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Preparation and evaluation of sodium alginate hydrogel sheet loaded with *Carica papaya* L. seed extract for wound healing

Ishan DURVASHA[1], Anil KUMAR[1], Shweta AGARWAL[1,2*]

1 LR Institute of Pharmacy, Department of Pharmaceutics, Solan, India
2 ICFAI University, Faculty of Pharmaceutical Sciences, Baddi, India

ABSTRACT

In this study, an innovation in the form of a hydrogel sheet containing *Carica papaya* seed extract was developed and assessed for wound healing activity. The hydrogel sheet was prepared by using sodium alginate, guar gum, glycerol, methyl, and propyl paraben as excipients. Ninehydrogel formulations were prepared by varying the amount of sodium alginate (1.5%, 2.5%, and 3.5%), extract (8%, 10%, and 12%), and guar gum (0.8%, 1.2%, and 1.5%). The formulations prepared were evaluated for physicochemical characteristics like colour, pH, weight variations, folding endurance, tensile strength. Surface characteristics were studied by scanning electron microscopy. Wound healing potential was studied on wistar rats with hydrogel sheet (12%) showing 97.5% wound closure. All hydrogels containing seed extracts showed superior healing performance vis-a-vis control hydrogel. It can be deduced from the *in vivo* evaluation that the *Carica papaya* seed extract hydrogel sheet has wound healing properties comparable to the marketed hydrogel sheet.

Keywords: sodium alginate, innovation, *Carica papaya* extract, hydrogel, wound healing

*Corresponding author: Shweta AGARWAL
E-mail: shweta_ag26@rediffmail.com
ORCIDs:
Ishan DURVASHA: 0000-0002-2784-1243
Anil KUMAR: 0000-0002-2742-0705
Shweta AGARWAL: 0000-0002-9412-0788
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INTRODUCTION

The dynamic process of wound healing involves inflammation, epithelisation, collagen synthesis, and remodelling of tissue[1]. The process of wound healing starts from the moment the tissue injury occurs. The contact of platelets with the exposed collagen initiates the healing cascade. Clotting factors are released as platelets aggregate, emanating in the formation of a clot of fibrin at the injury site. This clot acts as a temporary matrix and paves the way for further healing events[2].

Inflammatory cells appear at the site of injury along with platelets and provide key signals in the form of cytokines[3]. Fibroblasts, the connective-tissue cells leading to collagen deposition, are required for tissue restoration. Collagen imparts structural integrity and strength to normal tissues. When tissues are damaged as a result of an injury, collagen is required to rebuild the damaged anatomic structure and restore its function. A therapeutic system could alter the wound healing process by interfering with any of the stages of wound healing[4].

Carica papaya L. (Caricaceae) is widely used in conventional medicine as papain, a proteolytic enzyme, the active principal provides protection against ulcers[5]. The *C. papaya* seed extract has been reported to possess high phenolic and flavonoid content having free radical scavenging property which helps in reducing wound inflammation. The papain and chymopapain present in *Carica papaya* seed extract cause proteolytic wound debridement, facilitating wound healing[6,7]. The presence of these phytoconstituents enables *C. papaya* to possess antimicrobial, antioxidant, and anti-inflammatory activities[8] that may be valuable in the treatment of chronic skin ulcers[9]. It is extensively used as an efficacious and easily accessible substance for wounds, especially burns, in developing countries. Care of wounds and maintenance entails a variety of procedures, including dressing.

Alginate is a biopolymer with numerous biomedical uses owing to its bio compatibility, nontoxic nature, and ease of availability. These attributes are also favourable for wound healing application[10]. By virtue of hydrophilic nature of alginate polymer, alginate-based hydrogel dressing have the potential to soak up unnecessary wound fluid, maintain an optimum hydration level at the wound site and reduce bacterial load at wound bed. Maintenance of moist milieu reduces the risk of scar formation, facilitates epithelisation of tissue and cell migration for wound healing. Also, moist conditions allow for effective debridement by clearing away of necrotic tissue, and foreign elements like microbes because of the sorption potential of hydrogel. Their mechanical strength enables them to act as a barrier to entry of microbes and foreign

bodies. The porous polymeric network permits exchange of gases allowing the tissue to breathe as elaborated by Kohler et al. in their comprehensive review on Hydrogel based wound dressings[11].

The present study was undertaken with the aim of developing a hydrogel sheet of sodium alginate loaded with bioactives of seed extract of *C. papaya* to expedite the wound healing process. Sodium alginate was chosen as the polymer as sodium alginate itself has been shown to possess wound healing properties and the hydrogel sheet would further aid in wound healing by absorption of exudates, allowing oxygen permeability, providing a protective covering over the wound and providing an optimum moist environment for better healing. Several studies have reported the use of sodium alginate either alone or in combination with antibiotics[12] inorganic substances[13] and plant extracts[14] for wound healing dressing. In fact, some alginate-based dressings have been commercialized like Nu-Gel (Systanix), Tegagel (3M GmBh)[11]. The novelty of the study lies in the fact that although alginate wound dressings have been developed earlier but none of the studies used a combination of papaya seed extract and sodium alginate as wound dressing, both being components aiding in expediting wound healing.

