Investigation of crosslinked hydrogel comprising papain for lesion recovery acceleration

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Abstract

Papain is a common protease exhibiting antioxidant and anti-inflammatory activities. Papain can be extracted from seeds, leaves or latex of papaya (*Carica papaya* L.). It is involved in accelerating wound healing process. Hydrogels are widely used in biomedical applications such as tissue engineering, drug delivery system, and wound healing. Cross-linked hydrogels containing papain are more aqueous, durable and promising devices to minimize recovering time of lesions. Ingredients of HPMC hydrogels were investigated and immobilized with papain. The hydrogel thickness, moisture content, Fourier transform infrared, drug content and *in vitro* papain release were characterized. The formula of 0.5 g papain, 5 g HPMC, 5 g CA, 7.5 g glycerin, 5 g P407, 0.3 g nipagin M and EtOH quantum satis (q.s) 100 g resulted in hydrogels 0.8 mm of thickness, 3.83 % of moisture. FTIR analysis confirmed papain incorporation into prepared hydrogels. The FE-SEM examination revealed a good distribution of papain on the hydrogel and size of papain was (5-10) µm. In addition, from 2.6652 % to 5.1021 % of papain was released from the hydrogel within 60 minutes

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Keywords

Papain, hydrogel, physicochemical characteristics, wound healing

1 Introduction

Papain is a common protease that is widely studied, with its three-dimensional structure first being proposed in 1964 using chemical technique [1], then corrected in 1968 and 1971 using X-ray crystallography at 2.8 Å (Figure 1) [2, 3], which is refined at 1.65 angstroms resolution in 1984 [4] (Figure 2). This enzyme, comprising of two unmistakable complex particles with a cleavage between them that conveyed a functioning site for catalysis, also has a sub-atomic load of 23,406 Da and globular structure with 212 amino acids [5]. Papain is abundant in papaya latex but can also be found in papaya leaf [6] and seed [7]. Papain applications are diverse ranging from uses in food and beverage industries (beer brewing [7] and meat tenderizing [8], textile and leather industries (leather and silk treatment [7]), water recycling industry (wastewater treatment [9, 10]) to drug development (protease inhibitor development [11, 12], wound healing agent [13-15]) in pharmaceutical industries.

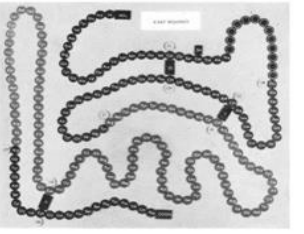

Figure 1 Amino-acid sequence as defused from the electron density map at 2.8 Å [3]

Figure 2 Amino-acid sequence as defused from the electron density map at 1.65 Å [4]

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Hydrogels are soft moist or jelly-like materials due to their three-dimensional network, developed by many cross-linked polymers, which enable to retain a high quantity of adsorbed water [12]. The cross-linked structure of hydrogels, which contain hydrophilic functional groups such as amine ($-NH_2$), hydroxyl ($-OH$), amide ($-CONH-$, $-CONH_2$), and sulphate ($-SO_3H$), allowing the hydrogel to absorb water or hydrophile fluids, preventing the dissolution and destruction of the cross-links during swelling [16, 17]. Upon application on wounds, hydrogels form effective dressings to prevent microorganisms from the environment, absorb tissue exudate while allowing gaseous exchange. These processes can involve boosting the migration of keratinocytes and fibroblasts during wound healing, modifying the immune cells within the wound, or encouraging angiogenesis (the development and expansion of blood vessels) in wounds with inadequate perfusion [18].

Although natural polymers, such as chitosan and gelatin, are the most frequently used due to their biocompatibility [19], other formulas such as Na carboxymethylcellulose-HPMC hydrogels for wound healing materials are proved to be effective and worth evaluated [20,21]. HPMC hydrogel has also been used with coumestrol which was originally found in *Glycine max* and *Medicago sativa*, also has anti-inflammatory, anti-herpes HSV-1, and estrogen-like properties, resulting in acceleration of wound size reduction by allowing the proliferative phase of wound healing to start earlier [22].

Numerous studies have reported the promising properties of papain in solving serious skin disorders [21], as well as anti-bacterial bioactive wound dressing [23]. These studies also agree with other studies that evaluate hydrogel loaded with papaya latex which is abundant of papain [24]. Various composites have also been tested for papain immobilization using pineapple peel carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA), and mesoporous silica SBA-15, which suggest that hydrogel formulas are attributed to temperature-sensitivity of the prepared hydrogel composites, resulting in the accessibility of the substrate to the active sites of papain [23], meaning that other hydrogel formulas should be evaluated as a delivery system for papain to utilize the enzyme wound healing property.

