



An *in vitro* evaluation of films prepared from gelatin – *Carica papaya* methanolic extract for wound healing

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Abstract

This paper is a part of research aiming at preparing biodegradable films containing gelatin and *Carica papaya* extract for wound healing purposes in rats. *Carica papaya* leaves were collected and extracted using methanol aqueous method (20% methanol: 80% water). Eleven films (F1–F11) of *Carica papaya* extract (0–500) and gelatin (1000 mg) were prepared and investigated for selected *in vitro* parameters which include pH, thickness, folding endurance, water absorption capacity, tensile strength and extract released studies. Increasing concentrations of the added *Carica papaya* extract dropped the pH value from 7.3 ± 0.1 to 5.2 ± 0.1 . Blank gelatin film had the highest value of the thickness ($22.8 \pm 0.9 \mu\text{m}$). Folding endurance increased significantly ($p < 0.05$) in both blank and composite films. In blank films the values of the folding endurance increased from 122.7 ± 3.5 – 187.6 ± 3.5 while in composite ones the values increased from 232.0 ± 8.8 to 258.3 ± 2.5 . Tensile strength value increased with increasing the addition of *Carica papaya* extract significantly ($p < 0.05$) from $100.4 \pm 3.4 \text{ N/cm}^2$ to $191.5 \pm 3.6 \text{ N/cm}^2$. In contrast, within the composite films the highest addition of *Carica papaya* decreased the tensile strength value significantly ($p < 0.05$) from 332.8 ± 2.2 to $84.10 \pm 6.1 \text{ N/cm}^2$. Significant differences ($p < 0.05$) were noticed in the values of extract release. The maximum percentage of extract release was 96.6% which was scored in formulation 7 of the films (F7) while the minimum percentage of the extract release was 69.8% which was recorded in formulation 9 of the films (F9). In conclusion, the films contained high concentrations of *Carica papaya* extract gave promising *in vitro* results and are recommended to be used for wound healing applications.

Keywords: *Carica papaya*, wound healing, gelatin, films, tensile strength, extract release

Introduction

Effective delivery systems for the purpose of carrying a drug specifically and safely to a desired site of action became the most challenging tasks of pharmaceutical formulation investigators [1]. Animal proteins such as albumin and gelatin are the most widely used polymers in drug delivery [2]. Gelatin and its derivatives have proven wound healing properties individually and in combination with other drug materials. It was used for surgical purposes as surgical tissue sealants and effectively sealed a wound in lung tissue from blood and air leakage [3]. Wound healing using gelatin sheet (2 x 2 cm) combined with basic fibroblast growth factor (bFGF) was studied in mice and the results revealed that gelatin sheet containing bFGF was effective for wound healing and gave promising future for clinical use [4]. Blending solution of gelatin with PCL (poly(- caprolactone) and 2,2,2-trifluoroethanol (TFE) by mixing 10% w/v PCL/TFE and 10% w/v gelatin/TFE in 50:50 provided great potential results in the

treatment of dermal wound healing application [5]. Gelatin is widely used in various pharmaceutical and medical applications [6 – 12] because it is cheap, biodegradable and demonstrates good biocompatibility [13 – 18].

Carica papaya is well documented with the ability of healing wound and was investigated for this purpose [19]. An application of aqueous extracts of *Carica papaya* in a dose of 100 mg/kg for 10 days on diabetic wound rats exhibited a 77% reduction in wound area compared with wound without treatment [20].

In vitro studies are needed for wound healing films to ensure the amount of drug available, to handle films easily and comfortably, to secure application of films and to measure the capacity of a film to absorb wound exudates [21]. Several studies investigated *in vitro* parameters of wound healing films using extracts of *Carica papaya* from different part of the plant such as leaves, ripe and unripe fruits [22–25]. *Carica papaya* has great opportunities as a source of natural drug which can be used successfully in formulating various types of films for different wound healing applications because of its availability, effectiveness and safety. This research is conducted



with the objective of studying selected *in vitro* parameters in wound healing films formulated from gelatin and *Carica papaya* combination.

Materials and methods

Materials

Carica papaya leaves were collected from papaya farm belongs to Asia Fruits Sdn. Bhd. Located in Negri Sembilan, Selangor, Malaysia. The leaves were transported to extraction laboratory, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM), washed with tap water to remove the dust and other strange materials and spread on clean table and left to dry at room temperature. Gelatin type B from bovine skin was purchased from Sigma Co., Malaysia.