Several studies have been carried out on therapeutic effects of seed, pulp, peel extracts either in crude form or the form of dosage form. For example, seed extract has been investigated for contraception, antiulcerogenic activity in crude form and in the form of jelly for anthelminthic activity. Ethanolic seed extract of papaya has been studied for excision wound healing in rats by[15]. The results demonstrated hastened wound healing in comparison to the standard taken. Papain, an enzyme from the *Carica papaya* extract, added to sodium alginate membrane improved the healing wounds by improving debridement of necrotic tissue[16]. Papaya leaf extract formulated in the form of tablets and syrup form have been studied and used for treatment of dengue. Spray gel of papaya leaf extract was investigated for wound healing by Wijaya et al. and it showed accelerated wound healing as compared to placebo[17]. The novelty of the work lies in the fact that no work has been reported on the wound healing potential of seed extract of papaya along with sodium alginate in a dosage form. It was hypothesized that papaya seed extract along with sodium alginate in the form of hydrogel sheet will promote healing of wounds as sodium alginate alone has been shown to assist wound healing.

Sodium alginate hydrogel sheets loaded with papaya seed extract were formulated and evaluated for physicochemical characteristics like appearance, thickness, pH, moisture content, gelation time, weight variation, tensile strength,

water vapour transmission rate, folding endurance and swelling capacity. Animal studies were also carried out on rat model to study the effectiveness of the formulated hydrogel sheet in wound healing against the commercially available sodium alginate based wound dressings.

It was envisaged that the seed extract loaded hydrogel sheet would be an effective alternative to the usually used synthetic wound healing formulations as it would have the advantage of presence of sodium alginate, *C. papaya* seed extract and hydrogel sheet formulation.

METHODOLOGY

Chemicals and reagents

Glycerol was obtained from Fisher Scientific Pvt Ltd. Sodium alginate methylparaben, propylparaben and calcium chloride were procured from SD Fine Chem LTD. Ethanol and guar gum was procured from Loba Chemie Pvt Ltd.

Collection of seeds

The seeds of *Carica papaya* were collected from the local vendor, Solan.

Preparation of seed extract:

For the extraction of seed extract, the method given by Nayak et al. was employed with some modifications[15]. The seeds of *Carica papaya* were collected, washed thoroughly under running water to remove dirt and finally washed with distilled water. Seeds were shade dried for 15 days at room temperature. Then the seeds were dried at 40°C to get a constant weight and were powdered. The dry powder (25 g) was exhaustively macerated with water and alcohol (1:1) as the solvent. The extract obtained was filtered using Whatman filter paper and then, the extract was partially concentrated at 60 ± 2°C in vacuum oven to obtain the semi dried form.

Phytochemical screening

Preliminary qualitative phytochemical screening of the hydroalcoholic extract was conducted to ascertain the presence of secondary metabolites like alkaloids, glycosides, saponins, tannins and phenolic compounds, carbohydrates, terpenoids, and amino acids by using standard phytochemical screening and identification tests. Variation in color and form or formation of characteristic precipitate was noted to determine the presence of these secondary metabolites[18-23]. Hager's test, Wagner's test, and Mayer's test were used to check for the presence of alkaloids. The presence of Saponin glycosides was checked for by the foam test and cardiac glycosides were determined by Keller Killiani

test and Legal test. Lead acetate and Ferric chloride test were utilized to establish the presence of tannins and phenolic compounds. Carbohydrates were checked by Molisch, Benedict and Fehling's test and amino acids by Ninhydrin test. Salkowski and lead acetate tests were used to establish the existence of Terpenoids and Flavonoids respectively in the extract.

Physicochemical characterization of extract

Extract was characterized for pH, density and percentage yield. pH meter was calibrated at pH 4, 7, 9.2, and then pH was determined by directly dipping the electrode into the semi-dried extract till a constant pH was obtained. Density was measured by pycnometer/specific gravity bottle. The percentage yield of the extract was determined as percentage of the weight of the extract to the original weight of the dried powdered sample used.

Fourier Transform Infra-Red spectroscopy (FTIR):

The FTIR spectra were obtained by using ATR FTIR Spectrophotometer (Agilent technologies; Model: CARY 630). FTIR spectra were recorded for hydroalcoholic seed extract of *Carica papaya* and optimized formulation. The spectra were recorded in the range of 4000-650 cm^{-1} and were used to study extract-excipient interactions by checking for major alterations in peaks.

