratinized mucosa (11). The characteristics of AUs are circular/oval with a grey-yellow floor and erythematous haloes on the edge as shown in Figure 3. AUs are a breakdown of the oral epithelial layer of the nerve endings in the connective tissue layer, which leads to pain and inconvenience in the oral cavity (7). From available data, AUs occur in females more often than males. Minor aphthous ulcers are found in females and males at a ratio of 1.3:1 (12). AUs usually have a prevalence of 5-50%, which depends on the demographics and population sampled. One out of every five people can be expected to experience a RAS episode as a child or in their early adulthood (13). Out of 3,106 participants from Mahidol University in Thailand, 1,450 (46.7%) were AU patients (14).

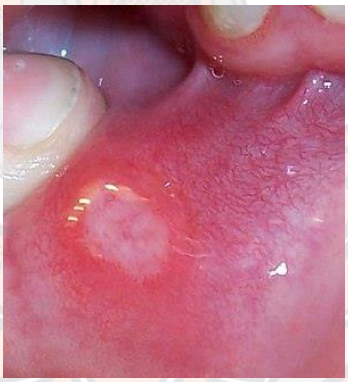

Figure 3 Aphthous ulcer

3. Categories of aphthous ulcers

Aphthous ulcers are classified into 3 categories (15) as follows:

Category 1: Minor aphthous ulcers (MiAUs)

MiAUs, which are shallow ulcers, are found to be around 80% of all aphthous ulcers categories. Approximately 1 to 5 ulcers with a diameter size of 0.1-1 cm can be found as shown in Figure 4A. MiAUs can spontaneously heal within 7-10 days. Moreover, they mainly occur on non-keratinized oral mucosa, e.g., the labial and buccal mucosa and around the lateral and ventral of the tongue (16).

Category 2: Major aphthous ulcers (MjAUs)

MjAUs that show deeper scarring compared to MiAUs which are 10-15 % of all aphthous ulcers categories. There are 1 to 3 ulcers and the subjects' ulcers and diameter size that is larger than 1 cm. MjAUs are more oval than minor aphthous ulcers, as shown in Figure 4B. MjAUs can spontaneously heal within 1 month. Additionally, they occur on both keratinized and non-keratinized oral mucosa, especially in the soft palate.

Category 3: Herpetiform ulcers (HUs)

HUs, which are multiple clustered lesions, are found to be 5-10% of all categories of all aphthous ulcers with 5 to 100 ulcers with a diameter size of 0.1-0.3 cm as shown in Figure 4C). HUs can spontaneously heal within 7-10 days. Moreover, HUs that are found in non-keratinized oral mucosa are especially found on the floor of the mouth, or the lateral and ventral of the tongue.

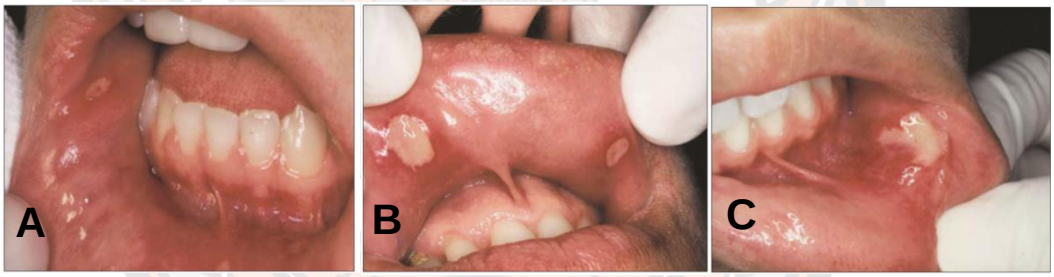

Figure 4 Photographs of Minor aphthous ulcers (A), Major aphthous ulcers (B), and Herpetiform ulcers (C) (15)

4. Causes and factors of aphthous ulcers

A common belief by some researchers is that aphthous ulcers arise from TNF-α, a pro-inflammatory cytokine, during the inflammation process, which is a result of the genetic basis and microbial aspects of the human body (17). Furthermore, many factors can stimulate aphthous ulcers for instance: emotional stress and mechanical forces in the oral cavity. Equally important, some diseases such as indigestion, constipation, and sodium lauryl sulfate, which is an ingredient in many

commercially manufactured toothpastes (18) , have been associated with the occurrence of these ulcers.

The clinical features of aphthous ulcers have been divided into 4 stages: the premonitory stage, the pre-ulcerative stage, the ulcer stage, and the healing stage as shown in Figure 5. The premonitory stage (0-24 hr) begins with paresthesia, a tingling burning sensation, which includes mononuclear inflammatory cells infiltration to the epithelium. The pre-ulcerative stage (18-72 hr) indicates that there is an existence of inflammatory erythema, macule, and increasing pain. The ulcer stage (1-16 days) occurs blanching necrosis on the superficial oral membrane, purulent exudate, and increasing erythematous halo, including reduction of pain. Finally, the healing stage (4-35 days), the stage where pain and inflammation begin to subside, the granulation of tissues with epithelial spanning of defect lesions, and fibroblast proliferation (13).

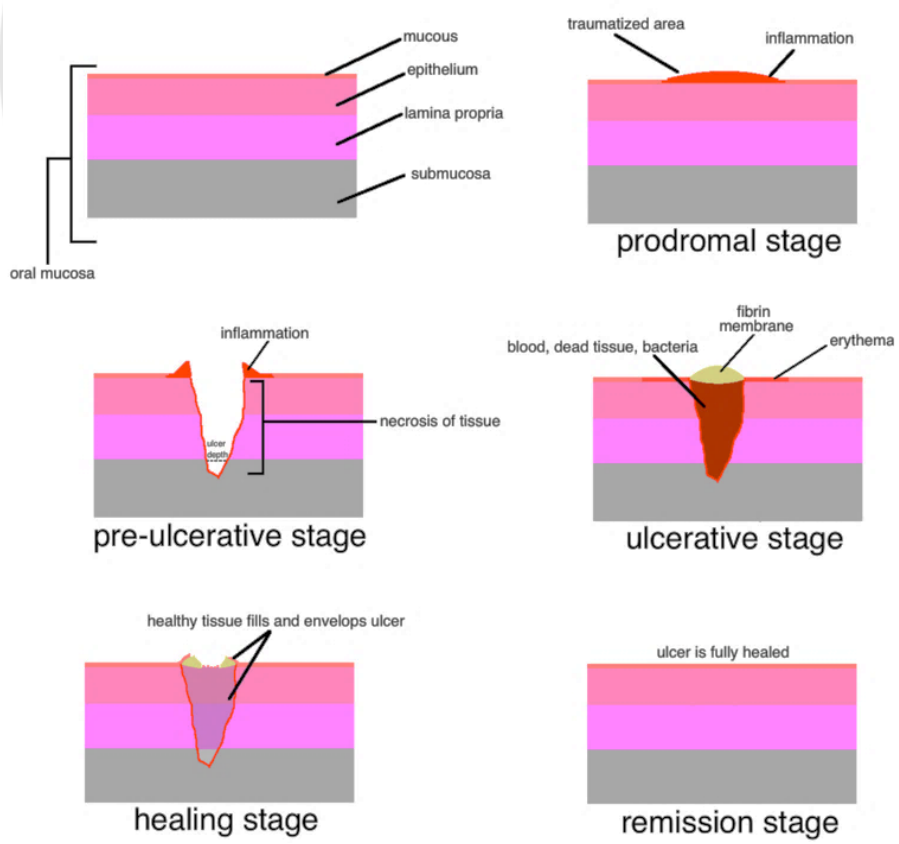

Figure 5 Wound healing process (19)

Inflammation

During the inflammatory process of AUs, TNF-α significantly affects endothelial cell adhesion and chemotaxis of neutrophils, as shown in Figure 6 (20). TNF-α and IL-1β may activate nociceptive sensory neurons causing pain and AUs (21). TNF-α immunoreactivity was tested in 4 cell types: endothelial cells, mast cells, macrophage-like cells, and lymphocytes. The number of cells that contain TNF-α in immunoreactive cells in aphthous ulcers (188±46 cells/0.2 mm^2) was higher than those in traumatic ulcers (52±14 cells/0.2 mm^2) (20). Also, the TNF-α level in the serum of 146 aphthous ulcer patients was 9.1 ± 1.0 pg/ml, 4.1 ± 0.5 pg/ml in 9 traumatic ulcer patients, and 3.8 ± 0.2 pg/ml in the 54 control patients. These results show conclusively that TNF-α levels in serum levels of AUs in the patients with ulcers were significantly greater than in the control subjects (22).

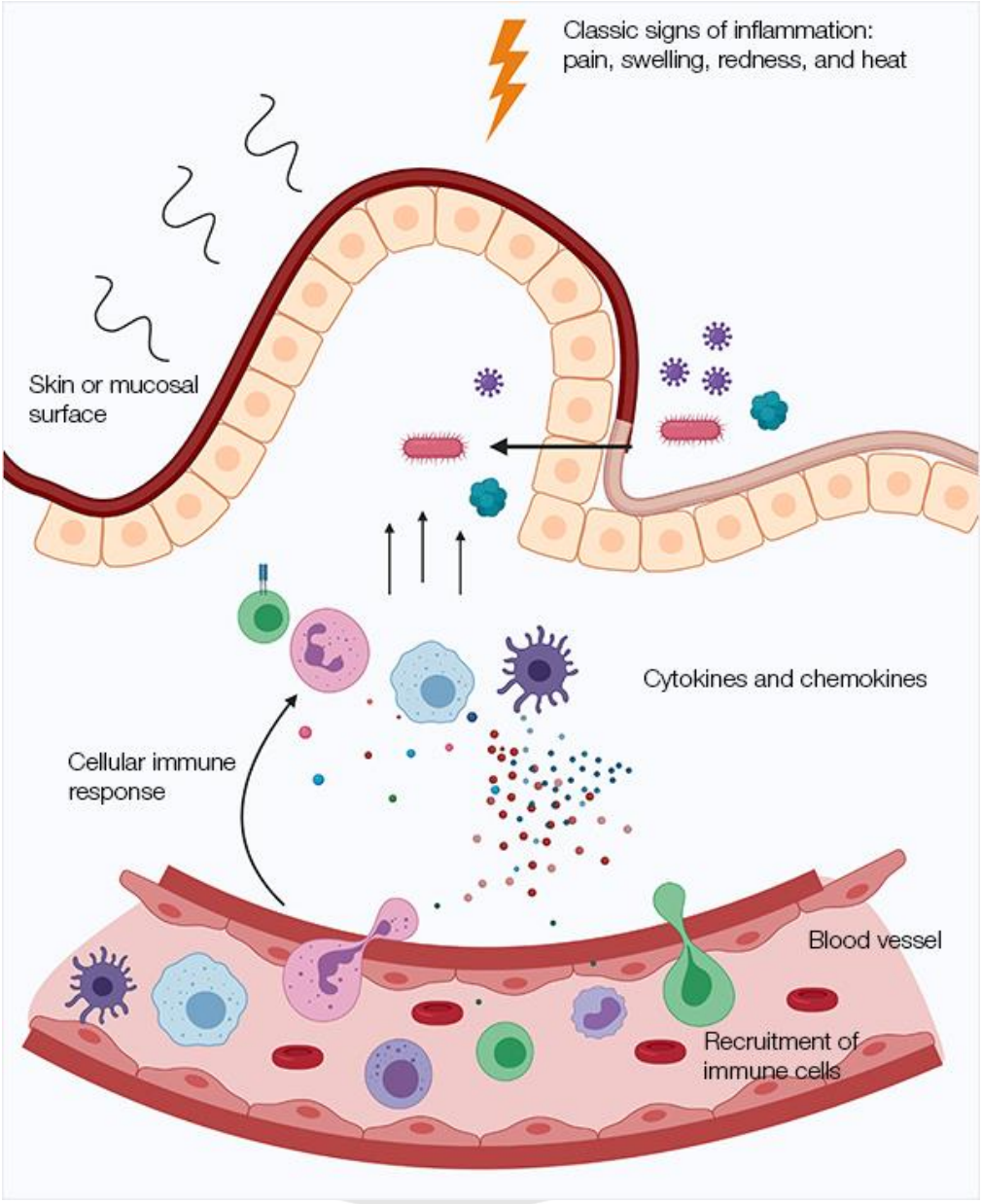

Figure 6 Initiation of inflammation (23)

Alpha-mangostin

Alpha-mangostin shown in Figure 7 is an active yellow matter that is found in the mangosteen pericarp (rind) as shown in Figure 8, especially found in mangosteen peel (*Garcinia mangostana* L.) in the Guttiferae family of plants (24). This active ingredient was first extracted from the mangosteen pericarp in 1855 (25). Additionally, there was a study of alpha-mangostin extraction using green solvents such as water, ethanol, ethyl acetate, and dichloromethane. The greatest quantity of alpha-mangostin was received from ethyl acetate extraction (75.66 mg/g dry matter) (26).