Methods

Preparation of *Carica papaya* extract

The dry leaves were placed in an oven set at 45°C and left for 5 – 7 days to fully dry. The dry leaves were broken by hands into small pieces and placed in a Waring blender set at high speed for 20 min. Modified method of aqueous extraction [22] was conducted using methanol and water in a ratio of 20: 80, respectively. 20 g of the dry blended leaves were placed in 1000 mL flask and the mixture of methanol in water was added in the ratio of 1:20 (blended leaves: mixture of methanol in water, respectively). The flask was covered with aluminum foil and left till the next day. The mixture was filtered through filter paper no.1 using a funnel. The filtrate was collected and rotor vaporized to remove the water. The extract was then freeze – dried using freeze dryer model (Scanvac Coolsafe, RZ 2.5, Germany) and the extract in form of powder of was obtained. The extract was placed in a clean dry bottle using paraffin lamination.

Preparation of blank and composite films

The blank and composite films were prepared by solvent casting technique [26] using the protocol shown in Table 1. The gelatin and extract of *Carica papaya* were mixed thoroughly individually or together using deionized water. 0.2 ml of polyethylene glycol (PEG) was added to all films formulations as plasticizer. To remove any entrapped air bubbles, filtration under vacuum technique was used. The solutions were then individually cast into the film on Petri dishes and dried at room temperature in sterilized environment.

pH measurement

The pH standard method was used to measure pH values of the films. The samples of the films were prepared individually using distilled water in a ratio of 1:10 (film sample: distilled water), respectively for 1 min. at low speed in a Waring blender. The pH meter (Tolledo 320 pH meter, Mettler – Instrument) was first standardized with two buffer solutions in pH 7.0 and 4.0 before used. The blank and composite films were sampled and the pH values were obtained.

Table 1: Composition of different wound healing films

Film no.	Film code	Formulation	
		Gelatin (mg)	Carica papaya (mg)
Blank film			
1	F1	1000	0
2	F2	0	100
3	F3	0	200
4	F4	0	300
5	F5	0	400
6	F6	0	500
Composite films			
7	F7	1000	100
8	F8	1000	200
9	F9	1000	300
10	F10	100	400
11	F11	1000	500

Film Thickness

The thickness of the films was measured using a micrometer (Mitutoyo, Kanagawa, Japan). The device was first calibrated to zero point and the measurements were taken with the smallest possible unit measurement count of 0.01 mm. The thickness was measured at five different locations of the films (centre and four areas around the edges) using the micrometer screw gauge, and the mean thickness was calculated.

Folding endurance

The folding endurance of the films was individually evaluated to determine flexibility of each film. The film was repeatedly folded manually at same place till it breaks or folded up to 300 times [21].

Water absorption capacity

An initial weight of 1 inch of dry film was recorded. Then the film was placed in 15 ml. of distilled water taken in Petri dish. The weight of the film was noticed periodically at first hour, second hour, third hour and 24th hour. Every time after the weight is taken, the film is placed in new fresh water.

Water absorption capacity of the film was calculated using the following formula:

$$\text{Water absorption capacity} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100 \%$$

Tensile strength

Tensile strength of the film was measured using texture analyzer TAXT2i (Stable Micro Systems, Surrey, UK) using the described method [27] with the following parameters:

1. 2 mm/s pre speed



2. 1 mm/s test – speed
3. 10 mm/s post speed with an acquisition rate of 50 points/s
4. 5 kg load cell

The resulting profiles were analyzed using Texture Expert, Version 1.22 (Stable Micro Systems, Surrey, UK).

Extract release studies

Extract release investigation was carried out using combination films coded from F7 – F11 in order to estimate the time taken to release the total *Carica papaya* extract from the films. A diffusion membrane was used in the determination. The samples were placed individually in a beaker. The measurement was done every 10 minutes interval by withdrawing 5 ml of the sample from the beaker. The concentration of *Carica papaya* in the samples was estimated using UV/Visible spectrophotometer at wavelength value of 260 nm. An equal volume of fresh distilled water was replaced after withdrawal of each sample.

Statistical Analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) test. Minitab version 14 statistical package (Minitab Inc., PA, USA) was used in the analysis. Statistical significance was set at p (0.05) level. The results are expressed as mean \pm S.D.