The addition of cross-links between polymer chains generates several notable effects, including enhanced elasticity, decreased viscosity, increased insolubility, improved strength and toughness, lowered melting temperature, and transition of thermoplastics into thermoset polymers [25]. Citric acid is a widely used cross-linker for HPMC [20,26]. The schematic representation of esterification reaction between HPMC hydroxyl groups and citric acid is shown in Figure 3. The citric acid molecule has three carboxylic acid groups; two of which possibly react with the HPMC hydroxyl groups and the other remain unreacted (as indicated by the circle) due to steric hindrance.

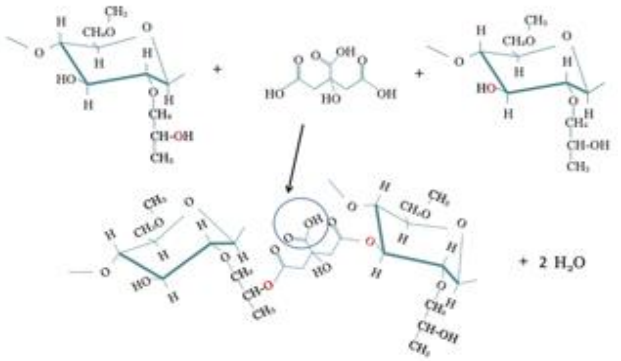

Figure 3 Schematic representation of esterification reaction between HPMC hydroxyl groups and citric acid [9]

Shoba et al. (2014) developed an encapsulation of papain and urea in PVA nanofiber to accelerate fibrogenesis and enhance wound healing [27]. Asanarong et al. (2021) immobilized papain on a bacterial cellulose film for wound dressing application. This bioactive material absolutely inhibited *E. coli* (ATCC25922), *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923) in the *in vitro* test [28]. In another study by Melo et al (2023), papain was loaded into chitosan membranes and released in a sustainable manner. This result indicates the potential of papain-loaded membrane for wounds application [29]. ?

In the present study, papain was immobilized on hydrogel film which had been formed cross-linking with citric acid. This kind of membrane was found to be slightly durable to water, promising a breathable but water-resistant wound dressing material.

2 Materials and methods

2.1 Materials

Papain was purchased from Yuanye Biology Co., Ltd (China). Citric acid (CA), CMC, ethanol, gelatin,

glycerin, HPMC, nipagin M, sodium alginate, trichloroacetic acid (TCA), sodium carbonate, and ethylendiamin tetraacetic acid (EDTA) was purchased from Guangdong Guanghua Sci-Tech Co., Ltd. (China). Poloxamer P407, casein, and cystein was purchased from Usolf (China). Folin-Ciocalteau reagent was purchased form Himedia (India).?

2.2 Enzymatic activity assay [30]

2.2.1 Standard curve of tyrosine

The enzymatic activity of the prepared papain-loaded hydrogel was investigated by using Folin-Ciocalteu assay, according to the procedure previously described by Gu et al. [30] with slight modifications. Briefly, a volume of (50, 100, 200, 400 and 500) µL of 1.1 mM tyrosine standard solution was added distilled water to achieve 2 mL. Then, 5 mL 500 mM Na_2CO_3 solution and 1 mL Folin Ciocalteau reagent were added. The tubes were mixed and incubated for 30 minutes at 37 °C. 2 mL of each mixture was filtered with a 0.45 µm filter, then the optical absorbance was measured by UV-Vis spectrophotometer (Shimadzu, Japan) at the wavelength of 660 nm. The blank control was prepared as previous without tyrosine solution. Each sample was carried out triplicate.

2.2.2 Papain activity determination

5 mL 0.65 % casein solution was added in 4 tubes and incubated for 5 minutes at 37 °C. Then, a total of (0, 20, 50 and 100) µL papain solution was added and tubes were incubated at 37 °C for 10 minutes. Afterwards, adding 5 mL TCA to each tube, (0.1, 0.08, 0.05, 0) mL the enzyme solution was filled in tubes. These tubes were incubated at 37 °C for 30 minutes and filtered with 0.45 µm filter. 5 mL of 500 mM Na_2CO_3 and 1 mL Folin Ciocalteau reagent were filled. The mixtures incubated at 37 °C for 30 minutes. 2 mL of each mixture was filtered with a 0.45 µm filter, then the optical absorbance was measured by UV-Vis spectrophotometer (Shimadzu, Japan) at the wavelength of 660 nm. Each sample was carried out triplicate.