Preparation of hydrogel sheet

Hydrogel sheet was prepared by ionotropic gelation method. Hydrogel sheets were prepared by using the composition as given in Table 1. The required quantity of sodium alginate was dissolved in 15ml of distilled water. The solution was then continuously stirred for 30 min using a magnetic stirrer set at 300 rpm. The guar gum mixture was prepared in 5ml distilled water with specified amount of glycerol, propyl paraben, methyl paraben, and seed extract. Guar gum mixture was then added into the sodium alginate solution and stirred continuously. All weight calculations were done considering 30g as the final weight of hydrogel. For removal of air bubbles, the solution was placed in a bath sonicator for 10 min and then was poured into the petri dishes with 19 cm^2 surface area. Then 30 ml of 0.5% calcium chloride solution was prepared and poured over the petri dish containing extract-excipient mixture for the crosslinking of the sodium alginate polymer. The petri dishes were left undisturbed at room temperature for 12 h. Then the hydrogel sheet was removed from the petri dish. Finally, the hydrogel sheet was placed in the desiccator for drying and a clear thin hydrogel sheet was obtained[24].

Table 1. Composition of the hydrogel sheets

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Plant extract (w/w)	10%	10%	10%	10%	10%	10%	10%	10%	10%
Glycerol (w/w)	21%	21%	21%	21%	21%	21%	21%	21 %	21%
Methyl paraben (w/w)	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%
Propyl paraben (w/w)	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Sodium Alginate (w/w)	2.5%	1.5%	3.5%	2.5%	1.5%	3.5%	2.5%	1.5%	3.5%
Guar Gum (w/w)	1.2%	1.5%	0.8%	1.5%	0.8%	1.2%	0.8%	1.2%	1.5%
Distilled water (q.s)	30 g	30 g	30 g	30 g	30 g	30 g	30 g	30 g	30g

Physico-chemical characterization of hydrogel sheets

The hydrogel sheets were characterized as the following parameters:

Physical appearance: Physical parameters like color and appearance were determined visually.

pH: pH of the sheet was checked after adding 1-2ml of distilled water to a small area of sheet to wet and swell the hydrogel. The pH was then determined by using digital pH meter after calibrating it with buffer pH 4, 7, 9.2.

Homogeneity: All the prepared hydrogel sheets were checked for homogeneity by visual inspection.

Gelation time: The gelation time of the hydrogel was observed by tilting the petri dish containing the formulation at an angle of 45° periodically. The time at which the hydrogel did not flow at the tilted angle for at least 30s was taken as the gelation time[25].

Weight Variations: Analytical weighing balance was used for the study of weight variation of the formulated sheets. The data obtained was averaged for obtaining weight variation values. Three patches from each batch were taken for weight variation and result reported as (mean ± SD)[26].

Thickness: Vernier caliper was used for the evaluation of thickness of sheets. Sheet thickness uniformity was ensured by measuring its thickness at 6 different places. The values obtained were averaged and reported (mean ± SD)[27].

Folding Endurance: This test is conducted to ensure mechanical strength and plasticizer efficacy in hydrogel sheet. The folding of formulated hydrogel sheets at same place was carried out until breaking and cracking in the sheet

was observed and the number of folds till the sheet broke gave the folding endurance (n=3)[28].

Swelling Index Evaluation: The individual weights of prepared hydrogel sheets were taken and then sheets were dipped in water until they started to almost disintegrate. The initial and final weight of formulated sheets was used for the calculation of swelling index for the formulated sheets. The following equation was used for calculating % swelling index:

$$SI = (w2 - w1)/w1 \times 100$$

Where w1 is the sheets initial weight, w2 is final weight. The result obtained were averaged and reported (mean ± SD, n=3).

Tensile Strength: The tensile strength was measured for formulated sheets using the tensiometer (UTM (servo & Vector Model). It consists of two load grips; the lower one being fixed and the upper one being mobile. Film strips 2*2cm were fixed between these grips, and force was gradually increased till the film broke. The tensile strength was read from the dial in kg (n=3)[29].

Water Vapor Transmission Rate: Oven-dried bottles and individually weighed formulated sheets were used in this study to make transmission cells. Saturated solution of potassium chloride was kept in desiccator to maintain an approximate humidity of 85%, and 1 g of anhydrous calcium chloride was put in each bottle. The transmission cells with their mouth covered with hydrogel sheet were weighed and kept in the desiccator. These were removed from the desiccator after specified intervals of time, i.e., 6, 12, 24, 36, 48, and 72 h. The transmission cells were reweighed at the end of the study to get the result as mean ± SD (n=3)[30].

$$Water\ vapor\ transmission\ rate = W/ST$$

W = Final weight- initial weight

W is the increase in weight in 24 h; S is area of film exposed (m^2); T is exposure time.

Percentage Moisture Content: Formulated sheets were weighed initially and then kept in desiccator. Silica was also placed in desiccator for 24 h. The patches were kept in the desiccator till they attained a constant weight. The % moisture content value was estimated from the difference in the initial and final weights of sheets. The following equation was used to calculate the value of % moisture content. (n=3)

$$\%\text{Moisture Content} = \frac{wi - wf}{wi}\ x\ 100 \quad (5)$$

Where wi represents the initial weights, and wf represents the final weight[31].