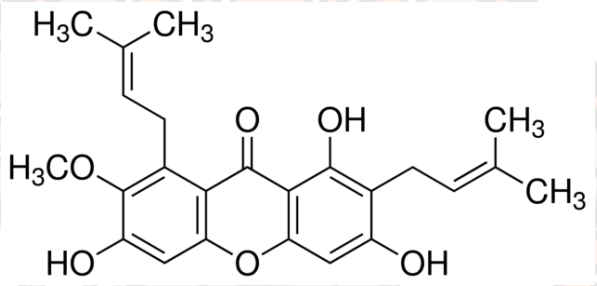

Figure 7 Alpha-mangostin

Figure 8 fruit and peel (27)

Several *in vitro* studies of the anti-inflammatory effects of alpha-mangostin showed that using 1 µg/ml of LPS induced RAW 264.7 cells. The results also showed that alpha-mangostin, in doses of 8 and 14 µg/ml, significantly inhibited cytokines nitric oxide, PGE_2, TNF-α, IL-4 and COX-2 which was inhibited by 31.5 ± 4.2 % at a dose of 8 µg/ml and 74.04 ± 5.8 % at a dose of 14 µg/ml. Studies regarding *in vivo* anti-inflammatory effects of alpha-mangostin using carrageenan-induced peritonitis in male ICR mice showed alpha-mangostin significantly inhibited leukocyte migration and neutrophils at inhibition percentages of 75% at a dose of 14 mg/kg and 82% at a dose of 25 mg/kg. Alpha-mangostin significantly reduced the cytokines, TNF-α and IL-1β in peritoneal fluids (p<0.05) (5), and reduced nitric oxide (NO) at IC_{50} 12.4 µM and prostaglandin E2 (PGE_2) at $IC_{50\ of}$ 11.08 µM (28).

An acute oral toxicity test of alpha-mangostin performed by (29) on Wistar rats showed that the rats did not show oral toxicity from alpha-mangostin until a dose of 1.25 g/kg was administered. In addition, in an LD_{50} study of the effect of alpha-mangostin and mangosteen extract from intraperitoneal administration on mice, the results were, for alpha-mangostin extract, an LD_{50} of 150 mg/kg, and an LD_{50} for mangosteen extract of 231.5 mg/kg (30). The alpha-mangostin results showed no cytotoxicity effect on the murine macrophage cell line or the RAW 264.7 cells (28). An *in vitro* study of the cytotoxicity of alpha-mangostin, at doses of 1 to 14 mg/ml, did not show any cytotoxicity in RAW 264.7 cells (5). As well, alpha-mangostin showed non-cytotoxic attenuation of the secretion of TNF-α and IL-8 in various human cell lines (31). Also, alpha-mangostin at a dose of 4 mg/ml did not show any toxicity in human gingival fibroblasts for 480 min in MTT assay.

Treatments for aphthous ulcers

Although the true cause of aphthous ulcers is still unclear, it is thought that causes of inflammation, pro-inflammatory cytokines, and TNF-α, Genetic basis, and Microbial aspects may cause aphthous ulcers (17).

Currently, there are many treatments for reducing inflammation, relieving pain and antiseptics of aphthous ulcers. These innovative treatments of aphthous ulcers aim to relieve the pain; reduce the healing time; and promote the healing process of these ulcers.

1. Anti-inflammatory agent

Corticosteroids

Corticosteroids used as anti-inflammatory agents can inhibit arachidonic acid release in phospholipids. Arachidonic acid inhibition leads to reduced pro-inflammatory cytokines, for example as shown in Figure 9: cyclooxygenase 2 is associated with inflammation (32).

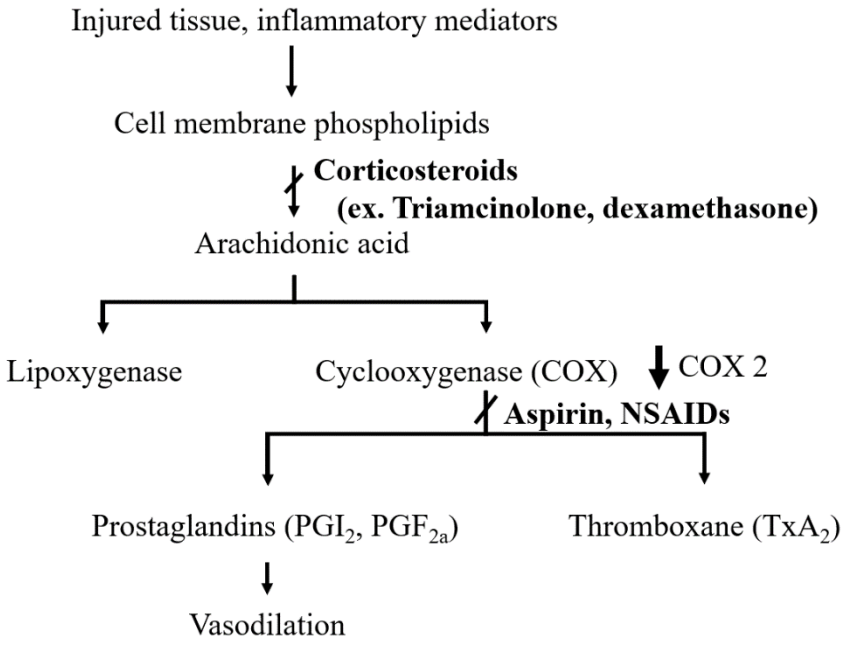

Figure 9 Corticosteroids inhibit arachidonic acid metabolites (33)

1.1 Triamcinolone

Triamcinolone acetonide (0.1%) in an oral paste form (gelatin, pectin, and Carboxymethylcellulose) was applied to the ulcers 2-3 times/day. However, it can lead to an overgrowth of *Candida Albicans* (Candidiasis) when used for a long period (34).

1.2 Dexamethasone

Dexamethasone at a concentration of 0.5 mg/5 mL in oral solution (mouthwash) form (Decadron®) is used to rinse the mouth for 2 minutes, 3 times/day. However, it may lead to an overgrowth of *Candida Albicans* (Candidiasis) when used for a long time (34).

1.3 Amlexanox

Amlexanox's mechanism inhibits the release of inflammatory mediators, histamine and leukotrienes, from mast cells, mononuclear cells, and neutrophils. However, aphthous ulcer treatment in clinical tests has not been quantified (35). Amlexanox 5% (Aphthasol®), an anti-inflammatory agent for healing ulcers in a paste form, is applied to an ulcer 4 times/day. However, it may cause minimal burning, rash, diarrhea, or nausea. (36)

2. Analgesic agents

Local anaesthetic binds to Na channel; thus, Na^+ influx, action potential and propagation are halted (37). Viscous lidocaine (2%Xylocaine® solution) 15 ml is used to rinse the oral cavity before meals and has been used as a local anesthetic agent that can relieve pain while eating. However, it may cause cardiotoxicity (36).

3. Antiseptics

Chlorhexidine gluconate mouthwash can reduce the severity and pain of aphthous ulcers (38). Chlorhexidine gluconate mouthwash (0.12%w/v) in commercial products, Peridex® or Periogard®, is used to rinse 15 ml of the solution 2 times/day. However, it may cause discoloration of the teeth and tongue after use for 7 continuous days: many individuals may also experience a burning sensation of the tongue (36).

4. Natural extracts for treatment aphthous ulcers

4.1 Alpha-mangostin

Alpha-mangostin is an active yellow matter in the mangosteen pericarp (rind of Garcinia mangostana L.) (24). It is extracted for the first time in 1855 (25). Several *in vitro* studies of the anti-inflammatory effects of alpha-mangostin have shown that using 1 µg/ml of LPS induces RAW 264.7 cells. As well, the results showed that alpha-mangostin at doses of 8 and 14 µg/ml significantly inhibited cytokines nitric oxide, PGE_2, TNF-α, IL-4, and COX-2 at a dose of 8 µg/ml was inhibited 31.5 ± 4.2 % and at a dose of 14 µg/ml was 74.04 ± 5.8 %. There have been other *in vivo* studies of alpha-mangostin for its anti-inflammatory effects on carrageenan-induced peritonitis in male ICR mice. The results of these studies showed that alpha-mangostin at doses of 14 and 25 mg/kg significantly inhibited leukocyte migration and neutrophils at an inhibition rate of 75% at a dose of 14 mg/kg and 82%

at a dose of 25 mg/kg. Alpha-mangostin significantly reduced the cytokines, TNF-α, and IL-1β in peritoneal fluids ($p<0.05$) (5). Additionally, alpha-mangostin reduced nitric oxide (NO) at IC_{50} of 12.4 μM and prostaglandin E2 (PGE_2) at IC_{50} of 11.08 μM (28). There was also a release study of alpha-mangostin from hydrogel film-based chitosan–alginate for treating aphthous ulcers that showed a release of 35% within 2 hours (39).

4.2 Acemannan

Bhalang et al. (40) reported that acemannan in Aloe Vera, which is one of the main active polysaccharides, reduced the size of aphthous ulcers by less than 0.1% triamcinolone acetonide on the 7^{th} day of the study, but there was no significant difference at p=0.05.

4.3 Ginger extract

Du et al. (41) compared the healing time using ginger extract that was given to 30 volunteers, with 29 volunteers given a placebo. The results showed the healing times were 6 days and 8 days, respectively.

4.4 Curcumin

Deshmukh et al. (42) compared the curcumin gel group with the triamcinolone acetonide gel group in the treatment of minor aphthous ulcers of 60 patients. There were 30 patients in the curcumin gel group with 30 patients in the triamcinolone acetonide gel group. The results showed a reduction of the aphthous ulcers after applying treatment daily for 7 days. In the curcumin gel group, the reduction was 3.8 units while in the triamcinolone acetonide gel group, the reduction was 3.7 units. Also, the reduction size of the aphthous ulcers after 7 days of treatment was 1.2 units for the curcumin gel group and 1.5 units for the triamcinolone acetonide gel group. (42).

Mucoadhesive patches

1. Conventional dosage forms versus mucoadhesive patches

Mucoadhesive dosage forms such as gels, ointments, mucoadhesive tablets, films, and patches (43) are readily available from pharmacies for individuals suffering from ulcer inflammation. Conventional dosage forms, gels and ointments have advantages because of the easiness of dispersing and applying on the ulcers.

However, the limitations of gels and ointments are inadequate residence time and drug release control covering effect, whereas mucoadhesive tablets, films, and patches have a much longer residence time potential. Mucoadhesive tablets lack flexibility and can cause discomfort when being applied to aphthous ulcers. Mucoadhesive films are the most suitable and are the preferred dosage form because of their flexibility, comfortable usage, and longer residence time on aphthous ulcers as compared to gels, ointments, and mucoadhesive tablets. Additionally, mucoadhesive films can be used to protect ulcers from stimulus factors that cause pain. Mucoadhesive patches are similar to mucoadhesive films since they are also flexible and demonstrate long residence time properties. Nevertheless, mucoadhesive patches' properties can be enhanced by adding an impermeable backing layer that protects the ulcers from mechanical stress in an oral cavity, and controls the direction of drug release, preventing drug loss, and reducing the deformation of the mucoadhesive layer of the mucoadhesive patches.

2. Mucoadhesion

Mucoadhesion is defined as 'the bonds of mucoadhesive polymer and a mucosal surface' (Mathiowitz et al., 2019). Mucus layer on an oral mucosa has the function of covering lower cells. The major component of mucus layer 95% is water, and the major little part 0.5-5% is mucin chains as shown in Figure 10. Others are lipids, inorganic salts, and cell debris (44). Mucin chains consist of glycoprotein, protein cores with oligosaccharide branches (Duchěne et al., 1988). Mucin chains show negative charges due to sialic acid (COO-) and sulphate groups (SO_4^{2-}). Furthermore, the data of appropriate pH values of saliva is between 6.2 and 7.6 (45) and the temperature in an oral cavity was 36.2-36.7°C in 1,100 tested samples in clinical studies (46). Mucoadhesion is the interaction of mucin chains and mucoadhesive polymers. Hydrophilic polymers can increase the lasting period at target sites in the oral cavity leading to controlling drug release from several polymers, and decreasing the frequency of treatment application (47).