Then, we can substitute the difference in absorbance between each test papain into the slope formula to calculate the corresponding amount of tyrosine (micromole) in this specific proteolysis reaction. Subsequently, the corresponding enzyme activity value in unit/mL is obtained using the following values:

11 = The total volume of the detection reaction (mL)

10 = Detection time per unit (min)

0.1 = Volume of enzyme used (mL)

2 = Volume used in colorimetric measurement (mL)

The number of micromoles of tyrosine divided by the number of minutes of time is the unit value of protease activity.

To determine the rate of an enzyme-catalyzed reaction, we utilize the Michaelis − Menten equation (Equation 1)

$$V_0 = \frac{V_{max} \times [S]}{[S] + K_m} \qquad \text{(Eq.1)}$$

K_m: the Michaelis constant

[S]: the substrate concentration (mol)

V_0: the velocity of enzyme-catalyzed reaction (mol/time)

V_{max}: the maximum velocity of enzyme catalyzed reaction (mol/time)

2.3 Investigation of hydrogel film forming components

Referring to the handbook of excipients [31], the roles and ratios of hydrogel film components, including papain, HPMC, CMC, sodium alginate, gelatin, citric acid, glycerin, P407, nipagin M, and EtOH 70 % were indicated in Table 1.

Table 1 Investigation of hydrogel film forming component

Ingredients	Uses	Theoretical percentage (%)	Survey percentage (%)	Ingredients	Uses	Theoretical percentage (%)	Survey percentage (%)
Papain	Medicinal substances	–	1, 2, 3, 5	Citric acid	Crosslinking agent	–	5, 10, 20
HPMC	Film-forming agent	2-20	2.5, 5, 7.5	Glycerin	Humectant	≤ 30	5, 7.5, 15
CMC	Gel-forming agent	3-6	5	P407	Surfactant	-	5, 10
Sodium Alginate	Gel-forming agent	–	2.5, 5, 7.5	Nipagin M	Preservative	0.02-0.3	0.3
Gelatin	Gel-forming agent	–	2.5, 5, 10	EtOH 70 %	Solvent	-	By volume

2.4 Investigation of engine capacity and microwave time on decay time of hydrogels

Various capacity (medium-low, medium, and medium high) and time of microwave (5 min and 10 min) were tested to find out which factors could prolong decay time of hydrogels.

2.5 Preparation of papain HPMC hydrogel films

HPMC hydrogel films were synthesized using CA as a cross-linker, as previously described by Dharmalingam and Anandalakshmi (2019). HPMC at 5 w/w concentration was prepared by dissolving 5 g of HPMC powder in ethanol 70 % at room temperature under magnetic stirring at 900 rpm for 30 minutes until a transparent solution was observed. Then, P407 5 %, glycerin 7.5 %, and CA 5 % of concentration were added and the mixture was stirred at 900 rpm for 1 h. After that, the mixture was placed in microwave for 5 minutes on medium-low power and let cool to room temperature. Papain was immobilized by adding 2 % papain solution (w/w) to the prepared hydrogel and stirred at 900 rpm for 30 minutes. Subsequently, the samples were poured onto a petri dish (diameter of 9 cm) and let to dry at room temperature overnight. The dish was then located in an oven at 45 °C for 8 h. The hydrogels without papain immobilization act as the control sample. Finally, all samples was stored in a desiccator for further analysis.

2.6 Characterization of papain loaded-HPMC hydrogel film [20]

2.6.1 Thickness

The film thickness was measured by an electronic digital caliper (500-182-30 model, Mitutoyo, Japan). All the films were pre-conditioned at 25 °C and (65 ± 5) % of relative humidity for 24 h. The measurement was taken on an average of 3 times.

2.6.2 Moisture content

The moisture content was determined using digital moisture meter (MB 45, Ohaus, USA). All the films were preconditioned at 25 °C and (65 ± 5) % relative humidity for 24 h. The measurement was taken on an average of 3 times.

2.6.3 Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was performed using FTIR Spectrometer (Shimadzu, model: IRAffinity-1, Japan) and attenuated total reflectance (ATR) method. Film samples were directly placed on ATR crystal. These films were investigated over an absorbance range of (4,000-400) cm^{-1} by 60 scans at a resolution of 4 cm^{-1}.