Study of surface morphology

The surface morphological study of the optimized sheet was carried out by scanning electron microscopy (SEM). The mesh structures of the sample were observed using field-emission SEM (S-3000 N, Hitachi, Japan).

Accelerated stability study

Accelerated stability study for the optimized formulation was performed at a temperature of 40 ± 2°C and relative humidity of 75 ± 5% RH for 3 months. The hydrogel sheet was stored for the stability study for 3 months and was checked for physical appearance, physicochemical properties like swelling index, tensile strength by the methods used for the evaluation of formulated sheets stated above at 0, 1, and 3 months.

Incision wound model

In vivo protocol bearing number (LRIP/IAEC/2022/PH-03) for carrying out wound healing study was passed by IAEC of the institute. CPCSEA guidelines were followed for carrying out animal studies. Wistar rats (RattusNorvegicus) were purchased from National Institute of Pharmaceutical Education and Research (NIPER) Mohali. The animals were anesthetized with Ketamine and Xylazine. The animals were kept on the operating table in the common position. One paravertebral strength incision of six cm was made on either side of the vertebral segment with the help of the scalpel blade. The wound was cleaned with methylated spirit. The animals were kept in independent cages. The rats were divided into six groups and each group contained 6 rats. The experimental design of *in vivo* study is given in Table 2. The first group was normal control group on which 0.9% normal saline was applied, second group was the blank hydrogel group in which blank sodium alginate hydrogel sheet was applied, third group was standard control group on which a commercially available hydrogel (Tegaderm) was applied, and the fourth group was experimental group on which formulated hydrogel sheet (8% extract) was applied. Two more groups for hydrogel sheets having 10% (fifth group) and 12% (sixth group) extract concentration were used. The wounds were created by following the method of incision wound model. Wounds of normal control group were covered with a simple gauze dipped in normal saline; wounds of the standard control group were treated with Tegaderm hydrogel (commercially available alginate dressing), and the wounds of experimental group were covered with formulated hydrogel sheets. Dressings were changed every day. Progressive changes in the wound length at the 0th, 3rd, 7th, and 11th days of the treatment were photographed with camera and measured with help of graph paper[32-33].

Table 2. *In vivo* experimental design

S. No	Group	Drug	Route	No. of animals
1	Control group	Normal Saline	Topical	6
2	Blank Hydrogel	Sodium Alginate Hydrogel	Topical	6
3	Marketed formulation	Tegaderm Hydrogel	Topical	6
4	Test group 1st	8% Ext Hydrogel sheet	Topical	6
5	Test group 2nd	10% Ext hydrogel sheet	Topical	6
6	Test group 3rd	12% Ext hydrogel sheet	Topical	6

RESULTS and DISCUSSION

Preparation of seed extract

Fresh seeds of the papaya fruit were collected, washed and sorted. The seeds were shade dried for approximately 15 days, to prevent the loss of active constituents and further dried in an oven at 40°C to get a constant weight and then powdered coarsely. The powder was sieved through sieve No. 40 and stored in an airtight container till used for extraction. The dry powder (25g) was exhaustively macerated with water and alcohol (1:1) and extract obtained was filtered using Whatman filter paper. The extract was then partially concentrated at 60 ± 2°C in vacuum oven to obtain the semi dried form.

Phytochemical screening

Phytochemical screening of the extract obtained was carried out to check for the presence of alkaloids, glycosides, saponin glycosides, tannins, phenolic compounds, carbohydrates, flavonoids, cardiac glycosides, amino acids, and terpenoids. It was established that alkaloids, tannins, phenolic compounds, carbohydrates, and flavonoids were present while saponins, amino acids, and terpenoids gave negative results as shown in Table 3[15].

Table 3. Phytochemical screening of the extract

S. No.	Phytochemical tests	Result
1	Alkaloids	
1.1	Hagers test	+
1.2	Wagners test	+
1.3	Mayers test	-
2	Saponin glycosides	
2.1	Foam test	_
3	Cardiac glycosides	
3.1	Killer killani test	-
3.2	Legal test	+
3.3	Kedde test	-
4	Tannins and phenolic compounds	
4.1	Lead acetate test	+
4.2	Ferric chloride test	+
5	Carbohydrates	
5.1	Molish test	-
5.2	Benedict test	+
5.3	Felhing test	+
6	Amino acids	
6.1	Ninhydrin test	-
7	Terpenoids	
7.1	Salkowski test	-
8	Flavonoids	
8.1	Lead acetate test	+

Physiochemical evaluation of the extract

Results of physicochemical evaluation have been illustrated in Table 4. The extract after partial dehydration, gave a semi-solid product with a yield of 5.1 ± 0.25%, pH 5.8 ± 0.31, and density 1.2 ± 0.13 g/ml. The extract appeared to be brownish in colour.

