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Original article

# Lidocaine loaded gelatin/gelatinized tapioca starch films for buccal delivery and the irritancy evaluation using chick chorioallantoic membrane

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ABSTRACT

The aim of this study was to confirm the feasibility of gelatin/gelatinized tapioca starch (α st) films for buccal delivery and to evaluate their irritancy. Lidocaine (LB) and lidocaine hydrochloride (LH) were used as model drugs and glycerin was used as the plasticizer. The scanning electron microscopy, atomic force electron microscopy, X-ray diffraction and thermogravimetric analysis results confirmed the compatibility of gelatin/α st/glycerin (Gαgly) films. Drug releases of LB- or LH-Gαgly films were evaluated. The drug release profiles of medicated films presented good patterns in both short time and 8 h drug release studies. The permeation study was examined through chick chorioallantoic membrane (CAM) by using modified Franz diffusion cells. Moreover, the irritancy study for buccal films was also examined by a hen's egg test on CAM model (HET-CAM). The results revealed that LB and LH could permeate through CAM, and these Gαgly films created no irritation on HET-CAM. This indicates that the LB- and LH-Gαgly films are possible to use as buccal films.



## 1. Introduction

Buccal drug delivery is one of interesting route to deliver drugs because it has a high total blood flow, can avoid gastrointestinal degradation and the first pass metabolism in the liver and intestines (Aungst, 2000; Harris and Robinson, 1992; Fonseca-Santos and Chorilli, 2018). In addition, it is easy to administer and remove (Senel et al., 2001). The buccal mucosa structure is similar to the skin and acts as an absorption barrier. The drugs can act either in the local area or absorb into systemic circulation. There are many dosage forms such as tablets, gels, ointments, patches and films which have been developed for buccal drug delivery (Peh and Wong, 1999; Kraisit et al., 2018).

Hydrophilic polymers are normally chosen to prepare the dissolving buccal films because the films can dissolve and deliver the drug after contact with liquid or saliva (Mahajan et al., 2011, Irfan et al., 2016). The polymers can be used alone or combined to gain a good film. There are many types of polymers used to make films, such as cellulose derivatives, pullulan, sodium alginate, methylmethacrylate copolymer, chitosan and gelatin (Nagar et al., 2011; Kadajji and Betageri, 2011). Gelatin is a natural polymer from skin or bones of animals. It has good properties such as biocompatibility, biodegradability, non-toxicity (Tao et al., 2018). Gelatin has been used in packaging, pharmaceutical, cosmetic, biomedical and food industries (Kumar et al., 2017). Gelatin has been prepared both as transdermal films (Jadhav et al., 2009) and edible films (Gómez-Estaca et al., 2009). In addition, gelatin can be blended with other polymers such as chitosan, polyvinyl pyrrolidone K30, sodium carboxymethylcellulose and polyvinyl alcohol (Shidhaye et al., 2008; Khairnar et al., 2009) as mucoadhesive patches. Starch is widely used in daily life as a food ingredient. Starch can be received from different by-products of harvesting. It is biodegradable and edible and has been used in many industries, for example, food, plastics, cosmetics and biomedical (Neelam

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et al., 2012). Sago starch can be applied as an edible film, an oral thin film or a controlled release film forming polymer (Bansal et al., 2017). Moreover, rice starch can be used as a film forming agent in mucoadhesive buccal films (Okonogi et al., 2014). Tapioca starch is also applied as a carrier for solid dispersion, suspending agent, matrix forming agent, film coating agent and carrier for mucoadhesive microspheres (Charoenthai et al., 2018). Starch-gelatin blend films have also been studied (Wannaphatchaiyong et al., 2019). These blended polymers might give advantages such as oxygen and water barrier properties, mechanical property and optical parameters (Acosta et al., 2015). In our previous study, a biopolymer blending gelatin and pregelatinized tapioca starch (alpha starch®; α st) was studied, and the effects of three water soluble plasticizers, i.e. polyethylene glycol 400 (PEG 400), propylene glycol (PG) or glycerin to improve flexibility of the film were observed. Glycerin at 25 parts per hundred of gelatin (phg) was chosen to mix with gelatin/α st (Wannaphatchaiyong et al., 2017). In this study, lidocaine base (LB) or its hydrochloride salts (LH) loaded gelatin/α st/glycerin (Gαgly) was further evaluated for use as an anesthetic film. The atomic force microscope (AFM), scanning electron microscope (SEM), thermogravimetric analysis (TGA) and X-ray diffraction (XRD) were further determined for their physical characteristics, thermal stability and decomposition, and compatibility of Gαgly films. For application to buccal mucosa, the anesthetic films should be non-toxic and non-irritant to the buccal membrane (Karki et al., 2016). Moreover, the films should release and permeate the drug to relieve pain. For this reason, the *in vitro* drug release study, *ex vivo* permeation study and irritancy evaluation were studied. Normally, the drug permeability via buccal tissue can be observed in animal buccal tissue such as rabbit (Dowty et al., 1992), hamster (Tsutsumi et al., 1999), dog (Zhang et al., 1994) and pig (Artusi et al., 2003; Marxen et al., 2018). The porcine buccal mucosa is reported as the nearest to human tissue; however, the cheek surface is too small and easily damaged during *ex vivo* membrane preparation. Moreover, it is difficult to acquire the fresh pig tissue from the farm at the right time for experiments. Therefore, chick chorioallantoic membrane (CAM) is used as an alternative membrane of porcine buccal mucosa and is easy to collect and prepare for use (Tay et al., 2011). In addition, CAM structure is quite similar to human buccal membrane, but has no mucus layer. CAM can be kept at −20 °C up to 14 days for permeation study with no changes in permeation properties (Tay et al., 2011). Furthermore, hen's egg test-CAM (HET-CAM) can also be provided to evaluate the irritancy of buccal films (Tay et al., 2012; Kaewbanjong et al., 2017).

Therefore, the aim of this study was to confirm the physicochemical properties, *in vitro* drug release and *ex vivo* permeation of Gαgly films for buccal delivery of both LB and LH, and the irritancy evaluation using HET-CAM.

## 2. Materials and methods

### 2.1. Materials

Gelatin (160 bloom) was bought from PB Gelatins (Tessenderlo, Belgium). The gelatinized tapioca starch (α st) was kindly gifted from Thaiwah (Bangkok, Thailand). Glycerin was purchased from Sigma-Aldrich (Munich, Germany). LB and LH were gained from Sigma-Aldrich (Shanghai, China). Methanol, ethanol (RCI Labscan Asia, Bangkok, Thailand), sodium hydroxide (Loba Chemie, Mumbai, India), sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride (Merck, Darmstadt, Germany) were used as supplied. Distilled water was used throughout the experiments. All other solvents and chemicals were pharmaceutical or analytical grade and used without further modification. The specific pathogen-free (SPF) chicken eggs of White Leghorn were collected from the Animal and Plant Health Center, Agri-Food and Veterinary Authority of Singapore for *ex vivo* permeation and irritancy studies.