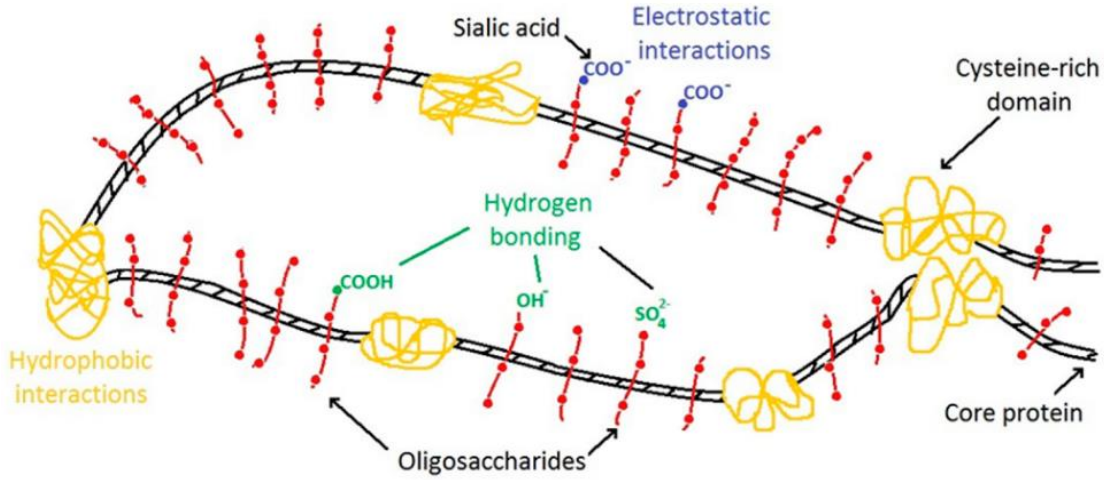

Figure 10 Mucin chain structure (48)

3. Major theories of mucoadhesion (44)

3.1 Electrostatic theory: opposite charges of mucoadhesive polymers which show positive charges i.e., chitosan and gelatin polymers attach to mucin chains which contain negative charges, as shown in Figure 11A.

3.2 Adsorption theory: There is wide acceptance of the theory of weak forces with many bonds such as van der Waals forces, hydrogen bonds (–OH, –COOH) and hydrophobic interactions for example, carbomer and polycarbophil attach to mucin chains by hydrogen bonds as shown in Figure 11B.

3.3 Diffusion theory: Is the interpenetration of mucoadhesive polymer chains that is appropriate in the range between 0.2 and 0.5 μm (49). The rate of interpenetration depends on the molecular weight (MW), flexibility and cross-linking density of the mucoadhesive polymer which, if it contains high MW, leads to entanglement with mucin chains, as shown in Figure 11C.

3.4 Wetting theory: States the affinity of mucoadhesive liquid polymers and the mucosal surface. Spreadability is measured by the contact angle: a lower contact angle means a greater affinity as shown in Figure 11D.

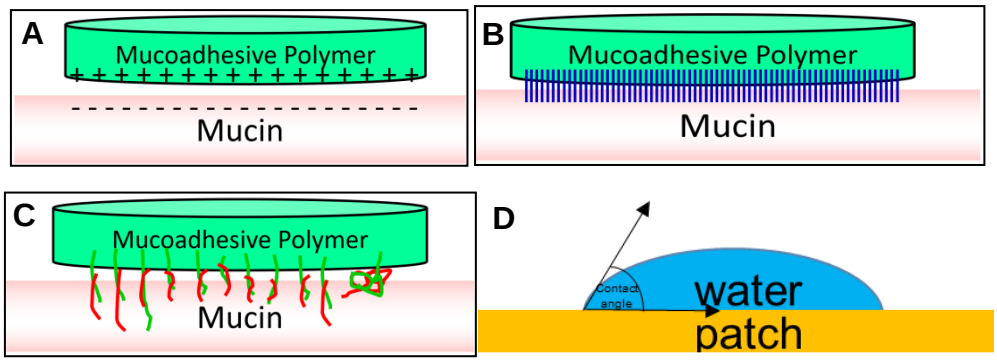

Figure 11 Major theories of mucoadhesion; Electrostatic theory (A), Adsorption theory (B), Diffusion theory (C), Wetting theory (D)

Conventional dosage forms for delivering drugs such as solutions, capsules, and adhesive tablets to the oral mucosa give short residence time (50). Mucoadhesive films and patches are noticeable choices that show ideal characteristics, have long-lasting properties up to 5 or 6 hr, and have soft and flexible characteristics making them strong enough to prevent breaks from mechanical stresses in the oral cavity, and provide suitable swelling of polymers. These characteristics also assist in patient compliance (51).

4. Patches preparation methods

The most widespread film preparation methods are solvent casting and hot melt extrusion methods. The solvent casting method is used to evaporate solvents leading to the formation of patches. The advantages of this method are easy to make, at low cost, and is practical. This makes these patches the preferred approach. However, there are some limitations depending on the type of solvent used (52, 53).

The hot-melt extrusion is used to melt drugs, polymers, and excipients together at a high temperature without organic solvents or a lesser quantity of organic solvent. However, a limitation of this process is that the active ingredients that are not stable at high temperatures are not suitable to be used (52).

5. Gelatin

Gelatin is a natural water-soluble protein which is normally obtained from collagen denaturation. Gelatin polymer has good characteristics including biodegradability, biocompatibility, and low antigenicity (54). It is used as a support

material for tissue engineering and gene delivery. Gelatin is divided into 2 classes. Type A gelatin is acquired from acid-treated collagen derived from pig skin, while type B gelatin is acquired from alkaline-treated collagen derived from beef skin (55). Gelatin polymer was used as a glue with good adhesive properties (56). Bloom strength or gel strength describes the stiffness and strength of gelatin referring to the average molecular weight that is usually found between 30 and 300-bloom. Low bloom strength is less than 150 bloom, while medium bloom is found between 150 and 220 bloom, and high bloom is between 220 and 300-bloom (55).

Abruzzo et al. (57) formulated chitosan/gelatin type B bovine skin (225 bloom) mucoadhesive film containing propranolol hydrochloride for buccal drug delivery. The results showed that the highest *in vivo* residence time was 4 hours.

Jovanović et al. (58) formulated a mucoadhesive gelatin-based film containing propranolol hydrochloride (PRH) for buccal drug delivery. They tested Type A gelatin from porcine skin with and without PRH and also Type B gelatin from bovine skin with and without PRH. The results showed that adding PRH to the 2 types of gelatin could increase adhesion strength (detachment force) greater than occurred in the gelatin without PRH formulation. Moreover, the force of adhesion for gelatin from bovine skin was 10% higher than gelatin from porcine skin.

Bonferoni et al. (59) formulated carrageenan-gelatin mucoadhesive systems for ion-exchange-based ophthalmic delivery. The ratios of carrageenan to gelatin (gelatin A with 75-100 bloom) were 50:50, 25:75, 100:0, and 0:100. The results showed that the relative difference $\Delta F/F$ values were 0.382, 0.617, -0.380, 1.036, respectively. From the results, they could conclude that gelatin had a role in mucoadhesive properties.

Oğur (60) tested the mechanical properties and light transmission (%) of edible protein films. The results showed that gelatin film had the highest tensile force values (5.267±0.559 N) and the highest light transmission values (63.30±0.01 %) of all the edible protein films tested.

Wannaphatchaiyong et al. (61) varied plasticizer types of gelatin film; glycerin (Gly), propylene glycol (PG), and polyethylene glycol 400 (PEG 400) in the range of 5 to 30% of gelatin content. Physical appearance results of gelatin/Gly and gelatin/PG films showed a transparent appearance. However, the gelatin/PEG 400

film showed an opaque appearance. Furthermore, the ultimate tensile strength values of gelatin/Gly were the lowest. The elongation at break values of gelatin/Gly film was the highest. Thus, gelatin/Gly was the most optimal plasticizer in this experiment (61).

Jongjareonrak et al. (62) reported fish gelatin films that contained glycerin from 25, 50, and 75% of gelatin content. The tensile strength results decreased and the elongation at the break values (%) values increased.

Thomazine et al. (63) evaluated gelatin films with glycerin 25 to 55% of 2%gelatin content. The puncture force values decreased from 16.39 N at a glycerin content of 25% to 8.31 N at a glycerin content of 55%. Gelatin films with glycerin used as a plasticizer showed more flexibility than sorbitol. Of the plasticizers with 55% of 2%gelatin content, the gelatin films with glycerin showed higher elongation at break values (127.61%) than gelatin films with sorbitol (74.20%) (63)

6. Ethyl cellulose

Ethyl cellulose polymer (EC) is widely used in cosmetics, pharmaceutical agents, and food products as oral or topical dosage forms, as illustrated in Figure 12. EC is practically insoluble in glycerin, propylene glycol, and water. EC is a non-toxic, non-irritant ingredient (64) that is usually compatible with a plasticizer, such as castor oil for its high softening effect and diphenyl phthalate, cyclohexyl phthalate, benzyl phthalate for their lower softening effect (65). Safety data of EC from oral rat tests showed that the LD_{50} value was more than 5g/kg (66). As well, EC gave high stability between pH values 3 and 11 (67).

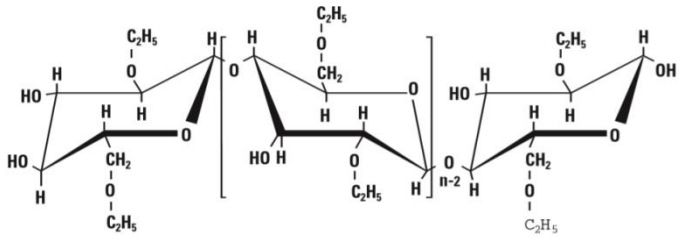

Figure 12 Chemical structure of ethyl cellulose (65)

Satishbabu, & Srinivasan used 5%EC in a mixture of acetone: isopropyl alcohol at a ratio of 65:35, 20% dried weight dibutyl phthalate (plasticizer) for mucoadhesive films (68).

Guo, & Cooklock (69) compared the effects of backing materials; ethyl cellulose (EC), polyvinylpyrrolidone and cellulose acetate mixture (PVP/CA), and Poly (ethylene-co-vinyl acetate) on hydration and adhesion of bioadhesive patches for controlling buccal drug delivery. The mucoadhesive layer contained carbopol 934 grade, polyisobutylene, polyisoprene and drug. Another layer, the backing layer, contained EC, PVP/CA, and poly (ethylene-co-vinyl acetate). The results of using EC with hydrophobic polymers showed low water permeability, moderate flexibility, and water uptake delayed by the patches for 24 hr. PVP/CA showed high water permeability properties and allowed the drug to pass through. Poly (ethylene-co-vinyl acetate), which is a highly hydrophobic polymer, showed elastic properties.

Sankar et al. (70) formulated 3%w/v ethyl cellulose by using chloroform as a solvent and castor oil and glycerin as plasticizers, resulting in EC that was thin, flexible, smooth, and transparent. The folding endurance results with castor oil (more than 277 times) were higher than with glycerin (less than 242) at plasticizer 40% of 3%EC. The moisture absorption result with castor oil (4.073%) was less than glycerin (6.336%) at plasticizer 40% of 3%EC.

Mukherjee, & Bharath (71) formulated mucoadhesive buccal films with the mucoadhesive layer containing HPMC, CS, propylene glycol (PG), and risedronate sodium. The backing layer contained ethylcellulose (EC), acetone, and diethyl phthalate. The films were tested for *ex vivo* mucoadhesion time by applying the films onto fresh porcine buccal mucosa and the mucoadhesive time of the total formulations was between 7.13 and 12.32 hr.

Roh et al. (72) prepared a fast-dissolving mucoadhesive bi-layered strip as topical anesthetics. The mucoadhesive layer contained 5% polyvinylpyrrolidone (PVP), 2% hydroxypropyl methylcellulose (HPMC), glycerin, and lidocaine. The backing layer contained ethylcellulose (EC) and 1%dibutyl phthalate. The result of *in vitro* adhesion time showed that an HPMC:PVP ratio of 1:9, showed that 2/3 of the strips were detached from the beaker wall after 5 hr while at an HPMC:PVP ratio of 100:0, all the strips were detached after 5 hr.

7. Mucoadhesive polymers that could increase residence time

7.1 Hydroxypropyl Methylcellulose (HPMC)

HPMC is a semi-synthetic. non-ionic, polymer with hydrophilic properties usually used in the pharmaceutical industry that is stable in solution at a pH between 3 and 11. HPMC interpenetrates mucin chains by forming hydrogen bonds. HPMC is non-toxic and reduces the time of adhesion (74). HPMC E15LV has a low molecular weight and shows a viscosity value of 15 cP at 2% in water (75). HPMC type K4M showed the maximum mucoadhesion at pH 5-6 (74) showing as Figure 13.

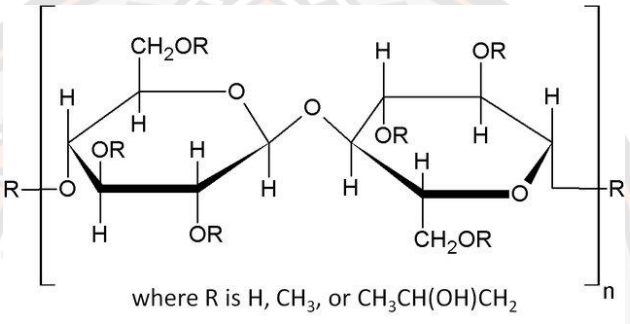

Figure 13 Chemical structure of HPMC (73)

7.2 Sodium polyacrylate

Polyacrylic acid and its derivatives gave high mucoadhesive values (76). Polyacrylic acid and its derivatives showed high intrinsic mucoadhesive properties showing as Figure 14.