Table 4. Results of physiochemical evaluation of the *C. papaya* seed extract

S. No.	Parameter	Observations (Mean ± SD*)
1	Percent yield	5.1 ± 0.25
2	Density	1.2 ± 0.13
3	pH	5.8 ± 0.31

*n=3

Preparation of hydrogel sheet

Hydrogel sheet was prepared by ionotropic gelation method by using a solution of calcium chloride. It was method of choice because the calcium ions absorbed during the process are exchanged for the sodium ions from the wound exudate and promote hemostasis by platelet activation. Also, this method is economical, simple and requires less equipment and time. Sodium alginate was selected because it was a bio compatible, non-toxic, non-immunogenic, biodegradable polymer and has an antimicrobial property with the advantage of gelation in presence of divalent cation[34]. Guar gum is a natural polysaccharide that had the ability to form hydrogen bonds with water. It is also used as a thickener and a stabilizer. Glycerol was used as a humectant, plasticizer, and bacteriostatic agent that allows the exudate to dry out and keeps hydrogel hydrated overlong periods of time. The combination of methylparaben and propylparaben was used as preservative, and it is commonly employed to increase the shelf life. Using the mentioned excipients and method, hydrogel sheet loaded with *Carica papaya* seed extract was successfully prepared.

Physicochemical evaluation of hydrogel sheets

The outcomes of physicochemical characterization of hydrogel sheet are revealed in Table 5. During the evaluation of the hydrogel sheet for different parameters, it was observed that the formulation F2, F5, and F8 were not suitable because minimal crosslinking has taken place in hydrogel due to the low concentration of polymer resulting in cracks and low mechanical strength[35]. Moreover, their gelation time was very long (48-49 h). It depends on the so-

dium alginate concentration and the concentration of divalent cations used for cross linking. When the sodium alginate solution is brought in contact with calcium chloride (or any other divalent cation), the calcium ions diffuse into the solution and crosslink the alginate. Gelation time varied from 12 h to 49 h. It was found to be longer in formulations composed of low concentration of sodium alginate[35].The thickness of the remaining hydrogel sheets was in the range of 2.1 0 ±.3 to 4.1 ± 0.15 mm[36]. Tensile strength indicates the strength and mechanical property of the sheet. The results for tensile strength were between 0.81 ± 0.18 to 1.82 ± 0.13 kg/cm^2 and the results of weight variation indicated uniformity of weight. The results revealed that change in the polymer concentration has effect on thickness and tensile strength[37]. With elevation in polymer concentration, thickness, and tensile strength were found to increase. The moisture content studies, showed less than 2.5% moisture in all formulations except F3, F6, and F9. Low moisture content is desirable as this results in stable formulation, reduced bulkiness and minimized microbial contamination during long term storage. The pH of all formulations was less than 7 (6.5-6.9) making them non-irritant to skin[22]. The folding endurance indicates mechanical strength of the formulations, and it varied from 162 ± 1.7 to 310 ± 1.8 for the various batches. These results indicate direct proportionality of folding endurance to polymer concentration[14]. F9 batch which has the highest polymer concentration (Sodium alginate + guar gum) has the maximum value of 310 while F5 batch having lowest concentration of polymers developed cracks at initial stages only. WVTR results (912-2110) show that it is inversely proportional to the polymer concentration in the formulation. The desirable range for WVTR is taken as 2000-2500 g/m^2/day as values within this range prevent maceration of wound by collection of exudates and also prevent excessive drying up of wound, thus providing an optimum hydration level for healing[38-39].

Table 5. Evaluation of hydrogel sheet formulations

Testing Parameter	F1	F2	F3	F4	F5	F6	F7	F8	F9
Physical Appearance	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
Color	Brown	Brown	Brown	Light brown	Dark brown	Light brown	Light brown	Dark brown	Light brown
pH	6.9 ± 0.2	6.5 ± 0.12	6.8 ± 0.2	6.7 ± 0.12	6.8 ± 0.2	6.5 ± 0.3	6.9 ± 0.3	6.8 ± 0.2	6.9 ± 0.12
Thickness** (mm)	2.4 ± 0.1	ND	3.5 ± 0.2	2.8 ± 0.3	ND	4.1 ± 0.15	2.2 ± 0.2	ND	4.5 ± 0.1
Weight uniformity*(g)	4.5 ± 0.02	ND	5.9 ± 0.25	5.5 ± 0.17	ND	6.1 ± 0.3	4.3 ± 0.3	ND	6.3 ± 0.2
Folding Endurance	215.67 ± 1.7	ND	254.33 ± 1.0	225 ± 1.89	ND	301 ± 1.7	192.67 ± 4.1	ND	310 ± 1.89
Swelling Index*(%)	79 ± 0.1	ND	71 ± 0.3	69 ± 0.2	ND	49 ± 0.12	33 ± 2.34	ND	60 ± 2.12
Moisture Content*(%)	1.7 ± 1.2	ND	3.2 ± 0.2	1.9 ± 2.1	ND	3.8 ± 0.2	2.1 ± 2.2	ND	4.5 ± 0.1
Tensile strength (kg/cm^2)	1.05 ± 0.20	ND	1.4 ± 0.02	1.21 ± 0.06	ND	1.54 ± 0.04	0.81 ± 0.18	ND	1.8 ± 0.13
Water vapor transmission* rate ($g/m^2/day$)	2110 ± 0.2	ND	1200 ± 1.2	2025 ± 2.2	ND	990 ± 1.2	1080 ± 1.1	ND	912 ± 2.2
Gelationtime* (h)	12	48	10	12	49	10	12	48	10

