RESEARCH ARTICLE

# Development of Vancomycin-Loaded Polysaccharide-Based Hydrogel Wound Dressings: *In Vitro* and *In Vivo* Evaluation

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## Abstract

**Aim:** The objective of this study was to develop and evaluate carboxymethylcellulose (CMC) and tamarind gum (TG)-based citric acid cross-linked vancomycin-loaded hydrogel dressing for wound healing. **Materials and Methods:** CMC and TG are known for its biocompatibility and biodegradability and hence selected for preparation of hydrogel dressings. The hydrogel films were characterized by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, solid-state $^{13}$C-nuclear magnetic resonance ($^{13}$C-NMR) spectroscopy, differential scanning calorimeter (DSC), and scanning electron microscopy (SEM). The prepared hydrogel films were evaluated for the carboxyl content and equilibrium swelling ratio. Vancomycin hydrochloride was loaded into hydrogels films and drug release was studied in the phosphate buffer pH 7.4. The hemolysis assay was used to study the biocompatibility of hydrogel films. The wound healing activity of optimized hydrogel film was studied in the albino mice of either sex. **Results and Discussion:** The results of ATR-FTIR, solid-state $^{13}$C-NMR, DSC, and SEM confirmed the formation of citric acid cross-linked hydrogel films. The total carboxyl content of hydrogel film was found to be increased when polymer ratio and amount of cross-linker was increased. The prepared hydrogel dressing showed pH-dependent swelling and swelling was decreased significantly with increase in the concentration of TG and cross-linker. Optimized hydrogel dressing batch HD1 exhibited highest drug loading with non-Fickian drug release. Hydrogel dressings were found to be hemocompatible and exhibited wound healing activity in mice. **Conclusion:** It can be concluded that the citric acid cross-linked polysaccharide-based hydrogel dressings have potential application in the development of topical drug delivery systems for treatment of the wound.

**Key words:** Carboxymethylcellulose, citric acid, hydrogel wound dressing, tamarind gum, vancomycin

## INTRODUCTION

Wound infection is a serious complication and requires specialized care.[1] The common problem associated with the wound infection is the local tissue damage. It is characterized by signs of redness pain, swelling, raised temperature, and fever. The wound infections may lead to life-threatening sepsis and multiple organ failure due to systemic inflammatory and immunological response. It is the major problem in the field of wound care management because such infections can cause the formation of wound exudates, delay in the wound healing, and facilitate improper collagen deposition, etc. In such cases, early and appropriate clinical treatments are necessary to reduce the mortality rates associated with the injury. Wound closure is an important goal in the treatment of deep and large wounds in which the mortality rate is high.[2,3] As per the report of the WHO, approximately 30,000 deaths have been reported due to wound complications. The annual cost of caring for chronic wounds in USA has reached to 25

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billion USD. In addition, the wound management markets are estimated to raise a value of 4.4 billion USD in 2019 compared to the value of 3.1 billion USD in 2012.[4] Traditional wound dressing such as gauze, cotton, and wool causes inflammation further leading to wound debridement, thus delaying the wound-healing process. Therefore, bio-based polymeric dressings have drawn the attention of researchers as an alternative approach to deal with the problems of traditional dressing materials.[5]

Polysaccharide-based hydrogel dressings are potential choice for development of wound dressings because of high fluid retention capacity and absorb high volume of wound exudate.[6] Polymeric hydrogel-based wound dressings provide an ideal moisture environment required for healing while protecting the wound. Such moisturous environment not only calms the patient by providing the cooling effect but also offers additional advantages such as non-adherence of dressing to wound tissue,[2] easy removal, accelerated healing, reduction in pain, and inflammation.[4] These moist dressings have the ability to heal the wound faster than the traditional dry dressings.[7] Hydrogels promote fibroblast proliferation and keratinocyte migration which are required for complete epithelialization of the wound. Biomolecules can be easily loaded into the hydrogels. When the hydrogel comes in contact with the wound exudates, it swells and releases the biomolecules.[2] Due to tight mesh size, hydrogels prevent bacterial entry to the wound and allow controlled transport of bioactive molecules to the wound bed. The use of synthetic polymers such as polyvinylpyrrolidone,[4] carbopol,[8] polyvinyl alcohol,[9] and natural polymers such as chitosan,[3] sodium alginate,[6] xanthan gum,[10] and gelatin[11] are reported in the literature for preparation of hydrogel wound dressings. Natural polymers are extensively used nowadays due to their biocompatible and biodegradable nature.[12]