2.6.4 Surface morphology

To visualize the appearance of the samples, a digital camera (Sony, RX 100M7 Japan) was used, and the morphology and structure of gold-coated (E1054, Hitachi ion sputter, Japan) samples were characterized by field emission – scanning electron microscopy (FE-SEM) (S-4800, Hitachi Co., Japan).

2.6.5 Drug Content determination

5 mL of free papain (100 mg/5 mL) was added into 5 mL enzyme activator (including 5 mM L-cysteine and 2 mM ethylenediamine tetraacetic acid-EDTA), and then blended with 5 % substrate casein solution (5 mL) and subsequently heated at 37 ºC for 10 min. The reaction was added with 5 % TCA solution (10 mL) and incubated at 37 ºC for 30 min. After filtration through 0.45 µm, the optical absorbance was measured at 284 nm with UV-Vis spectrophotometer (Shimadzu 1900i, Japan). Before the papain solution adding in the blank control group, 5 % of TCA (10 mL) was added into the reaction mixture. The drug content is determined by using Equation 2. One unit of enzyme activity is defined as the amount of the enzyme required to release 1 µg of tyrosine per minute at 37 ºC.

$$A_e = \frac{A \times K \times V \times N}{t \times M} \qquad \text{(Eq. 2)}$$

A_e: sample solution absorbance value;

V: total volume of reaction solution (mL);

T: reaction time (min);

N: dilution ratio;

K: L-tyrosine standard curve slope;

M: the weight of the sample (mg)

Papain - HPMC hydrogel film was dissolved in 100 mL of distilled water. Aliquot (5 mL) was withdrawn and added 5 mL casein (5 %), 5 mL cysteine/EDTA. The mixture was left for 10 minutes at 37 ºC, followed by the addition of TCA (10 mL, 5 % w/v) to prevent the enzymatic reaction. After 30 min, the mixture was filtered through 0.45 µm filter paper. The amount of papain released was determined by the amount of tyrosine produced in the reaction with casein through UV-Vis spectroscopy (Shimadzu 1900i, Japan) at the wavelength of 284 nm. All experiments were carried out in triplicate.

2.6.6 Papain hydrogel release

The study of papain release was carried out using a dissolution tester (Pharmatech, Germany), following

previously described protocol [32]. Papain loaded-HPMC hydrogels were tested in 500 mL distilled water at (32 ± 0.5) ºC with a paddle speed of 50 rpm. Aliquots (5 mL) were withdrawn at (5, 10, 15, 30, 45 and 60) minute, and immediately replaced with fresh dissolution medium. To determine the amount of papain released, the following were added to the 5 mL aliquot: 5 mL casein (5 %), 5 mL L-cysteine/ EDTA. The mixture was left for 10 minutes at 37 ºC, followed by the addition of TCA (10 mL, 5 % w/v) to prevent the enzymatic reaction. After 30 minutes, the mixture was filtered through 0.45 µm filter paper. The amount of papain release was determined by the amount of tyrosine produced in the reaction with casein through UV spectroscopy (Shimadzu 1900i, Japan) at the wavelength of 284 nm. Experiments were carried out in triplicate.

3 Results and discussion

3.1 Papain activity

3.1.1 Standard cure of tyrosine

To generate a tyrosine standard curve, tyrosine concentrations of (0.05, 0.1, 0.2, 0.4 and 0.5) mmol were measured for their absorbance at 660 nm of wavelength. The correlation between tyrosine concentration and absorbance at 660 nm was shown in Figure 4. This exhibited that fourth power polynomial regression equation is $y = 1.908x^4 - 2.1592x^3 + 1.09x^2 + 1.078x + 0.0008$ ($R^2 = 0.9999$).

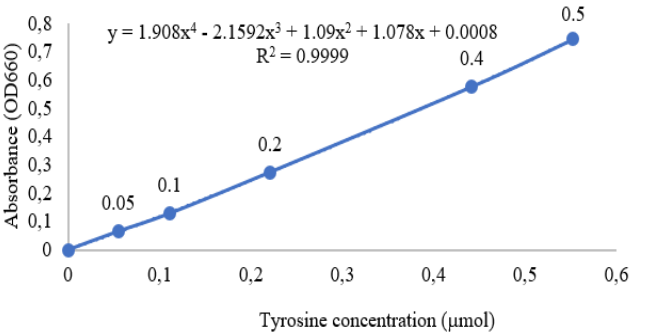

Figure 4 Standard curve of tyrosine at wavelength 660 nm

3.1.2 Papain activity value

The amount of tyrosine (mM) was calculated using the slope formula in the specific proteolysis reaction by substituting the difference in absorbance between each tested papain-containing samples and the blank sample. Subsequently, the enzyme activity value in unit/mL can be determined using Equation 1. Results are shown in Table 2. Table 3 displays the concentrations of papain and the corresponding velocities of the papain-catalyzed reaction.