ND=Not Determined, *n=3, **n=6

Selection of optimized formulation

Selection of optimized batch of hydrogel sheet was done on the basis of results of physiochemical characterization. Folding endurance, tensile strength, swelling index, WVTR were considered as important parameters during selection as they influence the performance of the sheet. The selected batch was subjected to stability study, surface morphological study by SEM and would further be formulated at 2 more extract levels (8%, 12%) for *in vivo* study. F2, F5, and F8 were not evaluated further after initial characterization as they exhibited poor mechanical integrity and had developed surface cracks during the gelation process. This could be due to the improper crosslinking because of low sodium alginate concentration[35].

F1 was selected as the final formulation for further stability, SEM and *in vivo* studies as it had optimum thickness (2.4 ± 0.1 mm) for the ease of application, retention on skin and acceptability. It had adequate mechanical integrity exhibited by the results of tensile strength (1.05 ± 0.20 kg/cm^2) and folding endurance (215.67 ± 1.7)[14]. It had excellent swelling and absorption capacity of 79% for absorption of wound exudates and for providing a moist physiological environment for wound healing. Its water vapour transmission rate was also optimum between 2000-2500 to prevent maceration of wounds and for oxygen exchange for rapid healing[40]. Its gelation time of 12 h was also optimum. F3, F6, and F9 had high alginate concentration and were thick with low WVTR and were therefore not considered appropriate. F1 was further formulated at 2 more extract levels for *in vivo* studies.

Fourier Transform Infra-red (FTIR) study of extract and optimized formulation

FTIR spectra of the extract Figure 1(a) showed a broad peak on 3337 cm^{-1} due to presence of hydroxyl group, 1637 cm^{-1} due to presence of C=C stretching, 1044 cm^{-1} manifested the presence of C-O stretching[41]. The spectrum obtained corroborated well with the spectrum obtained by Prasetya et al.[31]. In FTIR spectrum of optimized formulation, Figure 1(b), the characteristics peaks of seed extract were retained with only minor shifts and changes in intensity indicating compatibility with excipients.

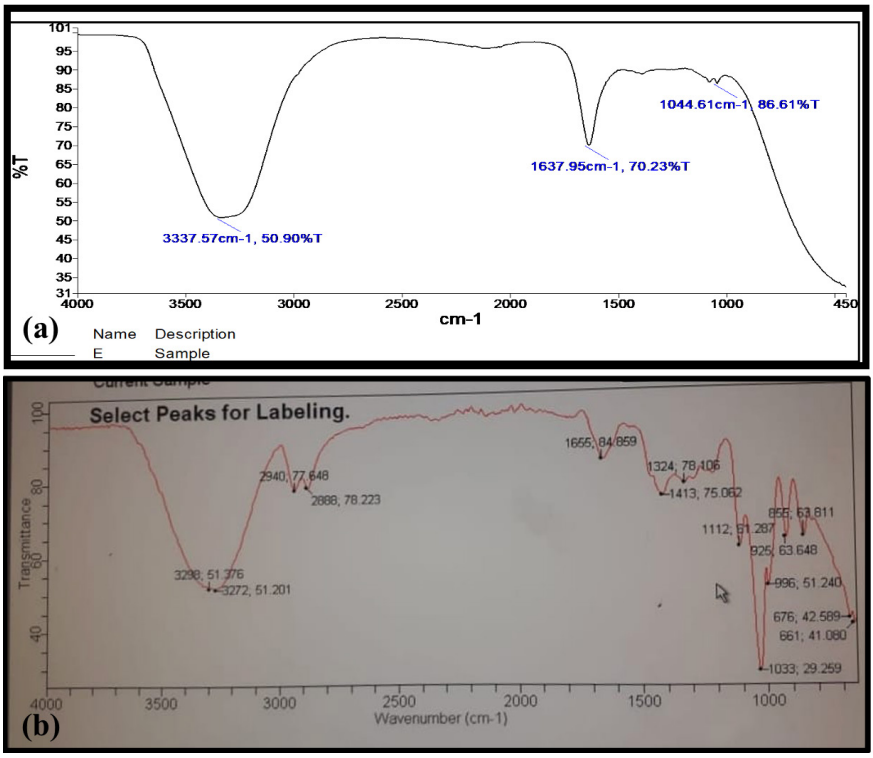
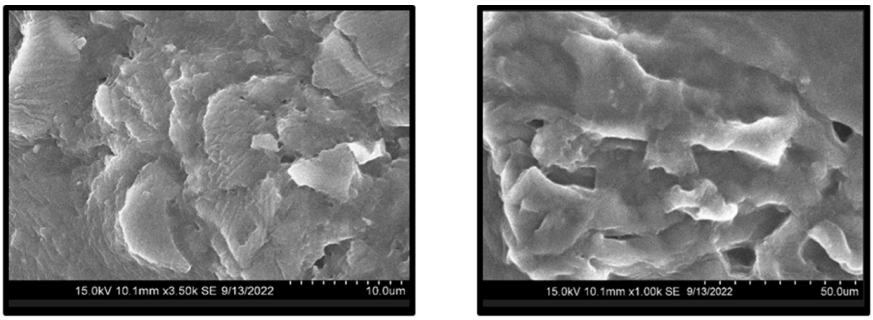