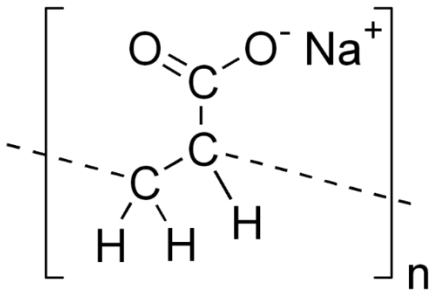

Figure 14 Chemical structure of Sodium polyacrylate

7.3 Hydroxyethyl Cellulose (HEC)

HEC is a hydroxyethyl ether of cellulose, mostly used as a thickening agent in many industries. HEC is a nonionic polymer with less sensitivity to changing pH values (77) showing as Figure 15.

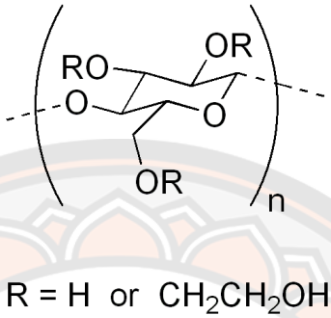

Figure 15 Chemical structure of HEC

CHAPTER III

MATERIALS AND METHODS

Materials

Gelatin type A with a bloom strength of 90-110 and 300 (Sigma-Aldrich Company, Germany) was used as a mucoadhesive layer, together with gelatin with a bloom strength of 250 (Union Chemical 1986 Co., Ltd., Thailand).

The backing layer used was ethylcellulose (EC) sourced from Dow Chemical Company, USA.

Glycerin (Namsiang by ArioMarketing Co., Ltd., Thailand) was used as the plasticizer for the gelatin.

Castor oil (Namsiang by ArioMarketing Co., Ltd., Thailand) was used as the plasticizer for the ethylcellulose.

Ethanol (95%) (RCI Labscan Co., Ltd., Thailand) was used as the solvent for ethylcellulose.

Co-solvent of α-MN (ChromaDex Co., Ltd, Irvine, USA). We obtained α-MN from ChromaDex Co., Ltd., located in Irvine, USA.

Time patch (Jiangsu Yessen Biotech Co., Ltd., China).

Taisho patches with a diameter of 1 cm (Taisho Pharmaceutical Co., Ltd., Japan), were purchased for comparison.

Equipment

Coating thickness gauge (936 FN, Protronics Co., Ltd., Thailand)

Scanning electron microscope (model 1455VP, LEO Co., Ltd., England) Texture analyzer (TA.XT plus, Charpa Techcenter Co., Ltd, Thailand)

Disintegration tester (ZT 221, Erweka Co., Ltd, Germany)

Drop shape analyzer (DSA25E, Co., Ltd., Germany)

UV-vis spectrophotometer (Evolution60, Thermo Fisher Scientific Co., Ltd., Thailand)

Methods

1. Preparation of mucoadhesive patches

Mucoadhesive patches were prepared using the solvent casting method. The patches consisted of a backing layer and a mucoadhesive layer. To prepare the backing layer, EC was mixed with 95% ethanol and castor oil as a plasticizer to 25% of the EC until it was homogeneous (Table 1). The mixed solution (6.54 g/cm^2 of EC) was then cast onto a Teflon sheet mold and dried in a hot air oven at 60°C for 4 hr. For the mucoadhesive layer, α-MN was dissolved in ethanol and mixed with a gelatin solution to form a mixture containing 1-7% w/w of gelatin. The resulting mixture was cast onto the EC backing layer and dried in a hot air oven at a temperature of 60°C for 24 hr (Table 2).

Table 1 The solution for preparing the backing layer of mucoadhesive patches in various formula

Ingredients	**(%w/w)**	**Function**
Ethyl cellulose (EC)	5	Backing polymer
Ethanol (95%)	93.75	Solvent for EC
Castor oil (25% of EC)	1.25	Plasticizer for EC

Table 2 The solution for preparing a mucoadhesive layer of mucoadhesive patches in various formula

Component (%w/w) \ Formula	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	Function
Gelatin 90/110 bloom	3.0	-	-	-	-	-	-	-	-	-	-	-	Mucoadhesive polymer
Gelatin 250-bloom	-	3.0	-	-	-	-	-	-	-	-	-	-	Mucoadhesive polymer
Gelatin 300-bloom	-	-	3.0	3.0	3.0	1.0	3.0	5.0	7.0	5.0	5.0	5.0	Mucoadhesive polymer
RO water	96.1	96.1	96.1	96.4	95.8	98.8	96.4	94.0	91.6	76.57	76.62	76.47	Solvent for gelatin
Glycerin	0.9	0.9	0.9	0.6	1.2	0.2	0.6	1.0	1.4	2.0	2.0	2.0	Plasticizer for gelatin
Alpha-mangostin	-	-	-	-	-	-	-	-	-	0.1	0.05	0.2	Anti-inflammatory agents
Ethanol (99%)	-	-	-	-	-	-	-	-	-	16.33	16.33	16.33	Co-solvent for alpha-mangostin

We varied formulation variables, including the type of gelatin varying bloom strength of 100, 250, 300-bloom gelatin fixing 3%gelatin (glycerin 30% of gelatin), quantity of gelatin 1, 3, 5, 7% fixing 300-bloom gelatin (glycerin 20% of gelatin), and plasticizer 5%gelatin 300-bloom with glycerin 40% (α-mangostin 0.108%).

2. Physicochemical properties of patches

2.1 Thickness

The thickness of the mucoadhesive patches was measured using a coating thickness gauge (936 FN, Protronics Co., Ltd., Thailand).

2.2 Scanning electron microscope (SEM)

To analyze the mucoadhesive patches, a 1 cm^2 sized patch was placed on an aluminum stub that had been coated with gold using the sputtering method for 10 sec. The cross-sections of the patches were then examined using a scanning electron microscope (model 1455VP, LEO Co., Ltd., England).

2.3 Puncture test

The puncture strength and elongation at the break of the patch were investigated using a texture analyzer (TA. XT, Stable Micro Systems Co., Ltd., England) through puncture tests. The experimental conditions included a load cell of 30 kg, spherical probe P/5S, contact force of 5 g, and a test speed of 0.30 mm/sec. Testing was conducted on patches of size 2.25 cm^2. The puncture strength and elongation at break (%) were calculated using equations [1] and [2] respectively (78; 79). The experiment was performed five times, and the results were recorded.

$$\text{Puncture Strength} = \frac{\text{Max force (N)}}{2\pi \times \text{Support radius (mm)} \times \text{Film thickness (mm)}} \quad [1]$$

$$\%\text{Elongation at break} = \frac{\sqrt{\text{Support radius}^2 + \text{Max distance}^2} - \text{Support radius}}{\text{Support radius}} \times 100 \quad [2]$$

3. *In vitro* residence time test

To simulate the residence time, which is the maximum length of time that the mucoadhesive patches remained on aphthous ulcers, mucin-coated cellophane was prepared to mimic oral mucosa. The cellophane was immersed in a 2% mucin solution (porcine stomach type II) and dried at 45°C for 4 hr. Artificial saliva was prepared, containing NaCl (0.084%), KCl (0.12%), K_2HPO_4 (0.026%), and water (99.77%), and its pH was adjusted to 7.0 using lactic acid. Figure 16 shows the simulation of the residence time test (Erweka ZT 221, Heusenstamm, Germany). The beaker containing the artificial saliva was maintained at 37±2°C, which mimics oral cavity temperature. The mucoadhesive patches were applied onto the mucin-coated cellophane with a controlled force of 20 N for 10 seconds. The basket containing the mucoadhesive patches was shaken at 30 ± 1 strokes/min. The detachment time of the 5 patches was recorded as the *in vitro* residence time.

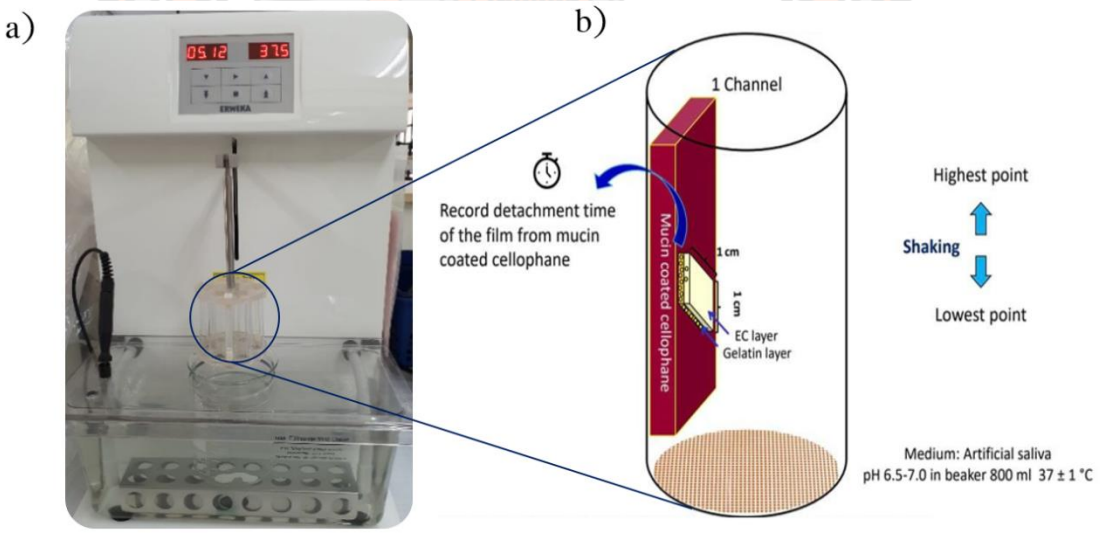

Figure 16 In vitro simulation of the residence time test. a) Disintegration tester, b) Mucin-coated cellophane cell used for assessing the residence time of a mucoadhesive patches

4. Wettability test

The wettability of the mucoadhesive patches was assessed using a drop shape analyzer model DSA25E with tilting device, Scientific Promotion Co., Ltd., Hamburg, Germany. The contact angle between droplets of purified water and the mucoadhesive layer surface of the patches was measured using a sessile drop protocol with the following parameters: substance: water, temperature: 25°C, volume: 10 μL, rate: 10 μL/s, and needle diameter: 0.5 mm (80). The experiment was performed five times, and the results were recorded and averaged.

5. Quantification of α-mangostin in the mucoadhesive patches

To quantify the total amount of α-MN in the patches, the thickness and weight of each patch were initially measured. Subsequently, the patches were cut into small pieces size 2x3 mm^2. To dissolve the gelatin layer, 6 mL of water was added, followed by the addition of 10 mL of ethanol to dissolve EC and α-MN. The mixture was then vortexed and sonicated at 25°C for 30 min and the sample underwent centrifugation at 31,514 × g for 5 min. The α-MN content in the supernatant was determined using a UV–vis spectrophotometer at its maximum absorbance wavelength of 320 nm. This current experiment is based on a previous study by Pham et al., 2019. In this experiment, patches were used, while in the previous experiment, nanoparticles were used, thus the form of drug entrapment and the dosages differed.

6. Alpha-mangostin release test

To indicate the release profile of alpha-mangostin to aphthous ulcers. Artificial saliva (1% Tween 80) used as a medium will be adjusted to pH 7 by 1M HCl. The temperature of the 7 ml of artificial saliva will be controlled at 37°C, then added to Franz diffusion cells which will remove the bubbles between the patches and the top of the receptor chamber. The patches will be placed onto the top of the receptor chamber of the Franz diffusion cells with no membrane between them because the aphthous ulcers are exposed. A volume of 0.5 ml of the samples will be collected by replacing an equal amount of the medium at predetermined times, 0.5, 1, 2, 4 and 6 hrs. Dilute and measure the absorbance of the alpha-mangostin using a UV-Vis spectrophotometer at 320 nm. The calibration curve of alpha-mangostin, ranging from 2-20 μg/ml will be used in the calculation of the artificial saliva (1% Tween80):

ethanol absolute at ratio 1: 1 as a solvent in the equation $y = 0.0529x - 0.0117$, $R^2 = 0.9999$ (modified from 81; 82).

Statistical analysis

The data were reported as the mean ± standard deviation (S.D.), derived from a minimum of three experiments. To compare the different groups of data, a one-way analysis of variance (ANOVA) was conducted using SPSS software. A significance level of * $p < 0.05$ was employed to establish statistical significance

CHAPTER IV

RESULTS AND DISCUSSION

The blank, 5% gelatin, 40% glycerin, and ethanol without α-MN were clear. Adding α-MN at 0.054%, 0.108%, and 0.216% conc. caused the patches to turn increasingly yellow (higher α-MN conc.) showing as Figure 17.