### 2.2. Preparation of blank and medicated LB- or LH-Gαgly films

The gelatin solution was prepared by dissolving gelatin powder in distilled water and heating at 45 °C until homogenous, cooling to room temperature and adjusting to 15% w/w. For the gelatin/glycerin (Ggly) film, glycerin at 25 phg was mixed in the gelatin solution. For the Gαgly film, the 5% w/w of α st solution was produced by dispersing and stirring slowly in distilled water. The starch solution was mixed with gelatin solution at the final concentration of 5 phg, and then 25 phg glycerin was added into the gelatin/α st solution. Either the Ggly or Gαgly mixture controlled between 16.5 and 18.5 g was poured into a 9.64 ± 0.10 cm in diameter petri dish lined with the aluminium foil and dried at 50 °C for 24 h. The dried weight of components in the mixture was first calculated to get a final dried film of 3 g/73 $cm^2$. The bottom of the petri dish was supported by aluminium foil in order to protect against the adherence of the film to the glass surface. The foil was easily peeled off from the dried film, resulting in a good dried film with no deformation caused by peeling. Both blank Ggly and Gαgly films were built, and both were used to evaluate their properties compared with medicated films.

For the medicated films, LB and LH were dissolved in (1:1) methanol:water mixture and distilled water to get 4% w/v of drug solution, respectively. LB or LH solution was slowly added into the Gαgly solution which was prepared as previously described. After that, the mixtures were stirred to get a homogenous solution, poured into the petri dish with the aluminium foil and dried at 50 °C for 24 h. The amount of either LB or LH loading was calculated in advance so that there was a final concentration of 5% drug in dry basis. The amount of mixture was controlled between 24.5 and 25.0 g for pouring into the petri dish and the final dried film still were controlled in the same amount at 3 g/73 $cm^2$.

All dried films were peeled off from the petri dish, the aluminium foil was then peeled off, and these films were stored in desiccators at room temperature before further evaluation.

### 2.3. Characterization of films

In the previous study, the thickness, weight uniformity, swelling and erosion, ultimate tensile strength, elongation at break, Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC) of Gαgly films were reported (Wannaphatchaiyong et al., 2017). In this study, the medicated Gαgly films were further evaluated for their morphology and thermal stability by using AFM, SEM and TGA. Moreover, the crystallinity of films was determined by XRD. These could confirm the compatibility of drug in Gαgly films.

The morphology of films was observed by using AFM (model nanosurf easyscan2, Switzerland). The non-contact static mode with the silicon probes, the resonance frequency of 160–225 KHz and a force constant of 36–90 N/m was used. The AFM results were calculated for the roughness of films by using easyscan2 control software and Gwyddion as a free program (GNU General Public License). The top surface, the bottom surface and cross section of films were also investigated by using SEM (model FEI: SEM-Quanta 400, USA.).

The thermal stability of films was evaluated by TGA (TGA 7, Perkin Elmer, USA.). The test was done under nitrogen atmosphere with a flow rate of 20 ml/min; the mass of samples ranged from 8 to 16 mg, and the temperature interval of 50–1000 °C at a heating rate of 10 °C/min. A function of temperature and weight loss was determined.

The XRD (Empyrean, PANalytical, the Netherlands) was used to study the compounds in materials and films. The parameters of the XRD study were 40 kV, 35 mA, scan range (2θ) of 5–90°, step size (2θ) of 0.026° and time/step of 70.125 sec.

### 2.4. Extraction of medicated films

In preliminary extraction, LH or LB was extracted from Gαgly films by using different solvents including methanol, methanol: water (1:1), isotonic phosphate buffer solution (PBS) pH 7.4 and water. The 1 cm × 2 cm medicated film was cut into small pieces and 10 ml of each solvent was added. The films were sonicated for 15 min and rested for 24 h. They were then sonicated for 1 h before being diluted with PBS. The suitable concentrations were analyzed by high performance liquid chromatography (HPLC) with Themo scientific BDS HYPERSIL C18 column. The HPLC conditions were as follows: the mobile phase was 50 mM ammonium acetate with 1% v/v acetic acid : methanol (60:40% v/v) and triethylamine was added as 0.1% v/v of the total volume, the injection volume was 50 μl, the flow rate was 0.8 ml/min, and UV detector wavelength was 254 nm. The drug content (%w/w) was calculated as Eq. (1) by using the ratio between drug extraction ($D_{analyze}$) and the accurate weight of film ($W_{accurate}$), and the percentage of drug entrapment efficiency (% Drug EE) was calculated by comparing between the drug extraction ($D_{analyze}$) and the theoretical drug loading ($D_{theory}$) as the Eq. (2).

$$\%\text{Drug content} = (D_{analyze}/W_{accurate}) \times 100 \quad (1)$$

$$\%\text{Drug EE} = (D_{analyze}/D_{theory}) \times 100 \quad (2)$$

### 2.5. Preparation of CAM

All SPF chicken eggs were wiped with povidone iodine and disinfectant (70% v/v ethanol) before being placed blunt end upwards into an egg incubator with an automatic rotator (Octagon® 20, North Somerset, UK) at 37 °C and 60% humidity. After 7 days, the embryo age (EA7) egg was punctured at the blunt end. Then, the egg shell and the internal shell membranes were removed in a sterile environment by the cleansphere CA 100 (Safetech Limited, USA.) to reveal the CAM. The egg was covered with parafilm and returned to the incubator without rotation. On day 15, the EA15 CAM was collected by cutting along the length of egg and pouring out of content. The CAM was washed until clean with normal saline, stored at −20 °C, and used within 14 days.

### 2.6. In vitro drug release and ex vivo permeation studies

*In vitro* drug release and *ex vivo* permeation studies of medicated Gαgly films were determined by using modified Franz diffusion cells (Hansen Research, Chatsworth, CA, USA.). The 11 ml receptor compartment had a controlled temperature at 37 ± 0.5 °C and was filled with PBS pH 7.4 and stirred with a magnetic stirrer for 200 rounds per min (rpm). The testing area between donor and receptor compartments was 1.87 ± 0.19 $cm^2$. In this testing area, the film containing 0.96 ± 0.10 mg of drugs was fitted in both drug release and permeation studies. In drug release, 2 patterns of study were observed, i.e. the short time release without barrier and the 8 h release with barrier. For the short time drug release study, the films were placed on the receptor compartment directly, and the aliquots of 1 ml sample were kept at 1, 2, 3, 5, 7, 11 and 15 min. For the 8 h drug release study, the films were put on the donor compartment which was divided from the receptor compartment by a dialysis membrane (Cellu Sep T4, USA). The molecular weight cut off and thickness of dialysis membrane were 12,000–14,000 and 20 μm, respectively. For the permeation study, the dialysis membrane was replaced with CAM, and the filter paper (Whatman No.1) was also used to support the CAM in the hole between donor and receptor compartments. In both 8 h drug release and permeation study, aliquots of 1 ml in receptor fluid were collected at 5, 10, 15, 30, 45 min and 1, 2, 3, 4, 6 and 8 h. After that, the equivalent aliquoted volumes of PBS were replaced in the receptor fluid. Each sample was evaluated for the drug concentration by HPLC at 254 nm. *In vitro* short time and 8 h drug release were done in triplicate, and *ex vivo* permeation was studied in quadruplicate. All of resulted studies were further analyzed into zero order, first order and Higuchi's kinetics (Habib et al., 2010; Rana and Murthy, 2013).