Table 2 Papain activity

	20 µL papain	**50 µL papain**	**100 µL papain**
OD 660	0.0322	0.0443	0.0466
Tyrosine concentration (µmol)	0.0366	0.0505	0.0532
Papain activity (Unit/mL)	0.2011	0.2778	0.2926

Table 3 Enzyme kinetic

[S] (molar)	**V_0 (mol/min)**
0.2	0.2011
0.5	0.2778
1	0.2926

By applying the Michaelis−Menten equation, the papain activity data obtained from the concentrations listed in Table 3, we have derived the following results for K_{min} and V_{max}: $\begin{cases} K_m = 0.11 \\ V_{max} = 0.3 \end{cases}$

Substitute K_{min} and V_{max} into the Michaelis−Menten equation. The activity of the papain is 0.29996 Unit/mL/min. To convert units per milliliter per minute (Unit/mL/min) to units per milligram (Unit/mg), we employ the following calculation:

$$E_A(Unit/mg) = \frac{E_A\left(Unit/_{mL}\right) \times Volume}{m_{papain}} = \frac{0.29996 \times 5}{20} = 0.07499$$

Papain enzyme activity was thus determined as previously described by measuring the amount of tyrosine hydrolyzed from casein in an appropriate medium using ultraviolet spectrophotometry [27]. The enzyme activity is defined as the amount of enzyme required to release 1 µg of tyrosine per minute at 37 ºC. The drug content analysis and *in vitro* release of papain in aqueous environment is also calculated based on this

papain activity data. The papain activity unit was changed from unit/mL/min into unit/mg to determine the drug content and release. The papain activity value is stable during these tests and helps in identifying papain concentration in a hydrogel film and evaluating amount of papain release from a hydrogel film.

3.2 Hydrogel film formulation

After investigating the film-forming abilities of HPMC, CMC, sodium alginate, and gelatin in EtOH 70 % solvent, HPMC demonstrated opaque solutions and established durable and transparent film after drying on petri dishes at appropriate conditions. As a result, HPMC was chosen to form hydrogel film. The solvent EtOH 70 % was accessed due to (1) fast and low-temperature evaporation after drying, (2) rapid solubility of HPMC in this solvent.

Among the tested HPMC concentrations of (2.5, 5, and 7.5) %, the results revealed that the presence of HPMC 2.5 % and 7.5 % yielded excessively liquid and sticky hydrogels, respectively; whereas HPMC 5 % resulted in a hydrogel with moderate consistency. Based on these findings, HPMC 5 % was selected.

After HPMC was selected as the film-forming agent, a survey of (2.5, 3.75 and 7.5) g of glycerin and (2.5 and 5) g of P407 was conducted. Glycerin is used to maintain humidity of hydrogel and P407 aims to increase the permeability of drugs through skin barrier. All ingredients' unit was g and total amount of one formulation was 50 g.

Table 4. Investigation of glycerin and P407 amount (g)

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
HPMC (g)	2.5	2.5	2.5	2.5	2.5	2.5
Glycerin (g)	**2.5**	**2.5**	**3.75**	**3.75**	**7.5**	**7.5**
P407 (g)	**2.5**	**5**	**2.5**	**5**	**2.5**	**5**
EtOH 70% (g)	42.5	40	40	37.5	37.5	35

According to the survey results, formulas F1 and F2 produced opaque films, while formulas F3, F4, F5, and F6 resulted in transparent films. Among these options, the film created by formula F3 exhibited desirable characteristics such as quick-drying, a soft texture, and tear resistance. Consequently, F3 was selected as the preferred choice.

Table 5. Investigation of CA amount (g)

Ingredients	**K1**	**K2**	**K3**	**K4**
HPMC (g)	5	5	5	5
CA (g)	**5**	**10**	**15**	**20**
Glycerin (g)	7.5	7.5	7.5	7.5
P407 (g)	5	5	5	5
EtOH 70% (g)	75	70	65	60

Continuing the investigation, as Table 5, different amounts of CA were examined to establish cross-linking with (5, 10, 15 and 20) g. At first, the cross-linking reaction was realized using simple hot temperature at about (70-80) ºC. However, decay time of hydrogel films after forming was short (about 3 minutes). Consequently, the mixture was microwaved at medium-low power for 5 minutes to ensure the cross-linking reactions. Then, it was poured into petri dishes weighing 5 g and 10 g, respectively.