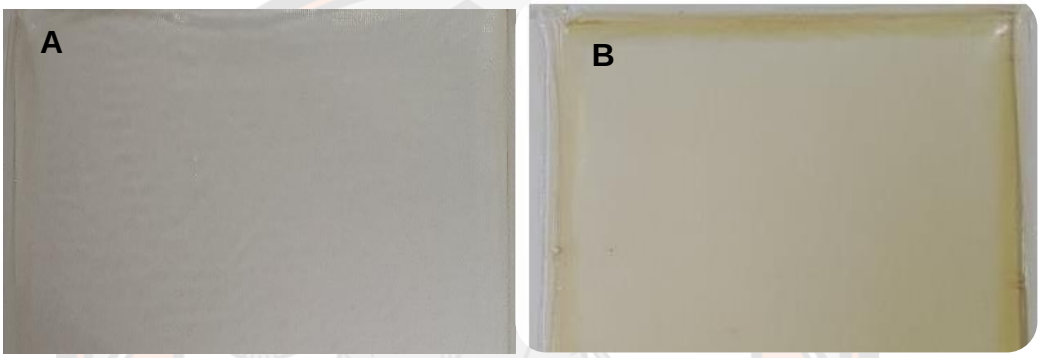

Figure 17 The mucoadhesive patch The plain patch (A), The patch containing α-MN (B)

The thickness of Ethylcellulose alone is approximately 60.17 ± 1.86 μm in a two-layer patch, where the second layer consists of gelatin. When the bloom strength is increased, the thickness also increases, ranging from 89 to 100 μm. Similarly, adding glycerin results in an increased thickness ranging from 87 to 124 μm. Furthermore, an increase in the amount of gelatin leads to a thickness increase ranging from 57 to 158 μm. The quantity of α-MN does not affect the thickness of the patch, remaining in the range of 102-104 μm showing as Table 3.

Table 3 Thickness of patches formulations

Formulations	Various factors	Mean (µm)	SD
1 layer of 5%Ethylcellulose (castor oil 25%)	-	60.17	1.86
Gelatin bloom strengths with 5%Ethylcellulose (castor oil 25%)	90-110 blooms	89.40	11.13
	250 blooms	97.00	7.67
	300 blooms	99.53	11.21
Glycerin levels at 3% gelatin with 300 bloom with 5%Ethylcellulose (castor oil 25%)	Glycerin 20%	87.40	2.69
	Glycerin 30%	89.37	1.30
	Glycerin 40%	123.67	2.08
Gelatin conc. at gelatin 300 bloom (20% glycerin) with 5%Ethylcellulose (castor oil 25%)	Gelatin 1%	57.73	2.19
	Gelatin 3%	94.53	4.11
	Gelatin 5%	129.00	5.00
	Gelatin 7%	157.33	8.33
Amount of α-MN at 5%Gelatin 300 blooms (40% glycerin) with 5%Ethylcellulose (castor oil 25%)	0.054	103.77	3.87
	0.108	103.70	5.80
	0.216	102.30	6.41

Mucoadhesive patches were prepared using the solvent-casting method. The blank patches, which contained 5% 300-bloom gelatin, 40% glycerin, and ethanol without α-MN, appeared clear. However, upon adding α-MN at concentrations of 0.054% (F11), 0.108% (F10), and 0.216% (F12), the patches turned yellow with increasing colour intensity observed at higher α-MN concentrations. When structural scanning of the patch was performed using scanning electron microscopy (SEM), the results showed the EC layer and the gelatin layer. The EC layer, shown in Figure 18, acts as a barrier that covers the ulcer and reduces pain and discomfort. The gelatin layer, shown in Figure 18, functions as the mucoadhesive layer and enables targeted and sustained drug delivery. The EC layer was observed to be thicker than the gelatin

layer, owing to the greater amount of ethylcellulose polymer solids present in comparison to the gelatin polymer.

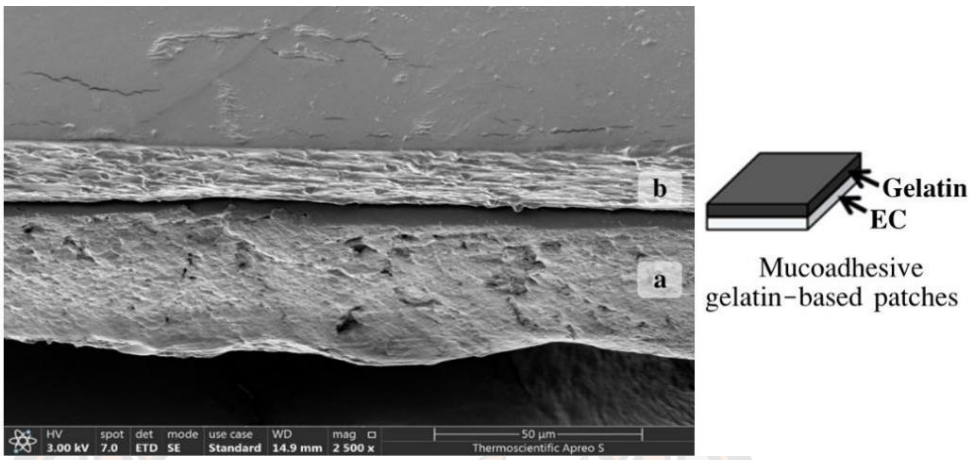

Figure 18 Cross-sectional scanning electron microscope images of the double-layer mucoadhesive patch, showing (a) the ethylcellulose layer and (b) the gelatin layer

Influence of gelatin bloom strength

Bloom strength is a parameter that measures the rigidity or firmness of gelatin. A higher bloom strength indicates a firmer gelatin. In this study, the patch's puncture strength and elongation at break were assessed using different bloom strengths to gain insight into the ability of the mucoadhesive patch to withstand damage and maintain its structural integrity during application and use, as shown in Figure 19. The results demonstrated that the strength of the two-layer film was noticeably reduced compared to the backing layer film, which may suggest that the casting of the mucoadhesive layer interfered with the formation of the backing layer film. The test results show that the puncture strength significantly increased with an increase in bloom strength, as seen in Figure 19a. The highest puncture strength value of 2.9 N/mm² was observed with 300-bloom gelatin (F3), which indicates the stiffness of the polymers due to their proline and hydroxyproline content (83). The 250-bloom gelatin (F2) also exhibited high puncture strength, but it was not significantly higher than the 300-bloom gelatin (F3). A similar result was observed by Ahmady & Abu

Samah (2021). Figure 19b shows that the elongation at break of 250-bloom gelatin was higher than all of the 90-110 and 300-bloom gelatin. The flexibility of the patches was primarily determined by the backing layer (EC layer), which is more brittle than the gelatin film.

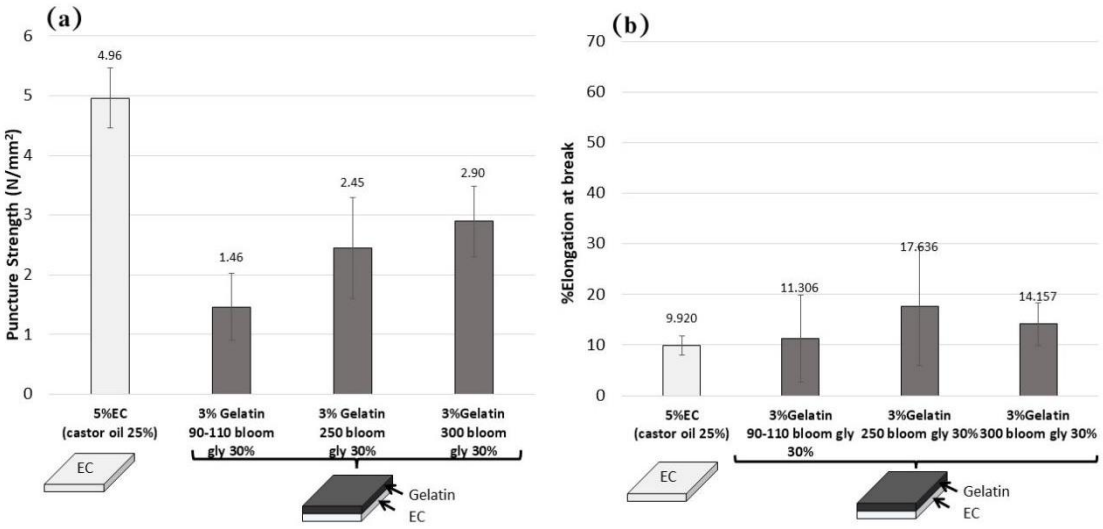

Figure 19 Effect of varying gelatin bloom strength on (a) puncture strength and (b) elongation at the break of mucoadhesive patche

Figure 20 shows the effect of varying gelatin bloom strength on the *in vitro* residence time of mucoadhesive patches. The *in vitro* residence time of the patches with 300-bloom gelatin (F3) was the longest, at 58 min, showing that the increasing bloom strength of gelatin increases the residence time. This is because of an increase in the molecular weight of the gelatin and the entanglement of the polymer from the bonds with mucin. The residence times of the commercial patches were 24 and 50 min. The viscosity of hydrated gelatin varies depending on the bloom strength of the gelatin used, with higher bloom strengths exhibiting higher viscosity than those with lower bloom strengths, as reported in previous studies (84; 85).

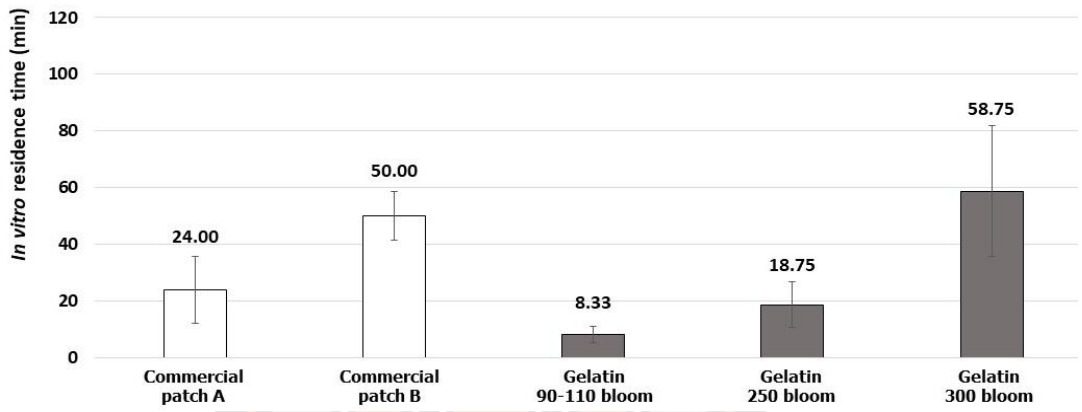

Figure 20 Effect of varying gelatin bloom strength on the in vitro residence time of mucoadhesive patches

Contact angle measurements were performed to evaluate the hydrophilicity of mucoadhesive patches prepared using different bloom strengths of gelatin. The results are shown in Figure 21A. The contact angle decreased with increasing bloom strength, with the 300-bloom (F3) gelatin exhibiting the lowest contact angle, indicating higher hydrophilicity compared to the 90-110 bloom (F1) and 250-bloom gelatin (F2). This result suggests that the gelatin with higher bloom strength has a higher affinity for the wetted mucosal surface, which could enhance the mucoadhesive property of the patches. The longer *in vitro* residence time observed for the mucoadhesive patches prepared using 300-bloom gelatin (F3), as illustrated in Figure 21, could be attributed to the lower contact angle value observed for this gelatin, indicating a stronger interaction with the mucosal surface and improved mucoadhesive properties.

Higher bloom strength gelatins exhibit improved hydrophilicity and potentially smoother surfaces due to their denser structures and more extensive hydrogen bonding networks (86) . These properties can contribute to enhanced wettability by facilitating better water absorption and promoting liquid spreading and adhesion on the surface.

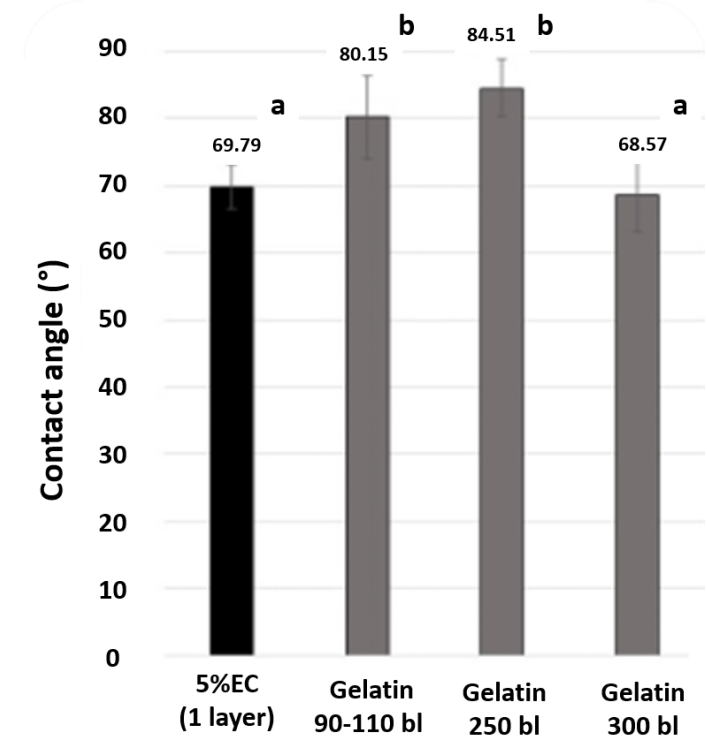

Figure 21 Effect of bloom strength of gelatin on the water contact angle

Influence of plasticizer concentration

The 300-bloom gelatin (F3) was chosen as the foundation for the mucoadhesive layer in subsequent experiments. The next experiment explored the impact of plasticizer concentration, specifically the quantity of glycerin, on the attributes of the mucoadhesive patches. The results are shown in Figure 22. An inverse relationship was observed between glycerin concentration and the puncture strength of the patches, as seen in Figure 22a. This phenomenon can be attributed to glycerin's interpenetration among gelatin polymer chains, consequently diminishing their entanglements. The integration of plasticizers into the polymer structure leads to a reduction in the glass transition temperature (Tg), subsequently increasing the amorphous nature and decreasing the rigidity of the material.