### 2.7. Stability study of medicated Gαgly films

The medicated Gαgly films were kept for 3 months at 4 ± 1 °C, ambient temperature (≈28 ± 4 °C), and 45 ± 1 °C to determine their stability. They were examined for changes in appearance, drug content and drug release. For permeation of stored films, they were studied only after being stored at room temperature. All the tests were done as previously described.

### 2.8. Irritancy evaluation using HET-CAM

The SPF chicken eggs were hatched and the shells opened at EA7 as previously described. HET-CAM was used on EA10 to study the irritation potential of the formulations. The medicated Gαgly film was applied on the CAM surface of the opened egg. The films were cut into 1.5 cm × 1.5 cm so the area of film could cover almost all of the CAM surface. The positive control and negative control were 0.1 M sodium hydroxide solutions and 0.9% sodium chloride solutions, respectively. Then, 3 ml of each solution was dropped onto the CAM. The irritation test started after placing the sample and irrigating 20 sec with warm water (37 °C). After application, the blood vessels were evaluated and scored for irritant effects at 0.5, 2 and 5 min. The pictures of HET-CAM were taken by a digital camera microscope (Olympus DP 71, Japan) and zoom stereo microscope (Olympus SZ 61, Japan). Hyperemia, hemorrhage and clotting of blood vessels were observed and the cumulative irritancy score was interpreted in terms of irritation potential as shown in Table 1 (Luepke, 1985; ICCVAM, 2010; Kaewbanjong et al., 2017). These scores were evaluated by five referees. Irritancy testing was done in triplicate.

### 2.9. Statistical analysis

The results of roughness, drug EE, drug release, drug permeation and kinetics of drug release and permeation were expressed as mean ± S.D. Comparisons between groups were performed by using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests with SPSS statistical software. Values of $^{a}$, $^{b}$, * and ** ($p < 0.05$) were considered significant.

## 3. Results and discussion

### 3.1. Preparation of films

Several plasticizers have been studied to blend in the films for improving the mechanical properties of glassy films. However, limitations to mixing the plasticizers into films may occur because some plasticizers are hygroscopic causing the films to become over-hydrating and decreasing the adhesive strength (Kaur et al., 2014). Therefore, the appropriated types and amounts of plasticizer should first be evaluated. A previous study reported the effect of plasticizers including PEG 400, PG and glycerin in gelatin/α st

**Table 1**
Score of irritancy testing and the interpretation as cumulative score for severity of irritation potential (Luepke, 1985; ICCVAM, 2010; Kaewbanjong et al., 2017).

| Irritation effect | Time and score | | | Interpretation | |
|---|---|---|---|---|---|
| | ≤0.5 | 0.5–2 | 2–5 | Cumulative score | Irritation potential |
| | min | min | min | <1.0 | Negligible |
| Hyperemia | 5 | 3 | 1 | 1.0–4.9 | Slight |
| Hemorrhage | 7 | 5 | 3 | 5.0–8.9 | Moderate |
| Clotting/coagulation | 9 | 7 | 5 | 9.0–21.0 | Strong |

(Gα) films (Wannaphatchaiyong et al., 2017). Among them, the glycerin blended Gα films showed transparency and other good properties. The 25 phg of glycerin was suitable to make the Gα films. It showed lower ultimate tensile strength and higher elongation at break than PG and PEG 400 plasticized Gα films. Moreover, the over-hydrated films were not found during storage. Therefore, the Gαgly films were further studied, and then, either LB or LH was chosen to study and prepare as the medicated Gαgly buccal films because both drugs have different properties which might present the different properties of Gαgly buccal films.

Similar to the previous study, the blank Ggly and Gαgly films, and the medicated LB- or LH-Gαgly films could be prepared with good visualization and high reproducibility. The transparent thin films were prepared with good physical and mechanical properties as described previously (Wannaphatchaiyong et al., 2017).

### 3.2. Physicochemical properties of films

The AFM images of blank Ggly and Gαgly films, and medicated Gαgly films are shown in Fig. 1. The images revealed that both upper and lower surfaces of films were slightly rough. The AFM data were calculated for their surface roughness as shown in Fig. 2. LH-Gαgly presented the lowest roughness in both lower and upper sides of film. In the upper side, the roughnesses of each film were significantly different (noted as ** in Fig. 2). While the roughnesses of lower side of each film were also significantly different (noted as * in Fig. 2), except only the lower side of LB-Gαgly film was not significantly different with Gαgly film. The comparison between the medicated films showed that the upper and lower surfaces of LH-Gαgly were significantly smoother than LB-Gαgly. The completed dissolving of starch or drug and the drying rate might result in roughness of upper side films. The peeling might also have an effect on the roughness of the lower side films. However, this roughness of films was too minimal to be observed by naked visualization. The SEM images of medicated Gαgly films are presented in Table 2. The upper sides of LB- and LH-Gαgly films were quite smooth. On the other hand, some roughness was found on the lower sides of LB- and LH-Gαgly films. This might be due to the peeling of the aluminium foil support from the films after the drying process. The cross-section images at 250X and 2000X presented that both LB- and LH-Gαgly films did not contain any particles inside the films. This indicated a good blend of all components in the medicated Gαgly films.

The thermal stability of the films observed by TGA is shown in Fig. 3. Most TGA thermograms of materials and medicated Gαgly films revealed two thermal events. The first decrease of weight occurred immediately after the temperature increase and ended at about 300 °C. This event occurred from evaporation or dehydration of remaining water or other low molecular weight compounds in the sample (Liu et al., 2009). The initial peak of gelatin also presented around 100–300 °C which was the degradation of gelatin chain, and the second peak at 300–600 °C referred to the breaking of peptide bonds from amino acids which indicated a more thermally stable structure (Hoque et al., 2011; Mu et al., 2012). For the medicated Gαgly films, they exhibited the curve between 50 and 250 °C which might be the degradation of water, glycerin, starch, gelatin or drug. The second curve was 250–500 °C which was attributed to the decomposition of polymers of films (Rodríguez-Castellanos et al., 2015). The LB and LH showed different decomposed temperatures, but they did not affect the TGA thermograms of their medicated Gαgly films. The TGA thermograms of medicated Gαgly films had shifted to a lower temperature from the gelatin curve. This might be because glycerin could adsorb the moisture (water) which affected the protein-protein interaction and decreased the stability of the gelatin system (Chuaynukul, et al., 2014). However, the trend of TGA curves was similar to the original gelatin curve. This could imply that gelatin was the main component in the films without any significant change. Therefore, these TGA results could also confirm the compatibility of all components in the medicated Gαgly films.