Carboxymethylcellulose sodium (CMC) is a biodegradable, easily available, and considered an ideal candidate for preparation of hydrogels. CMC is water-soluble anionic cellulose ether of commercial importance. It has hydrophilic carboxylate groups in their backbones. It is widely used in pharmaceuticals, cosmetics, and foods.[13] The hydrogels prepared using alone CMC have poor matrix integrity due to reduced interpolymer cross-linking. Therefore, the presence of another polysaccharide is necessary to promote cross-linking.[14]

Tamarind gum (TG) is a natural polysaccharide extracted from the endosperm of *Tamarindus indica* L. seeds belonging to the family Leguminosae. Indian production of tamarind is about 0.3 million tons per year.[15] TG is composed of (1→4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and β-D-galactopyranosyl (1→2)-α-D-xylopyranose linked (1→6) to glucose residues.[16] It is non-carcinogenic, non-toxic, biocompatible, and stable at wide range of pH. TG is used as binder, gelling agent, thickener, emulsifier, suspending agent, and release modifier in different pharmaceutical formulations. It also acts as stabilizer in food and pharmaceuticals.[17] Tamarind polysaccharide is promising excipient, which is being used and investigated for the preparation of various dosage forms.[15,18–22] The presence of hydroxyl groups in TG makes it suitable for the preparation of physically[23] or chemically[24] cross-linked hydrogels.

The use of alone CMC as functional materials for medical applications is limited because of their strong absorbent ability and the poor mechanical property. It has been used in the combination with the polymers such as hydroxyethylcellulose[25] and polyvinyl alcohol[26] to improve the matrix integrity of the hydrogels. However, no study has been reported till date, where CMC and TG have been used together for preparation of hydrogel wound dressings for drug delivery.

Vancomycin is a glycopeptide antibiotic which acts by inhibiting the bacterial cell wall synthesis. It is effective against Gram-positive bacteria. However, due to its toxicity after parenteral administration and high cost, its clinical applicability is limited.[27] To improve the therapeutic outcome of vancomycin, novel hydrogel dressing has been developed for topical delivery.

Hence, the present work was aimed to synthesize and characterize citric acid cross-linked polysaccharide-based hydrogel dressings. The hydrogel dressings were evaluated for swelling study and biocompatibility by the hemolytic assay. Vancomycin hydrochloride (VH) was used to study drug loading, drug release, and *in vivo* wound healing activity from the hydrogel dressings.

## MATERIALS AND METHODS

TG (MW-1.5 × $10^5$ g/mol) was kindly gifted by Chhaya Industries, Barshi, Maharashtra, India. VH was obtained as a gift sample from Cipla India Pvt., Ltd., Goa. Sodium CMC (degree of substitution - 0.7 and average molecular weight - ~250,000) and citric acid (anhydrous) were supplied by Sigma Aldrich, Mumbai, Maharashtra, India. All other chemicals were of analytical grade and supplied by Loba Chemie, Mumbai, Maharashtra, India.

### Preparation of citric acid cross-linked polysaccharide-based hydrogel dressing

Hydrogel dressings were prepared using previously reported method with slight modification.[28,29] In brief, 2% w/v aqueous solutions of the TG and CMC were prepared. Initially, TG was added to the water and stirred using mechanical stirrer (RQG126D, Remi, India) at room temperature. Then, CMC and citric acid were added into it and stirred for another 1h. The solutions were kept overnight to remove entrapped air bubbles. The aqueous solutions were casted into Petri dishes of uniform size (9 cm diameter) and dried at 50°C for 24 h. The resulting dressings were cured at 140°C for 5 min. The cured hydrogel

dressings were washed with distilled water and isopropyl alcohol for 1 h to remove the untreated entities. Then, the hydrogel dressings were dried in a hot air oven at 50°C for 24 h and stored in a desiccator until further use. For optimization, the parameters such as concentrations of polymer ratio and citric acid were varied to get the hydrogel wound dressings [Table 1].