Furthermore, plasticizers have been found to contribute to a decline in the entanglement density of polymer chains. This results in enhanced mobility of the polymer chains, which in turn diminishes the puncture strength of the material.

Analogous findings were documented in a study by 87) , wherein an increase in plasticizer concentration from 10% to 40% led to an 85.89% reduction in the puncture strength of glycerol-plasticized films. The enhanced flexibility of the gelatin layer film may be attributed to the elevated concentration of plasticizer. In contrast to the puncture strength, no significant increase in the elongation at the break of the patches was observed when the glycerin concentration was varied, as seen in Figure 22b. This can be ascribed to the rigidity of the EC layer.

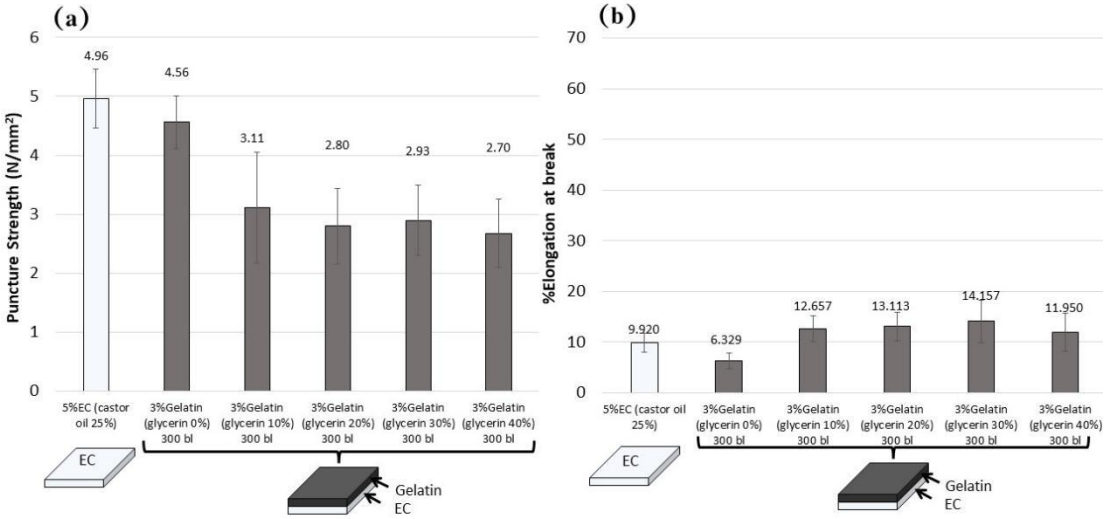

Figure 22 Effect of varying glycerin concentration on (a) puncture strength and (b) elongation at break of mucoadhesive patches

Figure 23 shows the effect of plasticizer concentration on the *in vitro* residence time of the mucoadhesive patches. The tested glycerin concentrations were: 20% w/w, 30% w/w, and 40% w/w. The results show that the formulation with 3% gelatin (300-bloom value) and 40% w/w glycerin (F5) yielded the longest *in vitro* residence time (107.50 ± 16. 67 minutes) . This outcome might be ascribed to glycerin's ability to absorb saliva within the mucoadhesive patch, consequently improving wettability and promoting polymer chain mobility.

The progressive increase in glycerin concentration correlated with a slight elevation in the *in vitro* residence time, possibly due to the humectant characteristics of the absorbed saliva. Previous research has demonstrated that interpenetration between gelatin and mucin chains, facilitated by bond formation, contributes to an increased *in vitro* residence time (88).

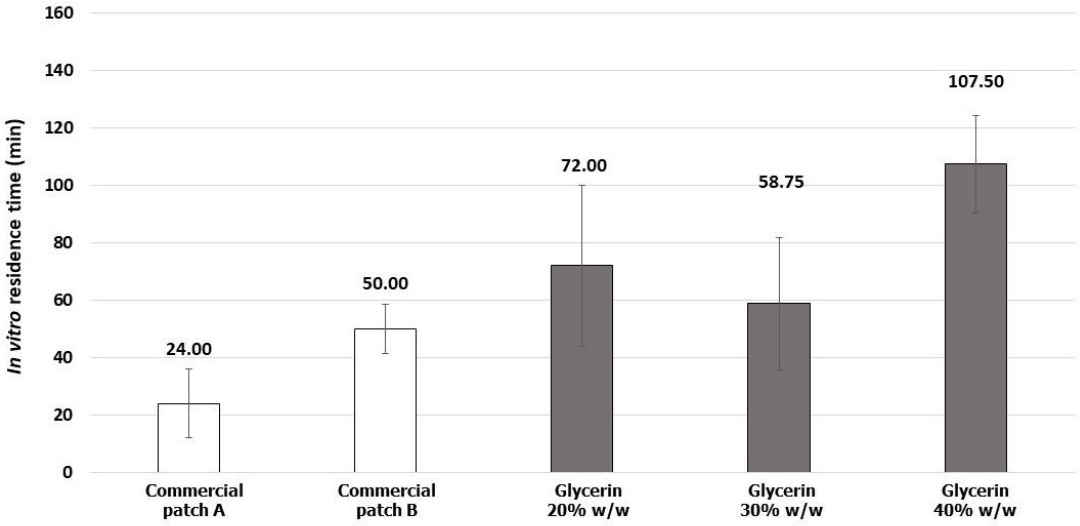

Figure 23 Effect of amount of glycerin on the in vitro residence time of mucoadhesive patches

Figure 24 shows the impact of varying glycerin levels on the contact angle. It is observed that an increase in glycerin concentration is associated with a slight elevation in the contact angle because of physicochemical properties of glycerin. Glycerin, being a hygroscopic substance, tends to interact with water molecules present at the solid-liquid interface. As the glycerin concentration rises, it competes with water for surface binding sites, leading to changes in the wetting behavior.

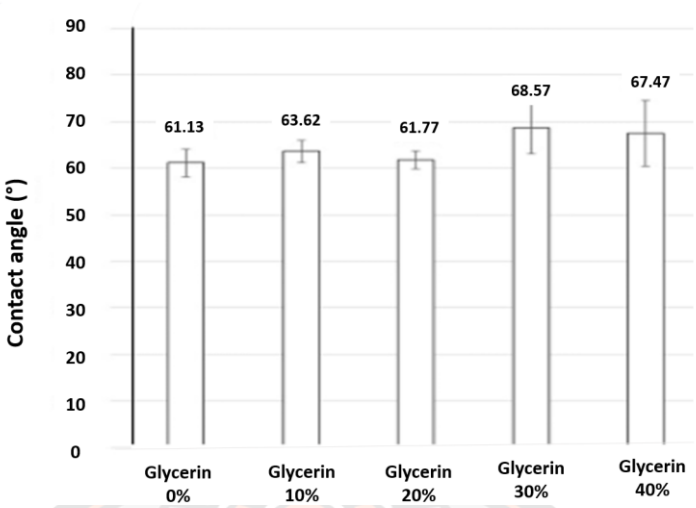

Figure 24 Effect of quantity of glycerin on the water contact angle of mucoadhesive patches

Influence on the amount of gelatin

Increasing the concentration of gelatin from 1% to 7% resulted in a notable increase in patch thickness due to the thicker mucoadhesive layer. This increase in gelatin concentration also led to an increase in puncture strength, as shown in Figure 25a. Moreover, the increase in gelatin concentration also resulted in a change in the ratio of EC and gelatin layers. This is illustrated in Figure 25b, which shows the increase in elongation at break for mucoadhesive patches with 7% gelatin concentration (F9). It is noteworthy that the flexibility of the patch was not solely determined by the backing layer as mentioned earlier. The solid content of the polymer in each layer per cm^2 was uniform. A study conducted by (89) found that increasing the thickness of a film resulted in a corresponding increase in its puncture strength and elongation. In thicker films, the polymer matrix is denser and richer in inter and intramolecular interactions and, consequently, more resistant to rupture.

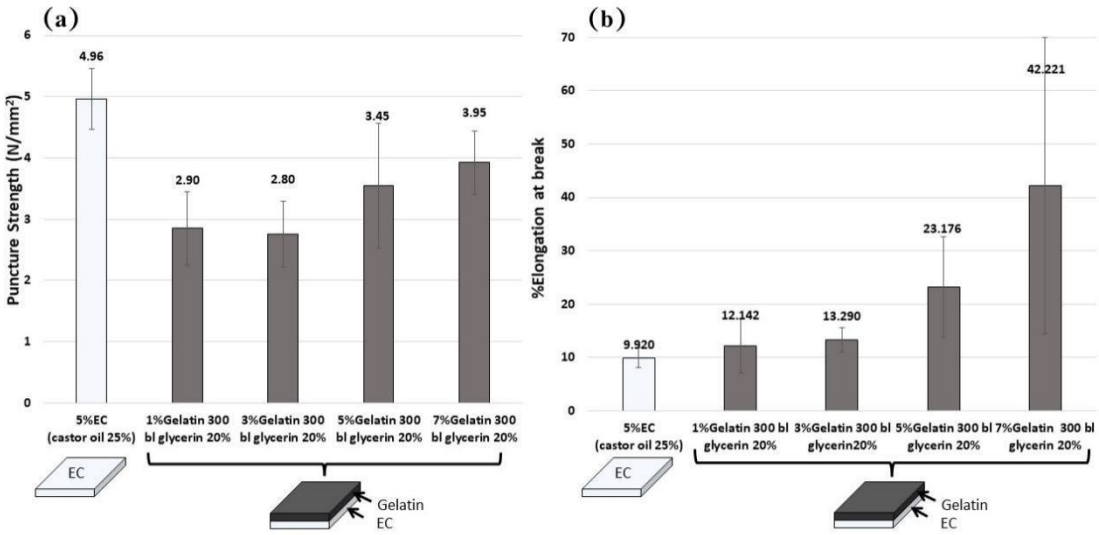

Figure 25 Effect of gelatin on (a) puncture strength and (b) elongation at the break of mucoadhesive patches

Figure 26 shows that as the concentrations of gelatin were increased from 1% , 3% , and 5% , to 7% w/w, the thickness of the gelatin increased, leading to an increase in the *in vitro* residence time of mucoadhesive patches. This suggests that patches with higher concentrations of gelatin may adhere to the oral mucosa longer. The increased concentration of gelatin led to an increase in the number of gelatin polymer chains that interpenetrated with mucin chains, building mucoadhesive bonds and ultimately increasing the *in vitro* residence time (90). However, it should be noted that film thickness has a greater impact on the overall mouthfeel and comfort of the patient compared to film mass (91) . Therefore, it is essential to strike a balance between increasing the gelatin concentration for improved *in vitro* residence time and maintaining a comfortable mouthfeel for the patient. Increasing the concentration of gelatin did not result in any significant change in the water contact angle. This is likely because the surface of the material remained the same, despite the increase in gelatin concentration.

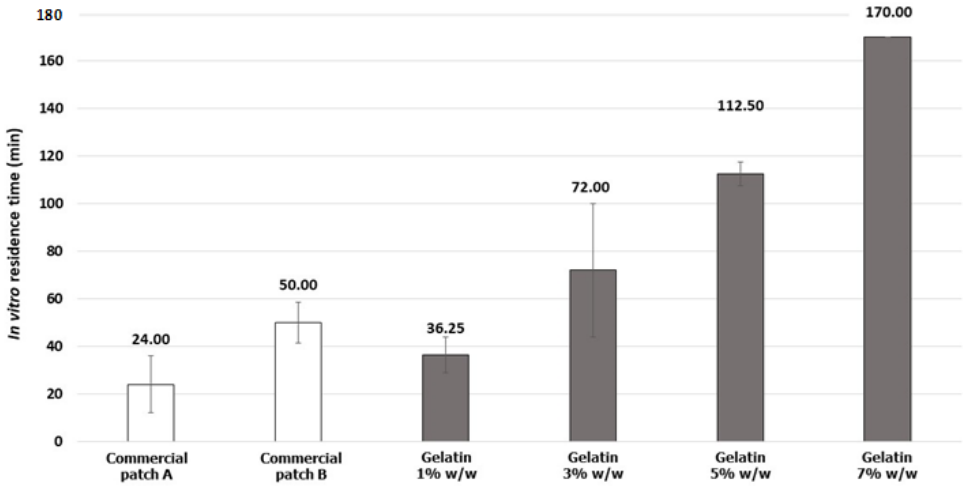

Figure 26 the impact of varying amounts of gelatin on the in vitro residence time of mucoadhesive patches

Figure 27 shows an increase in the amount of gelatin, the contact angle remains relatively constant. This phenomenon is attributed to the insignificant impact of the thickness of the mucoadhesive layer on the contact angle.