The XRD patterns of films are presented in Fig. 4 and can support the FT-IR and DSC results in the previous study (Wannaphatchaiyong et al., 2017). As in the former study, Gαgly film and medicated Gαgly films showed no new peak in FT-IR and the amorphous form of drug in DSC. In the results of this study, the XRD diffractogram of blank Gαgly films exhibited 2 broad peaks. Normally, the granular structure of starch can appear as a crystalline form in which the amylose of starch is still in granules and can form a complex structure (Nakorn et al., 2009). In this study, however, the pregelatinized starch is a soluble component that was dissolved completely before a film was formed. Therefore, the peak of α st in Gαgly film showed as a broad diffractogram which indicated the non-crystalline form of all components after the dried film was formed. These 2 broad peaks were also found in both blank Gαgly, and medicated LB-Gαgly and LH-Gαgly films. However, the crystalline patterns in medicated Gαgly films were observed, especially in LB-Gαgly films. LB has a low solubility in water, therefore, it might precipitate in the LB-Gαgly after drying and show 2 sharp crystalline characters in XRD diffractogram. Since LH has good solubility in water and might completely blend with the other soluble components such as gelatin and α st, a very slight crystalline form was observed in the XRD diffractogram. However, the crystalline peaks of both drugs were changeable from the raw drugs. These indicated the different crystalline forms of the drug after re-crystallization in the dried films. Moreover, this crystalline character of drug in Gαgly films was not observed by SEM technique as described previously. This indicated that the very small amount of crystalline drug remained in the medicated films. Although, there were some crystalline peaks in the medicated films, they might not affect the drug release behavior which had been already reported (Preis et al., 2014).

### 3.3. Drug extraction

The 5% w/w of the theoretical drug was loaded in each film during the preparation process. The results of drug extraction are presented in Table 3. The best solvents for LB and LH extractions from medicated Gαgly films were PBS pH 7.4 and water, respectively. For LB, PBS might be mixed with water and salts which the gelatin in film was swelled and dissolved, after that LB could be dissolved

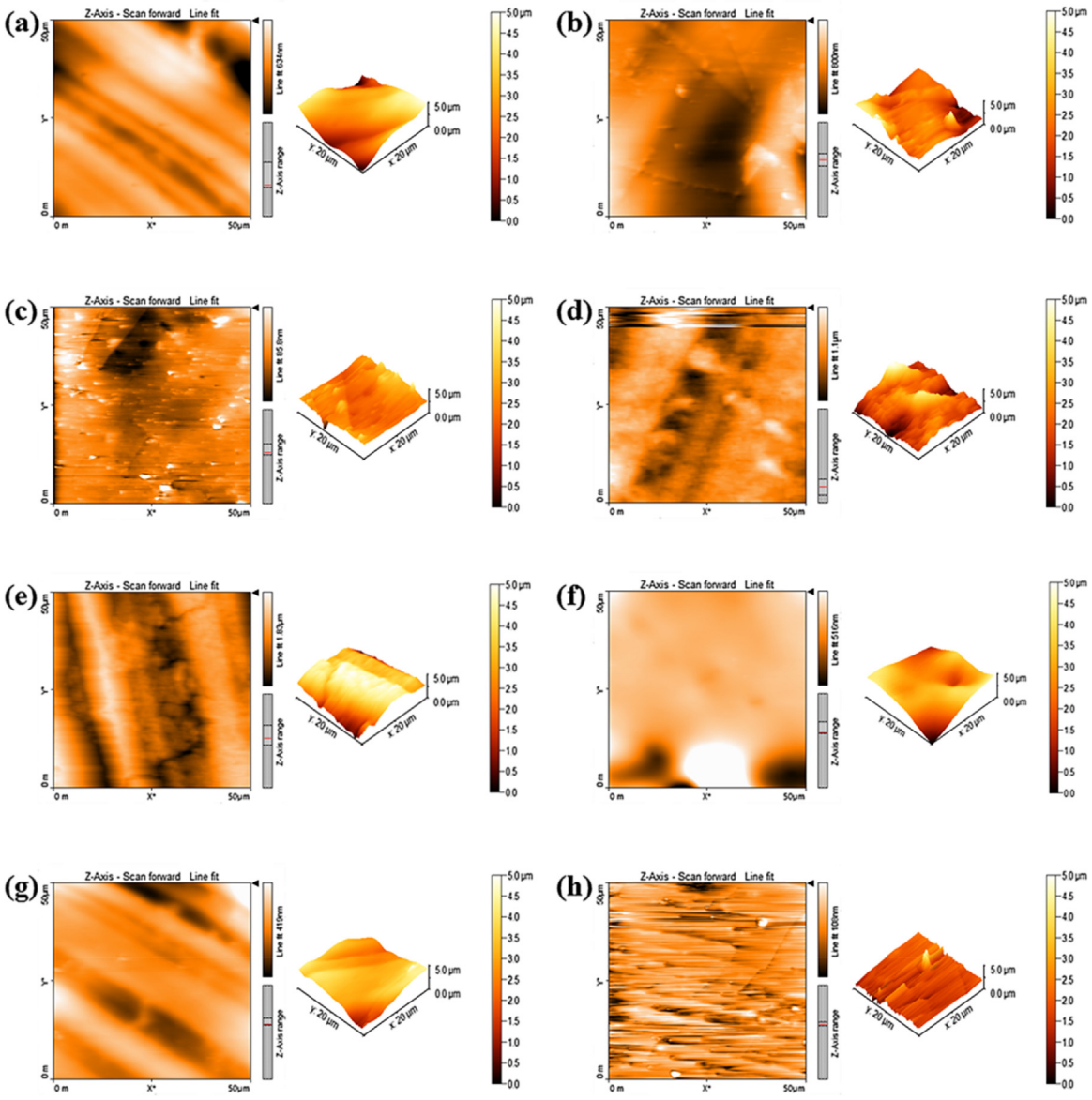



**Fig. 1.** The lower (a, c, e, g) and upper (b, d, f, h) AFM images of (a, b) Ggly films, (c, d) Gαgly films, (e, f) LB-Gαgly films and (g, h) LH-Gαgly films.

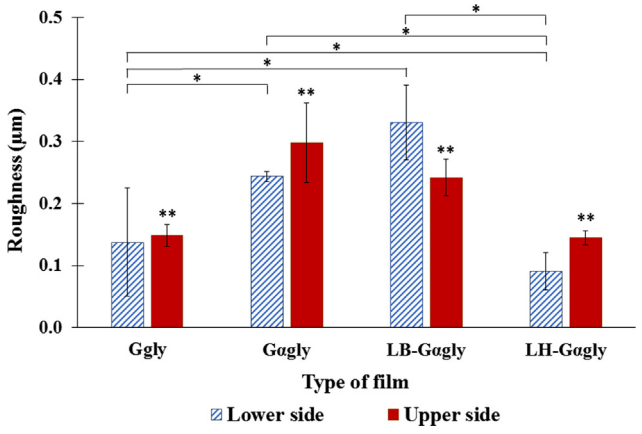


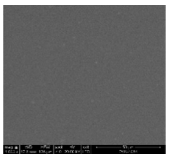
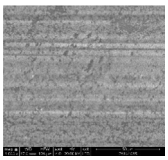
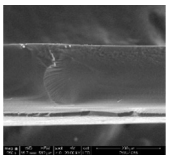
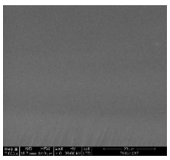
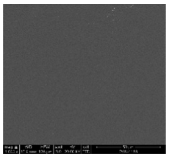
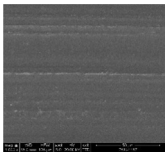
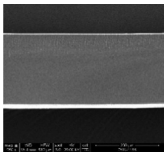
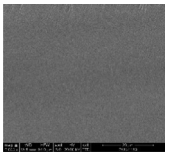

**Fig. 2.** The roughness values of the films calculated from AFM. * meant the significant difference ($p < 0.05$). ** meant each type of upper side film was significant difference ($p < 0.05$).