Results and Discussion

The pH values of different films are shown in Table 2. The highest pH value in this study was scored in the control sample prepared from gelatin only. Increasing the percentage of added *Carica papaya* caused noticeable decrease in the pH value, e.g. increasing *Carica papaya* from 0.0 to 500 mg dropped the pH value from 7.3 to 5.2. The pH value is an important factor within the wound–milieu which influences directly and indirectly all biochemical reactions taking place in this process of healing [28]. The effect of pH value on 20 wounds was investigated [29] over a two – week period and reported that wounds having a pH of 7.6 showed a 30% reduction in wound size after two weeks. They also reported that as the pH increased, the reduction in wound size decreased. It was found that both chronic and acute wounds with alkaline pH have demonstrated lower rates of healing than wounds in which the pH is closer to neutral [29, 30]. The pH value has great effect on toxicity of antibiotics in the presence of bacteria. For instance, it was observed that the toxicity of a new glycopeptide antibiotic (Oritavancin, LY333328) towards vancomycin resistant *Enterobacter* species (*E.faecium*) decreases significantly in an acidic milieu with a pH value of 6.4 compared to pH values of 7.4 and 8.4 [31]. They also noticed that the activity of the *Enterobacter* increased in an alkaline milieu as a pH value of 7.8 results in a 90–fold higher compared to pH 5.5. Many investigators reported that most relevant human– pathogenic bacteria present in wound need pH values above 6.0 and when pH milieu of 7.4 becomes exposed and their growth is inhibited pH value lower than 6 [32 – 34]. The thickness values of the film which may influence both the amount of drug available and the time required to absorb it into the body

are presented in Table 2. In blank films the increase in *Carica papaya* resulted in significant increase ($p < 0.05$) in thickness. The values range from 9.2 ± 0.3 – 22.8 ± 0.9 . Blank gelatin film had the highest value of the thickness. However, increasing concentration of *Carica papaya* extract in the formulation increased the values of thickness significantly ($p < 0.05$). In the composite films, the thickness values increased gradually then decreased significantly in the highest addition of *Carica papaya*. This phenomenon could be due to the competition of two polymers (gelatin and *Carica papaya*) to absorb water used in the formulations and caused increases in their swelling. Many researchers agreed with this fact and revealed that thickness of films significantly affected by their swelling due to the penetration of solvent into films [35 – 37]. Table 2 shows folding endurance measurement values generally increased significantly. The folding endurance information is needed to handle the film easily and for comfortable application on wound. It was noticed that there was significant ($p < 0.05$) increases with increasing the concentration of *Carica papaya* extract in the formulations. Within the blank samples, gelatin film scored the highest value of folding endurance. Increasing *Carica papaya* alone resulted in gradual and significant ($p < 0.05$) increase in folding endurance values. The values were increased from 122.7 ± 3.5 – 187.6 ± 3.5 . The composite films showed the same phenomenon. The values of the folding endurance increased significantly ($p < 0.05$) from 232.0 ± 8.8 to 258.3 ± 2.5 and highest addition of *Carica papaya* extract had the highest folding endurance. Table 2 shows the values of water absorption capacity of the films. It was observed that there was significant ($p < 0.05$) difference in water absorption capacity between the blank and the composite films. This could be due to hydrophilic and swelling properties of gelatin [38, 39]. The parameters of tensile strength of the films are given in Table 2. In the blank films it was noticed that as *Carica papaya* extract percentage is increased the value of tensile strength increased significantly ($p < 0.05$) from 100.4 (N/cm²) to 191.5 (N/cm²). However, the composite films showed significant ($p < 0.05$) individual tensile strength values. The highest value 445.17 (N/cm²) was found in F8 followed by 332.8 (N/cm²) in F7. The lowest value of tensile strength 84.10 (N/cm²) was recorded in F11. The tensile testing provides an indication of the strength of the film. It suggested that films suitable for wound dressing should preferably flexible and strong [40]. Table 3 presents the values of the cumulative percentage of extract release of selected films. The results were determined using *in vitro* diffusion studies in order to find the time taken by film to release the complete *Carica papaya* extract for the purpose of eliciting its antibacterial action on wound when *in vivo* studies will be conducted later. The values of extract release were significantly different ($p < 0.05$). The maximum percentage of extract release 96.6% was scored in the film coded F7 while the minimum percentage of 69.8% was found in the film F9. It was observed that within 1 ½ hour (90 minutes), the percentage of the extract released from selected films were 69.8, 73.2 ± 5 , 79.4 ± 5.5 , 87.3 and 96.6 for F9, F11, F10, F8 and F7, respectively. This phenomenon indicated that the films are not interfering in extract release on wound.