Among the formulas tested, both formula K1 (10 g) and formula K2 (10 g) yielded visually appealing and durable films, surpassing formulas K1 (5 g), K2 (5 g), and K3 (5 g and 10 g). However, formula K4 did not result in film formation. Based on these findings, formula K1 (10 g) and formula K2 (10 g) were chosen for further investigation.

Results of the effect of CA content (5 and 10) % and microwave time on hydrogel decay are presented in Table 6.

Table 6 Effect of CA concentration and microwave time on decay time

CA concentration	**5 %**		**10 %**	
Microwave time (min)	5	10	5	10
Decay time	11 min	4 min 30 s	6 min 30 s	4 min 30 s

As the formulation with 5 % CA and 5 minute of microwave resulted in the longest decay time (11 min), these conditions were selected for subsequent investigation on the impact of microwave power and time on the film-forming ability of the hydrogel.

Table 7 Effect of engine capacity and microwave time on hydrogel film formation

Engine capacity		Medium-low	Medium	Medium-high
Microwave time	5 min	11 min	5 min 47s	-
	10 min	4 min 30s	-	-

As shown in Table 5, microwaving the hydrogel at low power for 5 minutes developed in the longest disintegration time. Therefore, the decision was made to opt for a medium-low power setting.

An examination was conducted on the ratios of active ingredients and hydrogel combination, including 25 g of hydrogel and papain at ratios of (1, 2, 3, and 5) % respectively. It was observed that samples containing 3 % and 5 % papain dispersed unevenly within the hydrogel while Therefore, the formulation containing 2 % papain was selected for subsequent experiments.

The complete formula of papain - HPMC hydrogel is shown in Table 6.

Table 6 Composition of papain - HPMC hydrogel (g)

Ingredients	Papain	HPMC	CA	Glycerin	P407	Nipagin M	EtOH 70 %
Weigh (g)	0.5	5	5	7.5	5	0.3	76.7

In comparison to previous screened hydrogel formula which include NaCMC, HPMC and CA, the newly synthesized hydrogel using HPMC, CA, P407, glycerin and nipagin M is less durable and is deformed within 20 minutes. However, the presence of papain has strengthened hydrogel structure, thus remaining intact in aqueous environment for over 60 minutes. Further studies should focus on papain stability withing hydrogel structure as papain possibly interact with hydrogel to form a stronger structure, resulting in longer decay time.

HPMC is a soluble polymer and the swelling time in water is very short-live at about 3 minutes. The hydrogels are more stable in water and the tested swelling time is 15-minute period after HPMC and CA cross-linking process reaction under microwave condition with medium-low power. Although the hydrogel aqueous stability was improved, papain can be denatured at high temperature. To prevent denaturation, papain was initially diluted in water before it was added to the cross-linked hydrogel mixture. The enzyme activity and stability are unaffected while it is possible to create a homogeneous mixture of papain hydrogel. The weight percentage of papain in the formula is 2 % excluding solvents.

3.3 Thickness of papain - HPMC hydrogel

The average thickness of the papain - HPMC hydrogel was (0.80 ± 0.01) mm.

3.4 Moisture content

The average moisture content of papain – HPMC hydrogel was (3.83 ± 0.36) %.