(Østergaard et al., 2011) and extracted from the film higher than methanol:water, methanol and water, respectively. For LH, water could better extract the drug than PBS pH 7.4, methanol and methanol:water, respectively. The water could be adsorbed into the film and it could also dissolve LH from the film. The percentages of drug EE in different solvents of LB-Gαgly and LH-Gαgly were 80–95% and 89–98%, respectively. However, extraction values lower than 100% might be due to the entrapment of partial drug molecules in the structure of either gelatin or starch that could not be completely extracted by the solvents. These implied that the medicated Gαgly films prepared by casting method could preserve drugs in the film without any loss. After that, PBS pH 7.4 and water were used as solvent for determination of the drug EE in LB and LH loaded Gαgly films in the stability test, respectively.

### 3.4. Stability study of medicated LB or LH Gαgly films

The characteristic and color of medicated Gαgly films after the stability study at 1 and 3 months were quite similar to the initial preparations. The percentages of drug content were calculated

**Table 2**
SEM images of upper and lower medicated Gαgly films at 1000X and cross section at 250X and 2000X.

| Samples | Upper (1000X) | Lower (1000X) | Cross section | |
|---|---|---|---|---|
| | | | (250X) | (2000X) |
| LB-Gαgly |  |  |  |  |
| LH-Gαgly |  |  |  |  |

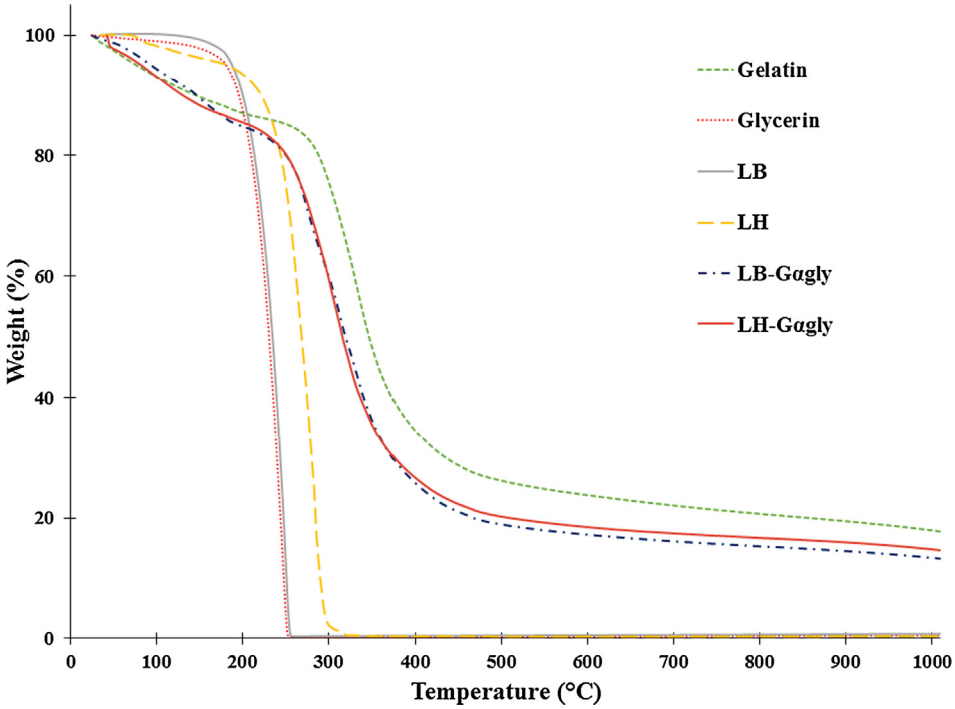



**Fig. 3.** TGA thermograms of materials and films.

after stability study at different temperatures (4 °C, ambient temperature and 45 °C) for 3 months (Fig. 5). The percentages of drug content of LB in Gαgly films were above 90% when stored at 4 °C for 3 months, and at room temperature and 45 °C for 2 months. In LH-Gαgly films, the percentages of LH content retained above 90% when stored at 4 °C and room temperature for 3 months, and at 45 °C for 2 months. The medicated Gαgly films were suitable for storage at 4 °C because the decrease of drugs was the lowest, indicating the highest stability of drug content in the films. The chemical instability of the drug was found after being kept at room temperature and 45 °C. The degradation of LB and LH would be explained with drug release and permeation study.

### 3.5. Drug release and permeation study

Normally, buccal films aim to deliver drug unidirectionally into the buccal surface. In this study, the medicated Gαgly films with no backing layer were tested in both drug release and permeation studies. In fact, the film with no backing layer could release the drug in multiple directions when used in the oral cavity. Only drug release in buccal side was determined as buccal delivery. The drug released in the other side was swallowed into the gastrointestinal tract. Therefore, in this study, the unidirectional drug release and permeation was observed by using Franz diffusion cells.

The drug release and permeation profiles of LB- and LH-Gαgly films are presented in Fig. 6. In the initial prepared films, both short time and 8 h drug release profiles of LH film were slightly higher than those of LB film. This might be due to the better solubility of LH in aqueous medium than in LB (Gröningsson et al., 1985); thus, LH could dissolve and release from the films easier than LB. LH blended in Gαgly films also increased the hydrophilicity of the film. Moreover, the drug permeation from LH-Gαgly film was also slightly higher than that of LB-Gαgly films, owing to the higher drug content and release from the Gαgly films, and a higher concentration gradient resulting in higher drug permeation. In fact, both LH and LB drugs could change their forms to the same lidocaine form when in PBS pH 7.4. Therefore, the permeability of both LB and LH in the same medium should be the same. Slight