Figure 1. FTIR spectrum of (a) extract (b) optimized formulation

Scanning electron microscopy

Scanning electron microscopy was performed for F1. It revealed the surface morphology of the hydrogel sheet to be porous, rough and irregular (Figure 2[a] and [b]). These characteristics corroborate with the shrinkage occurring during the drying process. Based on SEM, formulation F1 was observed to possess optimum porosity, which would help prevent accumulation of exudates at the wound site and aid in exchange of gases[42].



(a) (b)

Figure 2. SEM of optimized (F1) formulation at (a) 2500X (b) 700X magnification

Accelerated stability study for physical properties of hydrogel sheet

Stability study is performed to predict the shelf life of a product by hastening the rate of decomposition, ideally by increasing the temperature and relative humidity. The optimized formulation (F1) was put through stability study as per ICH guidelines by storing at 40°C/75% RH for 3 months and samples were analysed for changes in physiochemical properties at regular intervals. The results are shown in Table 6.

No major changes were found on the tested hydrogel sheet during stability study. The tested formulation was observed to be stable after exposure to accelerated humidity and temperature environment for a period of 3 months confirming the stability of the formulation.

Table 6. Results of accelerated stability study

Stability Conditions	Sampling interval (month)	Color	Thickness (mm)	pH	Moisture content (%)	Tensile strength (kg/cm²)	Swelling index (%)	Water vapour transmission rate (g/m²/day)
40 ± 2°C / 75 ± 5 % RH	0	No change	2.4 ± 0.1	6.5 ± 0.12	2.5 ± 1.2	1.05 ± 0.20	79 ± 0.1	2110 ± 0.2
	1	No change	2.3 ± 0.1	6.6 ± 0.23	2.5 ± 3.2	1.07 ± 0.20	77 ± 1.1	2107 ± 0.2
	3	No change	2.1 ± 0.1	6.6 ± 0.12	2.4 ± 0.2	1.09 ± 0.20	78 ± 1.2	2105 ± 0.2

In vivo evaluation (incision wound model)

The wound contraction rate was measured at every time interval. It is the percentage reduction of wound size. It can also be treated as a percentage of wound protection. By using a transparency paper and a suitable marker, progress of wound healing was assessed on 1st, 3rd,7th, and 11th day post wound creation. Length of the wound was traced by a placing a transparent tracing

paper over the wound and then this was placed on a sheet of graph paper (2 mm) to count the number of squares within the wound length.

$$\textit{Wound closure \%} = \frac{\text{(Wound length on day O – wound length on day N}}{\text{Wound length on day O}} \text{ X 100}$$