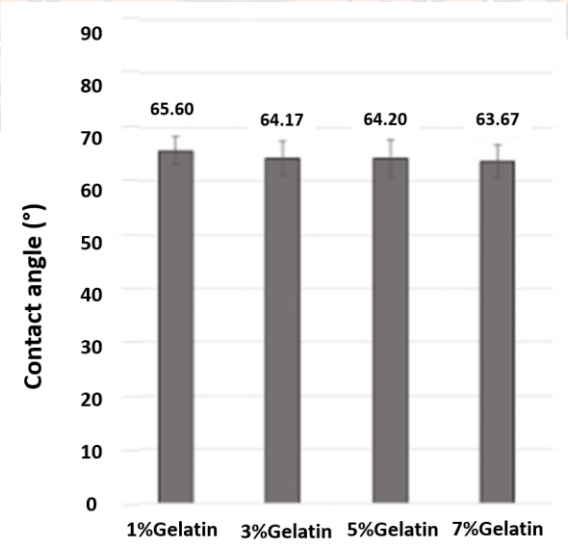

Figure 27 Effect of quantity of gelatin on the water contact angle of mucoadhesive patches

Loading of α-MN in mucoadhesive gelatin-based patches

The loading of α-MN in mucoadhesive gelatin-based patches was investigated for potential topical applications due to its potent anti-inflammatory and antibacterial properties. α-MN was incorporated into the gelatin layer at concentrations ranging from 0.054% to 0.216% w/w to achieve a theoretical concentration range of 142.12 to 568.48 μg/cm². The actual concentrations of α-MN in the patches were determined to be 144.12 ± 27.10 μg, 273.35 ± 17.20 μg, and 441.05 ± 94.79 μg for the patches containing 0.054%(F11), 0.108, (F10) and 0.216% α-MN (F12), respectively. However, a decrease in actual concentration compared to theoretical concentration was observed at higher concentrations, which may be attributed to the solubility limitations of α-MN.

The actual concentrations of α-MN in patches were 144.12 ± 27.10 μg, 273.35 ± 17.20 μg, and 441.05 ± 94.79 μg for patches containing 0.054%, 0.108%, and 0.216% α-MN, respectively as shown in Table 8. However, a decrease in actual concentration compared to the theoretical concentration was more prominent in higher concentrations, which can be attributed to the solubility limitations of α-MN showing as table 4

Table 4 Amount of α-MN loading in the mucoadhesive gelatin-based patches (5% gelatin, 300 bl, and glycerin 40%)

Formulation	Theoretical concentrations (μg/cm²)	Actual concentrations (μg/cm²)
α-MN 0.054%	142.12	144.12 ± 27.10
α-MN 0.108%	284.24	273.35 ± 17.20
α-MN 0.216%	568.48	441.05 ± 94.79

Alpha-mangostin release test

The alpha-mangostin (α-MN) release test showed that the cumulative release of α-MN increased with increasing α-MN concentration as shown in Figure 28. The release rate plateaued at higher α-MN concentrations, suggesting a maximum release rate. The drug delivery system was capable of releasing α-MN in a controlled manner, and the release rate could be controlled by adjusting the α-MN concentration. The release rate was relatively fast and sustained, and it was not significantly affected by the presence of other components in the drug delivery system. These results suggest that the drug delivery system has the potential to be used to deliver α-MN safely and effectively.

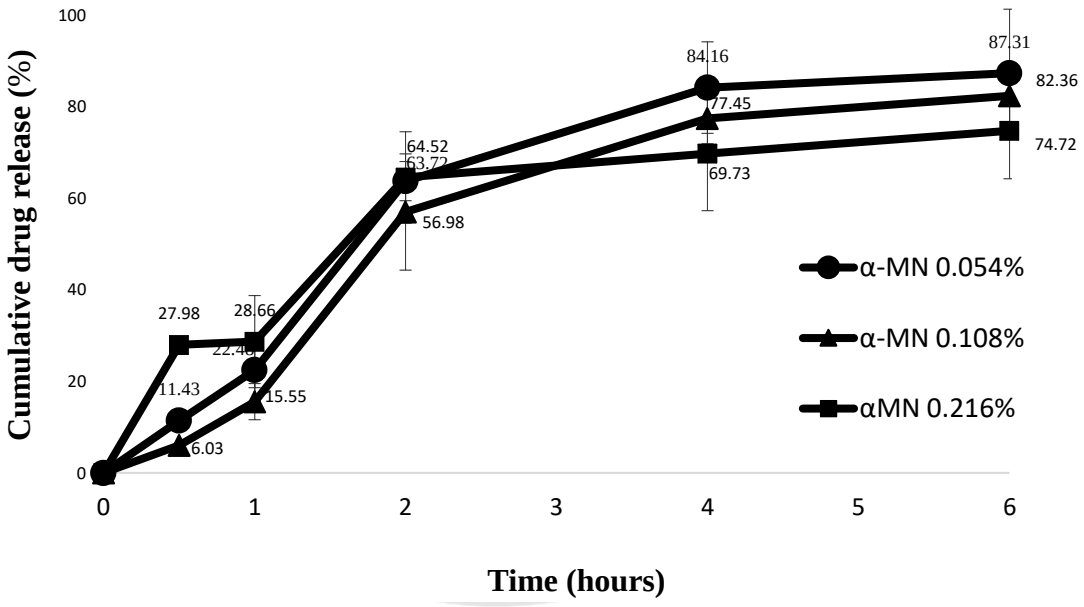

Figure 28 Effect of varying amount of α-MN on cumulative drug release (%)

CHAPTER V

CONCLUSION

In summary, mucoadhesive patches were fabricated using the solvent-casting method, with the addition of α-MN resulting in a noticeable color change, intensifying with higher concentrations. The best formulation is 5%Gelatin 300 blooms with glycerin 40% containing α-MN 0.108%. Because this formulation give the appropriate residence time and is easy to prepare.

Scanning electron microscopy revealed a layered structure, with ethylcellulose acting as a barrier and gelatin as the mucoadhesive component. Bloom strength of gelatin played a pivotal role in puncture strength and *in vitro* residence time, with 300-bloom gelatin exhibiting the highest puncture strength. Contact angle measurements supported these observations, showcasing increased hydrophilicity with higher bloom strength.

Glycerin concentration inversely affected puncture strength and directly impacted *in vitro* residence time, with higher concentrations leading to longer residence times due to enhanced wettability and polymer chain mobility. Increasing gelatin concentration yielded thicker patches, greater puncture strength, and extended *in vitro* residence times. However, maintaining patient comfort necessitates a balance between gelatin concentration and mouthfeel, with the water contact angle remaining relatively unaffected.

Loading α-MN in gelatin-based patches for topical applications exhibited decreased actual concentration compared to theoretical concentrations at higher doses, likely due to solubility limitations. These findings underscore the significance of gelatin properties, plasticizer concentration, and drug loading in the formulation and characteristics of mucoadhesive patches. This research holds promise for their potential application in drug delivery and topical treatments.

REFERENCES

REFERENCES

1. Chiang CP, Yu-Fong Chang J, Wang YP, Wu YH, Wu YC, Sun A. Recurrent aphthous stomatitis - Etiology, serum autoantibodies, anemia, hematinic deficiencies, and management. J Formos Med Assoc. 2019;118(9):1279-89.

2. Belenguer-Guallar I, Jimenez Y, Claramunt-Lozano A. Treatment of recurrent aphthous stomatitis. A literature review. Journal of clinical and experimental dentistry. 2014;6:e168-e74.

3. Taokaew S, Phonsee S, Woravut N, Pitaksuteepong T, Kriangkrai W. Characteristic Assessment of the Polymeric Films Used for Hair Gel Products in Thailand. Key Engineering Materials. 2019;819:98-103.

4. Bafi-Yeboa, N. F. A., Arnason, J. T., Baker, J., & Smith, M. L. (2005). Antifungal constituents of Northern prickly ash, Zanthoxylum americanum Mill. Phytomedicine, 12(5), 370-377. https://doi.org/10.1016/j.phymed.2003.12.005

5. Mohan S, Syam S, Abdelwahab S, Thangavel N. Anti-inflammatory molecular mechanism of action of α-Mangostin, the major xanthone from the pericarp of Garcinia mangostana; an in silico, *in vitro* and *in vivo* approach. Food & Function. 2018;9.

6. Goel AN, Long JL. Chapter 2 - The Oral Cavity. In: Chhetri DK, Dewan K, editors. Dysphagia Evaluation and Management in Otolaryngology: Elsevier; 2019. p. 5-12.

7. Moharamzadeh K. Oral mucosa tissue engineering. 2017. p. 223-44.

8. Atkinson, M.E., Jowett, A., White, F.H., 2000. Principles of Anatomy and Oral Anatomy for Dental Students. Cava Cadavers, Taddington.

9. Denecker G, Ovaere P, Vandenabeele P, Declercq W. Caspase-14 reveals its secrets. The Journal of Cell Biology. 2008;180:451-8.

10. Arthur R. Hand. (n.d.). Oral Structures and Tissues. Available February 10, 2022, from https://pocketdentistry.com/1-oral-structures-and-tissues/

11. Rogers RS. Recurrent Aphthous Stomatitis: Clinical Characteristics and Evidence for an Immunopathogenesis. Journal of Investigative Dermatology. 1977;69(6):499-509.

12. Lehner T. Autoimmunity in oral diseases, with special reference to recurrent oral ulceration. Proc R Soc Med. 1968;61(5):515-24.

13. Rogers R. Recurrent aphthous stomatitis: clinical characteristics and associated systemic disorders. Seminars in cutaneous medicine and surgery. 1997;16 4:278-83.

14. Pongissawaranun W, Laohapand P. Epidemiologic study on recurrent aphthous stomatitis in a Thai dental patient population. Community Dentistry and Oral Epidemiology. 1991;19(1):52-3.

15. Tarakji B, Gazal G, Al-Maweri SA, Azzeghaiby SN, Alaizari N. Guideline for the diagnosis and treatment of recurrent aphthous stomatitis for dental practitioners. J Int Oral Health. 2015;7(5):74-80.;

16. Porter SR, Scully C, Pedersen A. Recurrent Aphthous Stomatitis. Critical Reviews in Oral Biology & Medicine. 1998;9(3):306-21.

17. Judge T, Scott B, Ilies R. Hostility, Job Attitudes, and Workplace Deviance: Test of a Multilevel Model. The Journal of applied psychology. 2006;91:126-38.

18. Ngan V, Oakley A, DermNet NZ. Aphthous Ulcer [Internet];2016 [cited 2020 July 16]. Available from: https://www.dermnetnz.org/topics/aphthous-ulcer/

19. Ask2Health. Are Mouth Ulcers Hurting You? [Internet]; 2019. [cited 2020 Dec 14]. Available from https://www.ask2health.com/are-mouth-ulcers-hurting-you/

20. Natah SS, Häyrinen-Immonen R, Hietanen J, Malmström M, Konttinen YT. Immunolocalization of tumor necrosis factor-alpha expressing cells in recurrent aphthous ulcer lesions (RAU). J Oral Pathol Med. 2000;29(1):19-25.

21. Correia-Silva J, Sá A, Victória J, Diniz M, Costa F, Gomez R. Investigation of functional gene polymorphisms IL-1β, IL-6, IL-10 and TNF-α in individuals with recurrent aphthous stomatitis. Archives of oral biology. 2007;52:268-72.

22. Sun T, Youn S, Wu G, Kuntaraporn M. Online Word-of-Mouth (or Mouse): An Exploration of Its Antecedents and Consequences. J Computer-Mediated Communication. 2006;11:1104-27.

23. Bio-Rad Laboratories Co., Ltd. Inflammation Overview [Internet];2020 [cited 2020 Dec 14]. Available from https://www.bio-rad-antibodies.com/inflammation-antibodies.html

24. 1. Yates P, Stout GH. The Structure of Mangostin1. Journal of the American Chemical Society. 1958;80(7):1691-700.

25. Schmid W. Ueber das Mangostin. Justus Liebigs Annalen der Chemie. 1855;93(1):83-8.

26. Ghasemzadeh A, Jaafar HZ, Baghdadi A, Tayebi-Meigooni A. Alpha-Mangostin-Rich Extracts from Mangosteen Pericarp: Optimization of Green Extraction Protocol and Evaluation of Biological Activity. Molecules. 2018;23:1852.

27. Global Trading Corp Co., Ltd. Mangosteen [Internet];2019 [cited 2020 Feb 10] Available from: https://www.gtcthailand.com/product/21648-19146/mangosteen

28. Chen L-G, Yang L-L, Wang C-C. Anti-inflammatory activity of mangostins from Garcinia mangostana. Food and Chemical Toxicology. 2008;46(2):688-93.

29. Kumar P, Elsaidi H, Zorniak B, Laurens E, Yang J, Bacchu V, et al. Kumar et al-2016-ChemMedChem (1). 2016.

30. Choi Yh, Han S, You-Jin K, Kim Y-M, Chin Y-W. Absorption, tissue distribution, tissue metabolism and safety of α-mangostin in mangosteen extract using mouse models. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association. 2014;66.