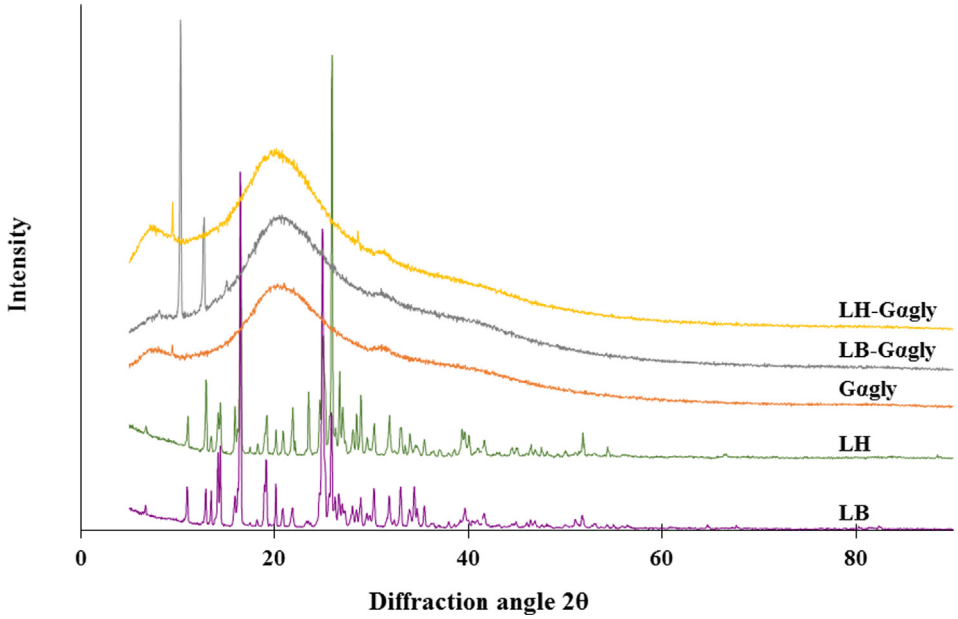



**Fig. 4.** XRD diffractograms of blank film and medicated films.

**Table 3**
The percentages of drug content and drug EE of medicated films extracted with different solvents (n = 5).

| Solvent | LB-Gαgly | | LH-Gαgly | |
|---|---|---|---|---|
| | % Drug content (mean ± S.D.) | % Drug EE (mean ± S.D.) | % Drug content (mean ± S.D.) | % Drug EE (mean ± S.D.) |
| Methanol | 4.039 ± 0.390 | 80.789 ± 7.795 | 4.606 ± 0.541 | 92.125 ± 10.824 |
| Methanol:Water (1:1) | 4.209 ± 0.175 | 84.183 ± 3.504 | 4.455 ± 0.730 | 89.109 ± 14.594 |
| PBS pH 7.4 | 4.768 ± 0.643 | 95.370 ± 12.854 | 4.662 ± 0.535 | 93.238 ± 10.708 |
| Water | 3.984 ± 0.347 | 79.676 ± 6.948 | 4.904 ± 0.301 | 98.098 ± 6.207 |

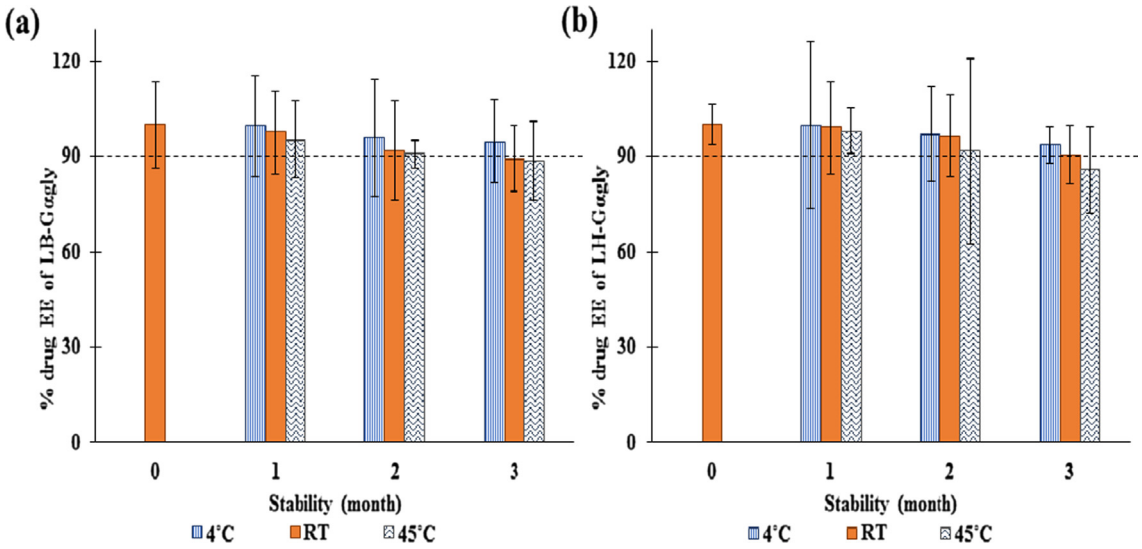



**Fig. 5.** The percentages of drug EE of (a) LB-Gαgly and (b) LH-Gαgly films (n = 5).

differences of permeation results should be displayed from different concentrations of drug release. After the films were stored at various temperatures, the drug release from films exposed to 45 °C for 3 months was the lowest than those exposed to 4 °C and room temperature for both LB- and LH-Gαgly films. Moreover, drug loaded Gαgly films at 4 °C presented the least change of drug release when compared to others. For the drug permeation profiles of films stored at room temperature, the longer the period of storage, the lower amount the permeation of drug. However, there was no significantly different permeation in LB-Gαgly films. Even though LB and LH are resistant to temperature and acid or base in aqueous solutions, the films were dried in an oven at 50 °C for 24 h to reduce the moisture content so the hydrolysis could occur in LB (Repka et al., 2005). A slight change of $^{1}$H NMR spectrum in