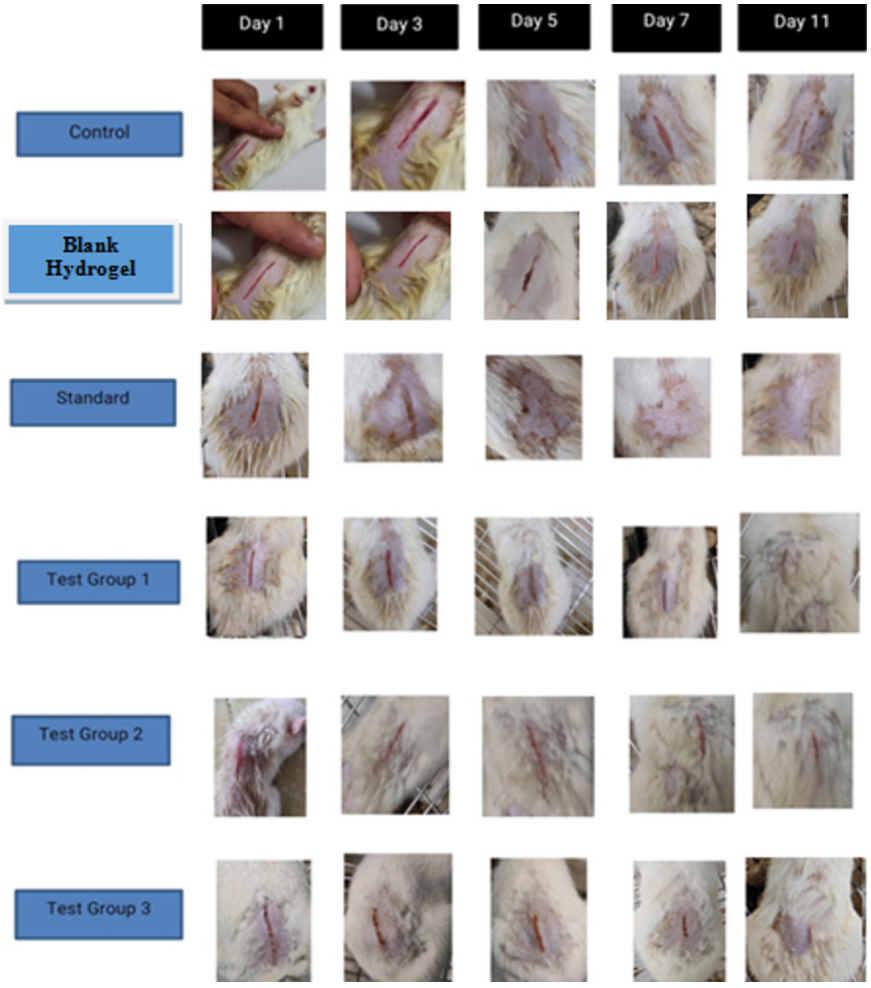


Figure 3. Progress of wound healing in different groups

Perusal of the data in Table 7 reveals that the rats treated with hydrogel sheet (12%) were found to possess accelerated wound recovery as compared to control, blank, test group 1 and test group 2 animals. The wound length of different groups was observed on 1st, 3rd, 5th ,7th, and 11th day for incision wound model and percentage wound contraction was calculated accordingly. The

standard group was treated with the commercially available Tegaderm™. The control group, blank hydrogel group, test group 1 and test group 2 rats showed lesser wound contraction while faster wound contraction was observed in the animals in test group 3 (12%) (Figure 3). The shorter epithelization period in test group 3 (12%) as compared to control and test group 1, 2, and blank hydrogel group might be due to rapid regeneration of epithelial cells. Therefore we can conclude that the *Carica papaya* hydrogel sheet with 12% extract accelerates wound healing more as compared to the control group, blank hydrogel, test group 1 and 2, and its wound healing potential was found to be insignificantly different from the standard group (Tegaderm) at 95% confidence interval (calculated p=0.9984) making it comparable or similar to the commercially available formulation Tegaderm™.

Table 7. Assessment of wound healing (Wound length in mm)

Groups	Day 1	Day 3	Day 5	Day 7	Day 11
Control Group (mm)	60 ± 0.2 (0%)	55 ± 1.1 (8.3%)	47 ± 1.2 (21.6%)	38 ± 0.2 (36.6%)	31 ± 0.1 (48.3%)
Blank Hydrogel	60 ± 0.2 (0%)	52 ± 1.8 (13.3%)	43 ± 0.2 (28.3%)	35 ± 0.2 (41.6%)	28 ± 0.1 (53.3%)
Standard (marketed) Group (mm)	60 ± 0.3 (0%)	50 ± 1.2 (16%)	41 ± 1.1 (31%)	20 ± 0.3 (66%)	1 ± 0.2 (98.3%)
Test group 1 (mm)	60 ± 0.1 (0%)	54 ± 0.2 (10%)	45 ± 1.4 (25%)	26 ± 1.1 (56%)	11 ± 0.2 (81.6%)
Test group 2 (mm)	60 ± 0.1 (0%)	53 ± 0.3 (11%)	44 ± 2.1 (26%)	25 ± 0.2 (58%)	8 ± 0.1 (86.6%)
Test group 3 (mm)	60 ± 0.2 (0%)	51 ± 0.2 (15%)	42 ± 0.1 (30%)	21 ± 0.2 (65%)	1.5 ± 0.1 (97.5%)

n=6 for each group

STATEMENT OF ETHICS

In vivo studies were approved by IAEC of L.R Institute of Pharmacy, Solan vide protocol number LRIP/IAEC/2022/PH-03.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept – I.D., S.A.; Design – İ.D., S.A.; Supervision – S.A., A.K.; Resource – S.A.; Materials – I.D., S.A.; Data Collection and/or Processing – I.D., S.A.,

A.K.; Analysis and/or Interpretation - I.D., S.A., A.K.; Literature Search – I.D., S.A.; Writing - I.D., S.A., A.K; Critical Reviews – S.A., A.K.

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