### Total carboxyl content of hydrogel dressing

Acid–base titration was used to determine total carboxyl content of the hydrogel dressings. Hydrogel dressing (100 mg) was dispersed in 20 ml 0.1N NaOH and stirred on a magnetic stirrer (Remi, India) for 2 h. Sodium hydroxide breaks down the ester linkages and reacts with the free carboxyl groups to form sodium carboxylate (citrate). The excess amount of 0.1 N NaOH was titrated with 0.1 N HCl using phenolphthalein as an indicator.[30] The carboxyl content in milliequivalent per 100 g of hydrogel dressing was calculated as given below:

$$\text{Carboxyl content} = \frac{(V_b - V_a) \times N \times 100}{W} \quad (1)$$

where N is the normality of HCl (eq/L), Vb and Va are the volumes of HCl in the absence and presence of a sample, and W is the weight of the sample (g).

### Characterization of the hydrogel wound dressings

The infrared spectra of TG, CMC, citric acid, and citric acid cross-linked hydrogel dressings were obtained using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectrophotometer (Shimadzu, Miracle 10, IR Affinity, Japan). The samples to be analyzed were placed onto the ATR and spectra were recorded in the range of 600–4000 $cm^{-1}$ at an average of 25 scans and resolution of 4 $cm^{-1}$.

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of TG, CMC, citric acid, and cross-linked hydrogel dressing were performed using Mettler-Toledo TGA/DSC1 thermogravimetric analyzer (Mettler-Toledo, Switzerland). Samples were heated from 30°C to 300°C at the rate of 10°C/min, under a nitrogen atmosphere (flow rate: 10 ml/min).

**Table 1:** Composition of hydrogel dressings

| Parameter | Batch code | | | | |
|---|---|---|---|---|---|
| | HD1 | HD2 | HD3 | HD4 | HD5 |
| CMC: TG * | 1:1 | 1:2 | 1:3 | 1:1 | 1:1 |
| Citric acid (mg) | 200 | 200 | 200 | 300 | 400 |
| Curing temperature (°C) | 140 | 140 | 140 | 140 | 140 |
| Curing Time (min) | 5 | 5 | 5 | 5 | 5 |

TG: Tamarind gum, CMC: Carboxymethyl cellulose (sodium salt), *Total polymer concentration equivalent to 2% w/v

Solid-state $^{13}C$ cross-polarization-magic angle spinning NMR spectra of TG, CMC, and citric acid cross-linked hydrogel dressing were measured using JEOL-ECX400 spectrometer operating at 400 MHz (contact time of 3.5 ms, a relaxation delay of 5s, sweep width of 35 kHz, and spinning speed of 10 KHz). The chemical shifts were calibrated with the external hexamethylbenzene standard methyl resonance at 17.3 ppm.

The surface morphology of citric acid cross-linked hydrogel dressings was studied using scanning electron microscopy (SEM), JEOL 6386®, Japan. Samples were placed onto double-sided tape, sputter coated with platinum, and examined under the microscope at 18 kV.

### Swelling and wound fluid absorption study of hydrogel dressing

The swelling of hydrogel dressing was determined in phosphate buffer pH 7.4. The known quantity (~0.2 g) of hydrogels was immersed in the swelling medium (10 ml) at room temperature. The swollen samples were removed at predetermined time intervals for 24 h, the excess medium was blotted using tissue paper and weight (AX 120, Shimadzu, Japan) of swollen hydrogel was determined.[31] The equilibrium swelling was calculated using following formula:

$$\text{Equilibrium swelling (\%)} = \frac{(W_T - W_0)}{W_T} \times 100 \quad (2)$$

where $W_T$ is the weight of swollen hydrogel at time T and $W_0$ is the weight of dry hydrogel dressings before the start of the study. All measurements were done in triplicate. Furthermore, swelling study was performed in 0.1N HCl, water, and 0.9% w/v NaCl. Similarly, wound fluid absorption capacity was carried out in simulated wound fluid (SWF).[32]

### Drug loading

The loading of a VH into hydrogel dressing was carried out by swelling equilibrium method. In brief, preweighed hydrogel dressings (200 mg) were placed in a 20 ml aqueous solution of VH (5 mg/ml) for 2h and hydrogel dressings were dried in hot air oven at 40°C for 24 h. To determine the amount of drug loading, VH-loaded hydrogel was cut into small pieces, weighed, and immersed in 50 ml water. The dispersion was stirred on a magnetic stirrer (Remi, India) at 100 rpm for 24 h and amount of VH was determined spectrophotometrically (Shimadzu, Japan) at 280 nm.