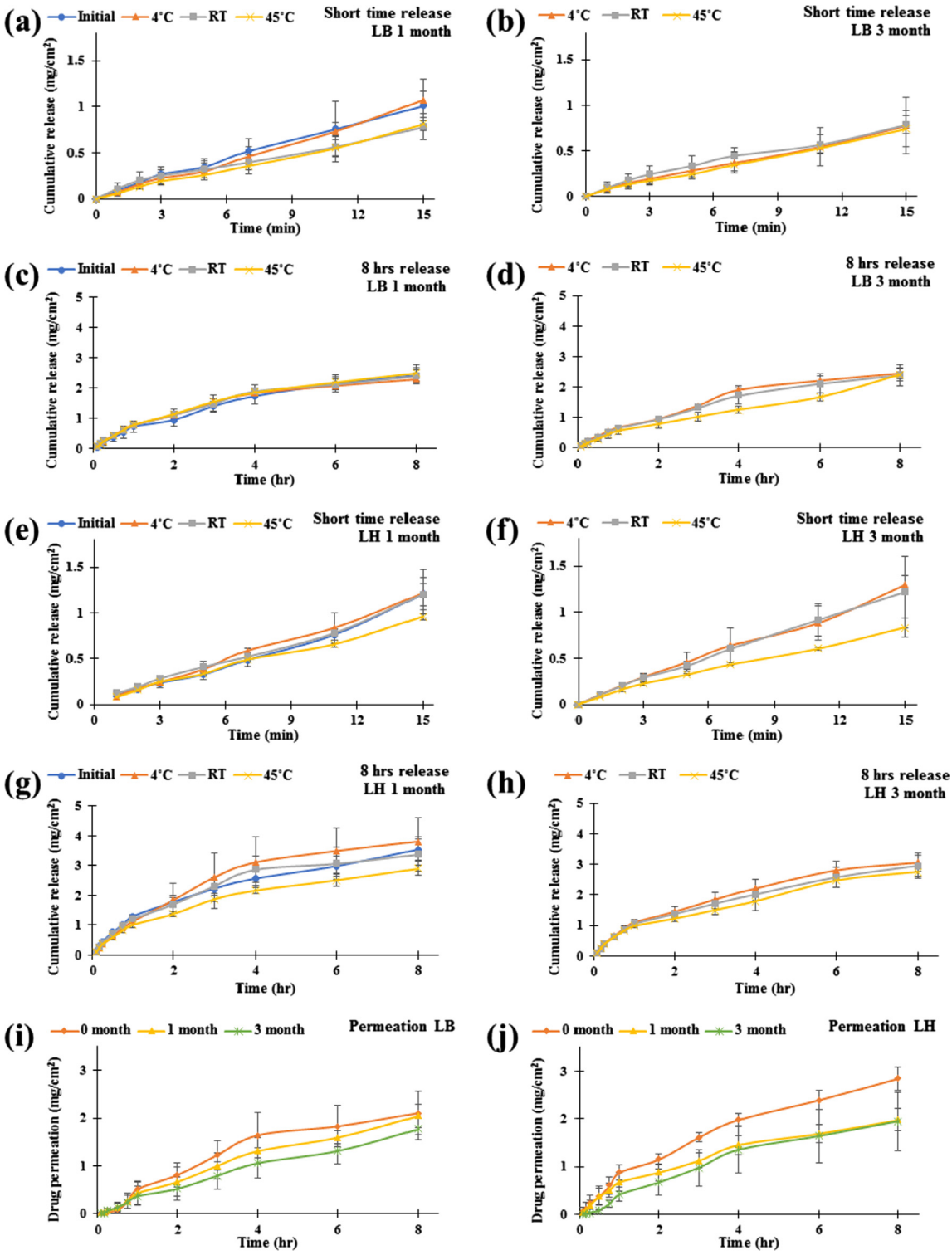



**Fig. 6.** The short time (n = 3) (a, b, e, f) and 8 hrs drug release profiles (n = 3) (c, d, g, h) of (a, c) LB-Gαgly 1 month, (b, d) LB-Gαgly 3 month, (e, g) LH- Gαgly 1 month, (f, h) LH-Gαgly 3 month and the permeation profiles (n = 4) (i-j) of (i) LB-Gαgly and (j) LH-Gαgly.

LH was also observed in oxidation reaction study at room temperature (Kadioglu et al., 2013). Thus, these reasons might affect the stability, drug release and permeation study.

### 3.6. Kinetics

The kinetics of drug release profiles (Table 4) in short time and 8 h drug release were different. For short time drug release, the releases from most of films fitted well to zero order kinetics. This could demonstrate that drug release was independent from concentration in the first 15 min, or the drug could be dissolved, partitioned and diffused from film (Bruschi, 2015). In this short time drug release study, the medicated Gαgly film was directly contacted to the receptor medium, and then, quick dissolving of whole films occurred. However, the drug release from LH-Gαgly films after 3 months fitted to the first order equation because the

**Table 4**
The kinetics of drug release (n = 3) in LB-Gαgly and LH-Gαgly films.

| Sample | Month | Temperature (°C) | Kinetics of short time drug release; $R^2$ (mean ± S.D.) | | | Kinetics of 8 hrs drug release; $R^2$ (mean ± S.D.) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Zero order | Higuchi's | First order | Zero order | Higuchi's | First order |
| LB-Gαgly | 0 | RT | 0.9902 ± 0.0010[a] | 0.9262 ± 0.0201[b] | 0.9675 ± 0.0146[a] | 0.9620 ± 0.0135 | 0.9782 ± 0.0128 | 0.9822 ± 0.0052 |
| | 1 | 4 | 0.9783 ± 0.0098 | 0.8979 ± 0.0669 | 0.9515 ± 0.0533 | 0.9263 ± 0.0258[a] | 0.9848 ± 0.0077[b] | 0.9929 ± 0.0062[b] |
| | | RT | 0.9500 ± 0.0339 | 0.9592 ± 0.0570 | 0.9705 ± 0.0260 | 0.9341 ± 0.0189[a] | 0.9818 ± 0.0061[b] | 0.9866 ± 0.0104[b] |
| | | 45 | 0.9879 ± 0.0100[a] | 0.9245 ± 0.0204[b] | 0.9737 ± 0.0223[a] | 0.9692 ± 0.0092 | 0.9805 ± 0.0085 | 0.9682 ± 0.0202 |
| | 3 | 4 | 0.9609 ± 0.0183 | 0.9413 ± 0.0428 | 0.9698 ± 0.0271 | 0.9678 ± 0.0066 | 0.9710 ± 0.0053 | 0.9720 ± 0.0052 |
| | | RT | 0.9301 ± 0.0596 | 0.9447 ± 0.0479 | 0.9626 ± 0.0303 | 0.9676 ± 0.0189 | 0.9775 ± 0.0112 | 0.9753 ± 0.0190 |
| | | 45 | 0.9614 ± 0.0285 | 0.9070 ± 0.0889 | 0.9554 ± 0.04821 | 0.9554 ± 0.0333 | 0.9722 ± 0.0330 | 0.9790 ± 0.0172 |
| LH-Gαgly | 0 | RT | 0.9576 ± 0.0241 | 0.8995 ± 0.1120 | 0.9588 ± 0.0630 | 0.9097 ± 0.0191[a] | 0.9907 ± 0.0031[b] | 0.9914 ± 0.0070[b] |
| | 1 | 4 | 0.9721 ± 0.0283 | 0.9105 ± 0.0588 | 0.9735 ± 0.0118 | 0.9382 ± 0.0126[a] | 0.9784 ± 0.0134[b] | 0.9880 ± 0.0003[b] |
| | | RT | 0.9705 ± 0.0288 | 0.9400 ± 0.0572 | 0.9774 ± 0.0166 | 0.9133 ± 0.0299[a] | 0.9803 ± 0.0063[b] | 0.9718 ± 0.0111[b] |
| | | 45 | 0.9788 ± 0.0171[a] | 0.9396 ± 0.0191[a, b] | 0.9852 ± 0.0121[b] | 0.9173 ± 0.0090[a] | 0.9866 ± 0.0092[b] | 0.9882 ± 0.0048[b] |
| | 3 | 4 | 0.9869 ± 0.0057[a] | 0.9392 ± 0.0169[b] | 0.9900 ± 0.0057[a] | 0.9358 ± 0.0339 | 0.9870 ± 0.0068 | 0.9597 ± 0.0410 |
| | | RT | 0.9788 ± 0.0242 | 0.9143 ± 0.0655 | 0.9605 ± 0.0314 | 0.9325 ± 0.0141[a] | 0.9926 ± 0.0004[b] | 0.9801 ± 0.0103[b] |
| | | 45 | 0.9841 ± 0.0110[a] | 0.9537 ± 0.0168[b] | 0.9894 ± 0.0060[a] | 0.9356 ± 0.0182[a] | 0.9831 ± 0.0074[b] | 0.9413 ± 0.0208[a] |

Each datum represents the mean ± S.D.
**a** and **b** in the same row meant the symbol of significant statistics. The different symbols meant the significant difference ($p < 0.05$).

condensed films might occur after storage and thereafter retard the film's dissolving. However, most short time drug release kinetics in medicated Gαgly films were not statistically different ($p > 0.05$). For 8 h drug release, most LB and LH Gαgly films fitted to first order kinetics; however, after 3 months, LH-Gαgly fitted to Higuchi's for all storage conditions. Most 8 h drug release kinetics were not statistically different from Higuchi's and from first order kinetics ($p > 0.05$). Fitting well to the first order or Higuchi's kinetics indicated that the drug release depended on concentration or diffusion taking place in the matrix (Bansal et al., 2013; Ramteke et al., 2014), respectively. For 8 h drug release study, the medicated Gαgly film and the receptor medium was separated by a dialysis membrane (MW cut-off 12000-14000), and then, the whole films could not be dissolved into the lower compartment. The drug release should occur by diffusion and some dissolution of matrix films. Moreover, the condensed films which occurred after storage