Table 2: pH, thickness, folding endurance, water absorption capacity and tensile strength parameters of blank and composite films

No	Code of the film	pH value	Thickness (μm)	Folding endurance (mean \pm S.D.)	Water absorption capacity % (mean \pm S.D.)	Tensile strength (N/cm^2)
Blank films						
1	F1	7.3 \pm 0.1 ^a	22.8 \pm 0.9 ^a	217.0 \pm 5.0 ^a	535.6 \pm 14.8 ^a	187.3 \pm 2.8 ^a
2	F2	5.4 \pm 0.2 ^b	9.2 \pm 0.3 ^b	122.7 \pm 3.5 ^b	562.2 \pm 15.1 ^b	100.4 \pm 3.4 ^b
3	F3	5.2 \pm 0.1 ^b	13.5 \pm 0.9 ^c	145.0 \pm 9.9 ^c	732.0 \pm 5.5 ^c	101.4 \pm 5.1 ^c
4	F4	6.7 \pm 0.2 ^c	14.1 \pm 0.5 ^d	159.7 \pm 7.0 ^d	919.2 \pm 18.7 ^d	108.6 \pm 3.8 ^d
5	F5	6.5 \pm 0.2 ^c	16.7 \pm 1.0 ^e	176.7 \pm 4.5 ^e	1156.5 \pm 8.7 ^e	161.5 \pm 4.5 ^e
6	F6	6.3 \pm 0.2 ^c	20.2 \pm 0.4 ^f	187.6 \pm 3.5 ^f	1059.1 \pm 22.7 ^f	191.5 \pm 3.6 ^f
Composite films						
7	F7	6.5 \pm 0.1 ^c	13.2 \pm 0.5 ^c	232.0 \pm 8.8 ^g	760.6 \pm 6.9 ^g	332.8 \pm 2.2 ^g
8	F8	6.2 \pm 0.2 ^c	19.2 \pm 0.5 ^g	239.7 \pm 2.5 ^h	816.6 \pm 9.4 ^h	445.17 \pm 8.1 ^h
9	F9	6.3 \pm 0.2 ^c	17.9 \pm 1.0 ^h	243.3 \pm 5.5 ⁱ	858.0 \pm 12.9 ⁱ	284.57 \pm 12.0 ⁱ
10	F10	6.7 \pm 0.1 ^c	27.5 \pm 0.9 ⁱ	248.3 \pm 3.5 ^j	980.2 \pm 13.9 ^j	211.76 \pm 11.0 ^j
11	F11	5.2 \pm 0.1 ^b	23.8 \pm 0.2 ^j	258.3 \pm 2.5 ^k	985.8 \pm 14.7 ^k	84.10 \pm 6.1 ^k

Means with the same superscript letter within a column were not significantly different at $p < 0.5$. Readings were means of triplicate measurements.

Table 3: Cumulative percentage of extract release from different film samples

Time(min)	Extract release (%) (Mean \pm S.D.)				
	F7	F8	F9	F10	F11
10	16.1 \pm 4.4 ^a	11.6 \pm 1.5 ^b	8.7 \pm 1.8 ^c	8.3 \pm 1.6 ^d	8.6 \pm 1.5 ^d
20	29.8 \pm 7.0 ^a	19.4 \pm 7.2 ^b	14.4 \pm 2.2 ^c	11.9 \pm 3.1 ^d	10.2 \pm 1.0 ^e
30	62.6 \pm 2.6 ^a	40.4 \pm 8.9 ^b	23.0 \pm 1.5 ^c	22.4 \pm 6.5 ^d	15.9 \pm 4.6 ^e
40	89.9 \pm 7.3 ^a	55.1 \pm 8.2 ^b	55.1 \pm 8.1 ^b	27.6 \pm 6.0 ^c	23.2 \pm 4.5 ^d
50	81.5 \pm 4.3 ^a	58.0 \pm 1.8 ^b	36.6 \pm 4.2 ^c	31.9 \pm 0.6 ^d	25.3 \pm 0.6 ^e
60	82.9 \pm 8.1 ^a	82.6 \pm 6.2 ^a	50.8 \pm 3.9 ^b	32.6 \pm 0.6 ^c	27.6 \pm 3.4 ^d
70	82.9 \pm 9.5 ^a	66.1 \pm 2.1 ^b	77.9 \pm 1.4 ^c	57.4 \pm 9.1 ^d	49.4 \pm 3.2 ^e
80	90.4 \pm 3.8 ^a	65.8 \pm 6.3 ^b	52.6 \pm 6.6 ^c	55.7 \pm 8.0 ^d	63.9 \pm 7.4 ^e
90	96.6 \pm 2.9 ^a	87.3 \pm 5.3 ^b	69.8 \pm 1.8 ^c	79.4 \pm 5.5 ^d	73.2 \pm 5.9 ^e

Means with the same superscript letter within a row were not significantly different at $p < 0.5$. Readings were means of triplicate measurements.

F7 – F11: Composite films formulations



Conclusion

In conclusion, new films formulated from gelatin and methanol aqueous (20% methanol: 80% water) were prepared. Selected properties of these films were characterized to determine their feasibility for use on skin wounds.

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