31. Gutierrez-Orozco F, Chitchumroonchokchai C, Lesinski G, Suksamrarn S, Failla M. α-Mangostin: Anti-Inflammatory Activity and Metabolism by Human Cells. Journal of agricultural and food chemistry. 2013;61.

32. Hong S-CL, Levine L. Inhibition of Arachidonic Acid Release from Cells as the Biochemical Action of Anti-Inflammatory Corticosteroids. Proceedings of the National Academy of Sciences of the United States of America. 1976;73(5):1730-4.

33. Laine Higa. Anti-inflammatories: Sort Out Your Many Steroids and NSAIDs [Internet];2017 [cited 2021 Feb 13]. Available from: https://www.reviewofoptometry.com/article/antiinflammatories-sort-out-your-many-steroids-and-nsaids

34. Regezi JA, Sciubba JJ, Jordan RCK. 2017, Patologia oral, correlações clinicopatológicas, 7th edn, Rio de Janeiro, Elsevier.

35. Bell J. Amlexanox for the treatment of recurrent aphthous ulcers. Clin Drug Investig. 2005;25(9):555-66.

36. David Rakel MD, in Integrative Medicine (Fourth Edition), 2018

37. Lirk P, Messner H, Deibl M, Mitterschiffthaler G, Colvin J, Steger B, et al. Accuracy in estimating the correct intervertebral space level during lumbar, thoracic and cervical epidural anaesthesia. Acta anaesthesiologica Scandinavica. 2004;48:347-9.

38. Scully C, Porter S. Oral mucosal disease: recurrent aphthous stomatitis. Br J Oral Maxillofac Surg. 2008;46(3):198-206.

39. Wathoni N, Shan C, Shan W, Rostinawati T, Indradi B, Pratiwi R, et al. Characterization and antioxidant activity of pectin from Indonesian mangosteen (Garcinia mangostana L.) rind. Heliyon. 2019;5:e02299.

40. Bhalang K, Thunyakitpisal P, Rungsirisatean N. Acemannan, a polysaccharide extracted from Aloe vera, is effective in the treatment of oral aphthous ulceration. J Altern Complement Med. 2013;19(5):429-34.

41. Zhou Y-z, Xue L-y, Gao L, Qin X-m, Du G-h. Ginger extract extends the lifespan of Drosophila melanogaster through antioxidation and ameliorating metabolic dysfunction. Journal of Functional Foods. 2018;49:295-305.

42. Deshmukh R, Bagewadi A. Comparison of effectiveness of curcumin with triamcinolone acetonide in the gel form in treatment of minor recurrent aphthous stomatitis: A randomized clinical trial. International journal of pharmaceutical investigation. 2014;4:138-41.

43. Salamat-Miller N, Chittchang M, Johnston TP. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Deliv Rev. 2005;57(11):1666-91.

44. Mathiowitz E, Chickering DE, Lehr CM. Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches, and Development: Taylor & Francis Limited; 2019.

45. Baliga S, Muglikar S, Kale R. Salivary pH: A diagnostic biomarker. Journal of Indian Society of Periodontology. 2013;17:461-5.

46. Sund Levander M, Forsberg C, Mlt P. Normal oral, rectal, tympanic and axillary body temperature in adult men and women: A systematic literature review. Scandinavian Journal of Caring Sciences. 2002;16:122-8.

47. Woodley J. Bioadhesion: New possibilities for drug administration? Clinical pharmacokinetics. 2001;40:77-84.

48. Balabushevich N, Sholina E, Mikhalchik E, Filatova L, Vikulina A, Volodkin D. Self-Assembled Mucin-Containing Microcarriers via Hard Templating on CaCO3 Crystals. Micromachines. 2018;9:307.

49. Khutoryanskiy, 2014

50. Gandhi RB, Robinson JR. Oral cavity as a site for bioadhesive drug delivery. Advanced Drug Delivery Reviews. 1994;13(1):43-74

51. Shahiwala A. Applications of Polymers in Buccal Drug Delivery. 2014. p. 59-96.

52. Karki S, Kim H, Na S-J, Shin D, Jo K, Lee J. Thin films as an emerging platform for drug delivery. Asian Journal of Pharmaceutical Sciences. 2016;11.

53. Patel VF, Liu F, Brown MB. Advances in oral transmucosal drug delivery. Journal of Controlled Release. 2011;153(2):106-16.

54. Gorgieva S, Kokol V. Collagen- vs. Gelatine-Based Biomaterials and Their Biocompatibility: Review and Perspectives. 2011.

55. Hanani ZAN. Gelatine. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Oxford: Academic Press; 2016. p. 191-5.

56. Schellmann N. Animal Glues - their adhesive properties, longevity and suggested use for repairing taxidermy specimens. NatSCA News. 2009:36-40.

57. Abruzzo A, Nicoletta FP, Dalena F, Cerchiara T, Luppi B, Bigucci F. Bilayered buccal films as a child-appropriate dosage form for systemic administration of propranolol. International Journal of Pharmaceutics. 2017;531(1):257-65.

58. Jovanović M, Tomić N, Cvijić S, Stojanović D, Ibrić S, Uskoković P.Mucoadhesive Gelatine Buccal Films with Propranolol Hydrochloride: Evaluation of Mechanical, Mucoadhesive, and Biopharmaceutical Properties. Pharmaceutics. 2021;13(2):273.

59. Bonferoni MC, Chetoni P, Giunchedi P, Rossi S, Ferrari F, Burgalassi S, et al. Carrageenan-gelatine mucoadhesive systems for ion-exchange based ophthalmic delivery: *In vitro* and preliminary *in vivo* studies. European Journal of Pharmaceutics and Biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV. 2004;57:465-72.

60. Oğur S. The physicochemical properties of edible protein films. Italian Journal of Food Science. 2015;27.

61. Wannaphatchaiyong S, Boonme P, Pichayakorn W. Gelatine Films and its Pregelatineized Starch Blends: Effect of Plasticizers. Key Engineering Materials. 2017;751:230-5.

62. Jongjareonrak A, Benjakul S, Visessanguan W, Tanaka M. Effects of plasticizers on the properties of edible films from skin gelatine of bigeye snapper and brownstripe red snapper. European Food Research and Technology. 2006;222:229-35.

63. Thomazine M, Carvalho R, Sobral P. Physical Properties of Gelatin Films Plasticized by Blends of Glycerol and Sorbitol. Journal of Food Science. 2006;70:E172-E6.

64. Rowe RC, Sheskey PJ, Owen SC, American Pharmacists A. Handbook of pharmaceutical excipients. London; Greyslake, IL; Washington, DC: Pharmaceutical Press ; American Pharmacists Association; 2006.

65. Dow cellulosics. ETHOCEL ethylcellulose polymers technical handbook [Internet];2005 [cited 2020 Dec 3]. Available from: https://www.industrialcellulosics.com/products/ethocel

66. Lewis RJ, Sax NI. Sax's dangerous properties of industrial materials. 2004.

67. Wasilewska K, Winnicka K. Ethylcellulose–A Pharmaceutical Excipient with Multidirectional Application in Drug Dosage Forms Development. Materials. 2019;12:3386.

68. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccoadhesive films of atenolol. Indian journal of pharmaceutical sciences. 2008;70(2):175-9.

69. Guo J-H, Cooklock KM. The Effects of Backing Materials and Multilayered Systems on the Characteristics of Bioadhesive Buccal Patches. Journal of Pharmacy and Pharmacology. 1996;48(3):255-7.

70. Sankar V, Johnson D, Sivanand V, Veerasamy R, Raghuraman S, Velrajan G, et al. Design and evaluation of nifedipine transdermal patches. Indian Journal of Pharmaceutical Sciences. 2003;65:510-5.

71. Mukherjee D, Bharath S. Design and Characterization of Double Layered Mucoadhesive System Containing Bisphosphonate Derivative. ISRN pharmaceutics. 2013;2013:604690.

72. Roh J, Han M, Kim K-N, Kim K-M. The *in vitro* and *in vivo* effects of a fast-dissolving mucoadhesive bi-layered strip as topical anesthetics. Dental Materials Journal. 2016;35:601-5.

73. Quinten T, De Beer T, Remon j, Vervaet C. Overview of injection molding as a manufacturing technique for pharmaceutical applications. Injection Molding: Process, Design, and Applications. 2011:1-42.

74. Mašková E, Kubová K, Raimi-Abraham BT, Vllasaliu D, Vohlídalová E, Turánek J, et al. Hypromellose – A traditional pharmaceutical excipient with modern applications in oral and oromucosal drug delivery. Journal of Controlled Release. 2020;324:695-727.

75. DuPont. METHOCEL™ E15 Premium LV. [Internet];2022 [cited 2022 April 8]. Available from: https://www.chempoint.com/en-emea/products/dupont/methocel-water-soluble-cellulose-ethers/methocel-water-soluble-cellulose-ethers/methocel-e15-premium-lv-1

76. Lam H, Zupančič O, Laffleur F, Bernkop-Schnürch A. Mucoadhesive properties of polyacrylates: Structure – Function relationship. International Journal of Adhesion and Adhesives. 2021;107:102857.

77. Di Giuseppe E. Analogue Materials in Experimental Tectonics. Reference Module in Earth Systems and Environmental Sciences: Elsevier; 2018.

78. Kriangkrai W, Puttipipatkhachorn S, Sriamornsak P, Pongjanyakul T, Sungthongjeen S. Magnesium Stearate as Anti-Tacking Agent in Acrylic Polymer Films Intended for Gas-Entrapped Floating Delivery System. Advanced Materials Research. 2012;506.

79. Taokaew S, Phonsee S, Woravut N, Pitaksuteepong T, Kriangkrai W. Characteristic Assessment of the Polymeric Films Used for Hair Gel Products in Thailand. Key Engineering Materials. 2019;819:98-103.

80. Wattanaphraya N, Manchun S, Taokaew S, Kriangkrai W. Development of Mucoadhesive Film-Forming Systems Containing Black Ginger Extract for Aphthous Ulcers. Key Engineering Materials. 2021;901:104-10.

81. Pan-on S, Rujivipat S, Ounaroon A, Kongkaew C, Tiyaboonchai W. Development, characterization and skin irritation of mangosteen peel extract solid dispersion containing clay facial mask. International Journal of Applied Pharmaceutics. 2018;10:202.

82. Pham DT, Saelim N, Tiyaboonchai W. Alpha mangostin loaded crosslinked silk fibroin-based nanoparticles for cancer chemotherapy. Colloids and surfaces B: Biointerfaces. 2019;181:705-13.

83. Khoirunnisa A, Joni IM, Panatarani C, Rochima E, Praseptiangga D. UV-screening, transparency and water barrier properties of semi refined iota carrageenan packaging film incorporated with ZnO nanoparticles2018. 030041 p.

84. Kadam, Y., Pochat-Bohatier, C., Sanchez, J., & El Ghzaoui, A. (2015), Modulating Viscoelastic Properties of Physically Crosslinked Self-Assembled Gelatine Hydrogels through Optimized Solvent Conditions. J Dispers Sci Technol, 36(9), 1349-1356. https://doi.org/10.1080/01932691.2014.984721

85. Leuenberger, B. H. (1991). Investigation of viscosity and gelation properties of different mammalian and fish gelatines. Food Hydrocoll, 5(4), 353-361. https://doi.org/10.1016/S0268-005X(09)80047-7

86. Peppas, N. A., Hilt, J. Z., Khademhosseini, A., & Langer, R. (2006). Hydrogels in Biology and Medicine: From Molecular Principles to Bionanotechnology. Adv Mater, 18(11), 1345-1360. https://doi.org/10.1002/adma.200501612

87. Ballesteros-Mártinez, L., Pérez-Cervera, C., & Andrade-Pizarro, R. (2020). Effect of glycerol and sorbitol concentrations on mechanical, optical, and barrier properties of sweet potato starch film. NFS Journal, 20, 1-9. https://doi.org/10.1016/j.nfs.2020.06.002

88. Hägerström H, Paulsson M, Edsman K. Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. European Journal of Pharmaceutical Sciences: Official journal of the European Federation for Pharmaceutical Sciences. 2000;9:301-9.

89. Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N., & Zaritzky, N. E. (2005). Mechanical and thermal properties of yam starch films. Food Hydrocoll, 19(1), 157-164. https://doi.org/10.1016/j.foodhyd.2004.05.002

90. Tiwari, D., Sause, R., Madan, P. L., & Goldman, D. (1999). Evaluation of polyoxyethylene homopolymers for buccal bioadhesive drug delivery device formulations. AAPS PharmSci, 1, 50-57. https://doi.org/10.1208/ps010313

91. Tzanova, M. M., Hagesaether, E., & Tho, I. (2021). Solid lipid nanoparticle-loaded mucoadhesive buccal films – Critical quality attributes and *in vitro* safety & efficacy. Int J Pharm, 592, 120100. https://doi.org/10.1016/j.ijpharm.2020.120100

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