**Table 5**
The kinetics of drug permeation (n = 4) in LB-Gαgly and LH-Gαgly films.

| Sample | Month | Temperature (°C) | Kinetics of short time drug release; $R^2$ (mean ± S.D.) | | |
|---|---|---|---|---|---|
| | | | Zero order | Higuchi's | First order |
| LB-Gαgly | 0 | RT | 0.9262 ± 0.0332 | 0.9528 ± 0.0217 | 0.9763 ± 0.0245 |
| | 1 | 4 | N.D. | N.D. | N.D. |
| | | RT | 0.9683 ± 0.0234 | 0.9549 ± 0.0322 | 0.9697 ± 0.0290 |
| | | 45 | N.D. | N.D. | N.D. |
| | 3 | 4 | N.D. | N.D. | N.D. |
| | | RT | 0.9700 ± 0.0272 | 0.9500 ± 0.0382 | 0.9828 ± 0.0172 |
| | | 45 | N.D. | N.D. | N.D. |
| LH-Gαgly | 0 | RT | 0.9517 ± 0.0134[a] | 0.9830 ± 0.0110[b] | 0.9926 ± 0.0039[b] |
| | 1 | 4 | N.D. | N.D. | N.D. |
| | | RT | 0.9233 ± 0.0402[a] | 0.9834 ± 0.0043[b] | 0.9825 ± 0.0081[a, b] |
| | | 45 | N.D. | N.D. | N.D. |
| | 3 | 4 | N.D. | N.D. | N.D. |
| | | RT | 0.9582 ± 0.0180 | 0.9702 ± 0.0130 | 0.9861 ± 0.0079 |
| | | 45 | N.D. | N.D. | N.D. |

Abbreviation: N.D. = not determined. Each datum represents the mean ± S.D.
**a** and **b** in the same row meant the symbol of significant statistics. The different symbols meant the significant difference ($p < 0.05$).

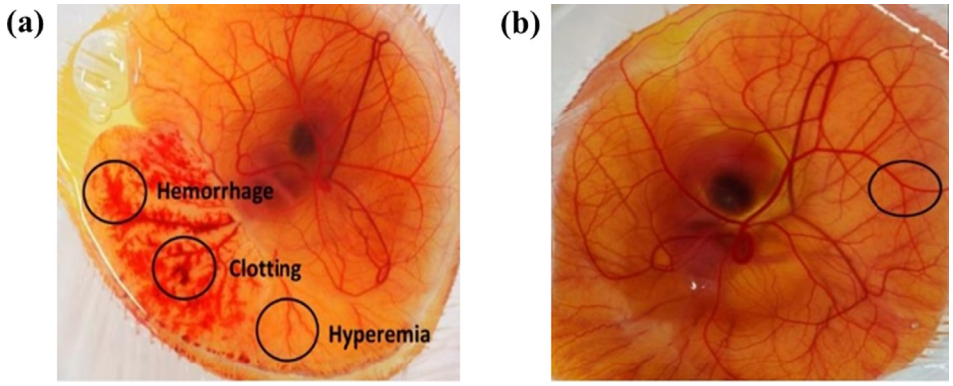



**Fig. 7.** HET-CAM model (a) positive control with hyperemia, hemorrhage and clotting and (b) negative control (Al-Kinani et al., 2018).

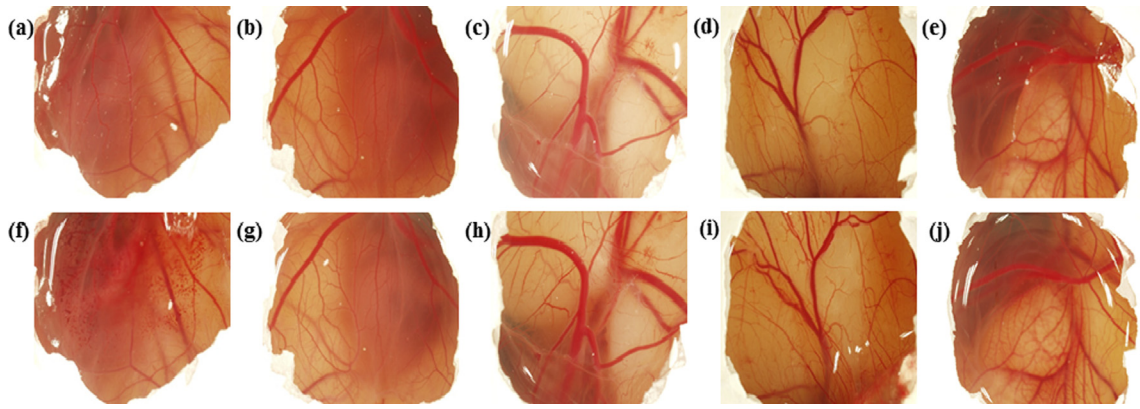



**Fig. 8.** The blood vessels of HET-CAM at EA10 (a-e) before applying the formulation or chemical and after applying (f) 0.1 M sodium hydroxide solution at 0.5 min as positive control, (g) 0.9% sodium chloride solution at 5 min as negative control, (h) LB-Gαgly at 5 min, (i) LH-Gαgly at 5 min, (j) GLY at 2 min.

could also retard the film's dissolving, and the diffusion kinetics was dominant. For the permeation profiles (Table 5), most of drug permeation kinetics was not statistically different in three types of kinetics. Some films were appropriated with first order or Higuchi's kinetic indicating that the drug permeation depended on the concentration of drug or diffusion from matrix that referred to the drug release from the films.

### *3.7. Irritancy evaluation using HET-CAM*

The irritancy results of the HET-CAM model of positive and negative controls are presented in Fig. 7. The levels of hyperemia, hemorrhage, and clotting were found in the positive test, but there was no change in the negative test. The irritancy results of medicated Gαgly films are shown in Fig. 8. The irritation potential of medicated Gαgly films was negligible. However, hyperemia was found in one sample of LB-Gαgly film. This might be due to the formulation being mixed with glycerin which slightly irritated HET-CAM and has been reported as a moderate irritant chemical (Sindhu et al., 2014). Moreover, the CAM is very sensitive, and glycerin also has hygroscopic property. The glycerin blended film was tightly attached with CAM in some experiments, and peeling off the film from CAM might affect or damage CAM. However, no observation of hemorrhage and clotting was found in LB- and LH-Gαgly films. This demonstrated LB- and LH-Gαgly films were safe to use. This could be used as buccal delivery systems.

## 4. Conclusion

LB- and LH-Gαgly films gave good properties for buccal drug delivery. The AFM results showed that the surface of medicated Gαgly films was slightly rough on upper and lower sides. However, the SEM images in the upper side and cross section of both LB and LH Gαgly films presented a smooth surface. The TGA thermograms of medicated Gαgly films revealed the same trend as the original gelatin curve. This confirmed the compatibility of all components in the medicated Gαgly films, same as the FT-IR patterns and DSC thermograms as reported previously. However, very slight crystalline form of drugs was observed in XRD diffractograms, especially in LB-Gαgly films. LB and LH could release from Gαgly films and permeate through CAM used as the buccal model. The stability test implied that medicated Gαgly films should be stored at low temperatures. Moreover, the irritation test in HET-CAM indicated that the medicated Gαgly films were safe and could be used for buccal delivery. In conclusion, gelatin and pregelatinized tapioca starch could be prepared as the transparent thin film using glycerin as plasticizer, and LB and LH could be loaded into Gαgly with good properties to use as buccal films.

## Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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