3.5 FTIR analysis

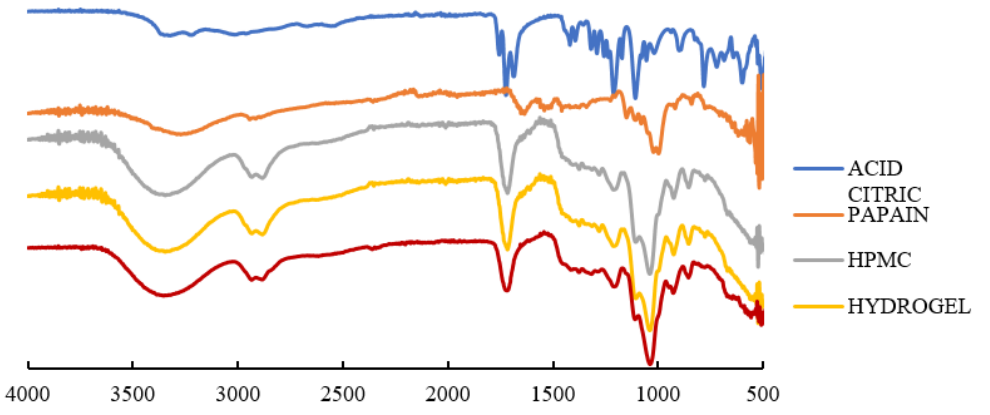

Figure 5 FTIR spectra of CA, papain, HPMC, hydrogel and papain-hydrogel

FTIR spectroscopy is one of the conventional experimental methods providing information on substances' structure. Figure 5 showed the FTIR spectra of CA, papain, HPMC, hydrogel and papain-hydrogel. In papain, it is important to study structure of proteins by the most informative primary amides, second amides and amide groups. Primary amides present the N–H stretching vibration at (3,300-3,400) cm^{-1} while second amides band represent the N−H stretching at (3,310-3,350) cm^{-1}, C–N stretching at (1,456-1,527) cm^{-1}. Amide groups present the C=O stretching vibration in the region (1,725-1,630) cm^{-1}.

The IR spectra of HPMC revealed absorption bands assigned to the O−H stretches at (3,300-3,400) cm^{-1}, C−H asymmetric and symmetric stretches assigned to the methyl and hydroxypropyl groups arising from the substitution of O−H groups at (2,800-2,900) cm^{-1}. Carbonyl compound gives a strong signal at (1,710-1,740) cm^{-1}. CO−C bonds in ether cellulose derivatives show bands at (1,000-1,300) cm^{-1}.

The IR spectra of papain − HPMC hydrogel exists all signals found in HPMC spectra because every band of papain is covered by bands of HPMC.

3.6 Surface morphology

The surface of papain - HPMC hydrogel films was shown by FESEM (Figure 6). Papain particles are round, white, small and distributed relatively evenly on the surface of the hydrogel prepared by HPMC and CA as a cross-linker. The addition of CA as cross-linker shows relatively more smooth and uniform surface morphology, as CA creates covalent and intermolecular hydrogen bonds between polymer chains by cross-linking.

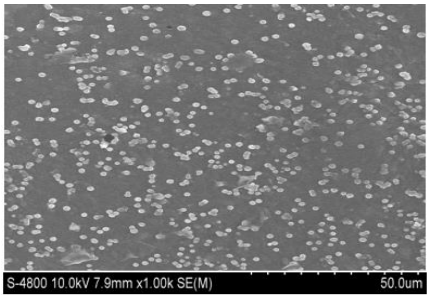

Figure 6 Papain particles distributed on the surface of HPMC hydrogel films by FESEM

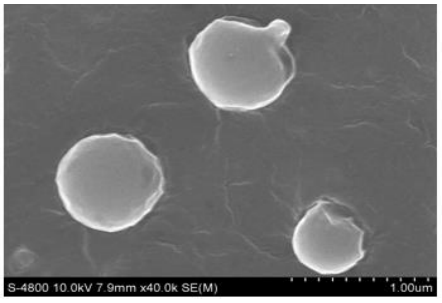

Figure 7 Papain particles size

Figure 7 reveals the size of papain particles which is round, white, and about (5 to 10) mm. The lines appeared among papain particles are glycerin which add moisture to the surface.

Table 7 Drug content of papain − HPMC hydrogel

Sample	Enzyme activity (Unit/mg)	Absorbance	Real $m_{papain/hydrogel}$ (mg)	$m_{hydrogel}$ (mg)	Theorical $m_{papain/hydrogel}$ (mg)	Papain %
1.1	0.07499	0.4542	74.1998	5250.4	100.9862	73.48
1.2	0.07499	0.4828	78.8683	5264.7	101.2612	77.89
1.3	0.07499	0.4052	66.1919	5234.6	100.6823	65.74

3.8 Papain hydrogel released

three times at intervals of (5, 10, 15, 30, 45 and 60) minutes. Based on the percentage of papain accumulation and the standard deviation of the released papain amount, the accumulation of papain over time is illustrated in Figure 8.

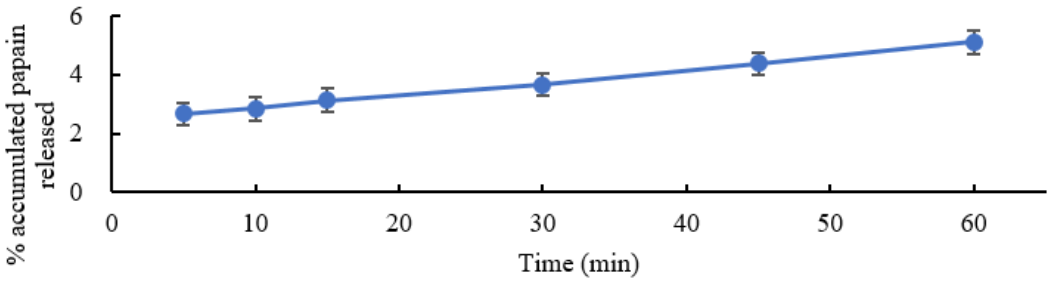

Figure 8 Percentage of papain accumulated released from hydrogel

The provided data indicated that the released papain amount from hydrogel gradually increased from (2.6652 to 5.1021) % over a period of 60 minutes. This trend indicates a progressive rise in the released amount of papain over time.

The drug content analysis and *in vitro* release of papain in aqueous environment is also calculated based on this papain activity data shown in Figure 8. Papain was quickly release immediately after the hydrogel was placed into aqueous environment with a little above 2.5 % which is more than 50 % of the total papain released within the course of the study of 60 minutes. Papain release increases steadily and linearly throughout the experiment and was recorded at from 2.8373 % at minute 10, 3.6493 % at minute 30, 4.3682 % at minute 45 to 5.1021 % at the end of the experiment. The temperature condition of *in vitro* released test was (32 ± 0.5) ºC, corresponding to physical skin temperature because the papain-hydrogel film was designed to minimize healing time of dermal wounds.

4 Conclusion and recommendation

4.1 Conclusion

The enzymatic activity of papain was evaluated by determining the amount of enzyme necessary to produce 1 µg of tyrosine per minute at 37 ºC. The standard curve of tyrosine was $y = 1.908x^4 – 2.1592x^3 + 1.09x^2 + 1.078x + 0.0008$ with $R^2 = 0.9999$ and the papain activity was 0.29996 Unit/mL/min or 0.0749 Unit/mg.

Papain-hydrogel films formula was accessed including 0.5 g papain, 5 g HPMC, 5 g CA, 7.5 g glycerin, 5 g P407, 0.3g nipagin M and EtOH q.s 100 g. The cross-linking between HPMC and CA was performed using a microwave at medium-low power to lengthen decay time of hydrogel films.

The physico-chemical examinations exhibited that the hydrogel films were 0.8 mm of thickness, 3.83 % of containing moisture. FTIR spectra confirmed the papain incorporation into hydrogels. The FE-SEM figures revealed a good distribution of papain on the hydrogel and size of papain was (5-10) µm. Also, papain released from the hydrogel was (2.6652 to 5.1021) % over 60 minutes duration. These experiments' characteristics highlight the possibility of developing a newly modified release method based on hydrogels and papain for wound healing.

4.2 Recommendation

Following the preparation and physicochemical characterization of hydrogel containing papain, it is essential to demonstrate the wound healing acceleration potential *in vivo* studies on mice. Determination of stability of the obtained papain-hydrogel films is also necessary during 28 days.

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Nghiên cứu hydrogel liên kết chéo chứa papain giúp mau lành vết thương

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Tóm tắt Papain là một protease phổ biến có tác dụng chống oxy hóa và chống viêm, đẩy nhanh quá trình chữa lành vết thương. Papain được chiết xuất từ hạt, lá hoặc mủ của cây đu đủ (Carica papaya L.). Hydrogel được sử dụng rộng rãi trong các ứng dụng vật liệu y sinh như kỹ thuật mô, hệ phân phối thuốc và chữa lành vết thương. Hydrogel liên kết chéo có chứa papain bền hơn trong môi trường nước và giúp giảm thiểu thời gian phục hồi các tổn thương. Công thức hydrogel HPMC đã được nghiên cứu và papain được cố định trong hydrogel này. Papain hydrogel được đánh giá độ dày, độ ẩm, hồng ngoại biến đổi Fourier, hàm lượng thuốc và khả năng phóng thích papain in vitro. Công thức Papain-hydrogel gồm 0,5 g papain, 5 g HPMC, 5 g CA, 7,5 g glycerin, 5 g P407, 0,3 g nipagin M và EtOH vđ 100 g. Kết quả cho thấy màng hydrogel có độ dày 0,8 mm, chứa 3,83 % độ ẩm. FTIR đã chứng minh sự kết hợp papain vào hydrogel. Số liệu FESEM cho thấy papain phân bố tốt trên hydrogel và kích thước của papain là (5-10) µm. Ngoài ra, papain được giải phóng từ hydrogel là 2,6652 % đến 5,1021 % trong thời gian 60 phút.

Từ khóa Papain, hydrogel, đặc tính lý hóa, chữa lành vết thương