### *In vitro* release

*In vitro* release studies of the VH were carried out by keeping the VH-loaded hydrogel dressings (100 mg) in 10 ml of phosphate buffer pH 7.4 at 37°C. The samples were

withdrawn at a predetermined time interval and replaced with fresh dissolution medium to maintain sink condition. The amount of VH release was determined using an ultraviolet-visible spectrophotometer (Shimadzu, Japan) at 280 nm after 15 min up to 300 min. The experiments were performed in triplicate.[27] *In vitro* release of VH was also performed in the 0.1N HCl.

### Hemolysis assay

Hydrogel dressings (2 $cm^2$) were equilibrated in phosphate buffer saline for 60 min at 37°C and human citrate-phosphate-dextrose (CPD) blood (0.5 ml) was added on dressings. After 20 min, 4.0 ml of 0.9% sodium chloride (NaC1) saline was added to each sample to stop hemolysis and the samples were incubated for 60 min at 37°C. Positive and negative controls were obtained by adding 0.5 ml of human CPD blood and 0.9% NaCl saline, respectively, to 4.0 ml of double-distilled water. The incubated samples were centrifuged for 10 min at 3500 rpm, the supernatant was taken, and its absorbance was measured on a spectrophotometer (Shimadzu, Japan) at 545 nm.[29] The percent of hemolysis was calculated using the following relationship:

$$\text{Hemolysis (\%)} = \frac{(A_{\text{Test sample}} - A_{\text{-ve control}})}{(A_{\text{+ve control}} - A_{\text{-ve control}})} \times 100 \quad (3)$$

where A is the absorbance. The absorbance of positive and negative controls was found to be 3.614% and 0.011%, respectively.

### *In vivo* wound healing activity of hydrogel dressing

Swiss albino mice (25–30 g) of either sex were procured from the National Institute of Bioscience, Pune, after getting ethical approval from Institutional Animal Ethics Committee, 7 days before the commencement of the study. Animals were placed in polypropylene cages in a controlled room temperature (22 ± 1°C) with a relative humidity of 60–70% in the registered animal house (1915/PO/ReBi/CPCSEA dated 04/11/2016). They were maintained with standard pellet diet (Amrut, Sangali, India), water *ad libitum* with 12:12 light and dark cycle. Mice were randomly divided into three groups:

- Negative control group in which wound was left to heal spontaneously,
- Group I in which wound was treated with blank hydrogel dressing,
- Group II in which wound was treated with VH-loaded hydrogel dressing.

Mice were anesthetized with diethyl ether and the surgical area was shaved. The wound-specific size was created on the dorsal side of the mouse using surgical scissors. The wound was cleaned with a cotton swab soaked in 70% alcohol. The contraction of the wound was measured on the 4th, 8th, and 12th day by tracing the raw wound area on transparent paper. Then, wound tracings were retraced on a millimeter scale graph paper, to determine the wound area. Wound contraction was measured using following formula: [32,33]

$$\text{Wound contraction (\%)} = \frac{(\text{Initial wound size} - \text{wound size on specific day})}{\text{Initial wound size}} \times 100 \quad (4)$$

Wound contraction of negative control, Group I, and Group II were compared.

### Statistical analysis

The numerical data were statistically analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered as statistically significant.

## RESULTS AND DISCUSSION

Preparation of citric acid cross-linked polysaccharide-based hydrogel dressing

From the preliminary studies, we found that the hydrogel dressings prepared using CMC or TG alone led to the formation of hydrogels with poor integrity (CMC hydrogels) or poor swellability (TG hydrogels). Hence, it was decided to use the CMC and TG in combination to overcome the problems of individual polymers. The total polymer concentration was fixed to 2% and CMC: TG ratio was varied from 1:1 to 1:3. The preformulation batches were prepared in order to optimize the concentration of citric acid, curing temperature, and curing time. The prepared hydrogel dressings were washed with distilled water to remove unreacted citric acid and polymer.

At a very low concentration of citric acid, the hydrogel dressings were not formed due to poor cross-linking which may have caused a loss in the integrity of dressing. At high concentration of citric acid, the hydrogel dressing formed was rigid which reduced the ability of film to absorb water.[34] This rigidity and poor ability to absorb water might be due to increase in cross-linking density which leads to the reduction in the mobility of polymer chains and reduced the free volume of the hydrogel network.[8] The curing temperature was varied from 130°C to 150°C. At lower temperature, poor cross-linked gel was formed, whereas at elevated temperature firm and tough hydrogel film was formed. This may be due to the formation of strong hydrogen bond interactions between polysaccharides (CMC and TG) and citric acid, leading to a reduction in expansion and relaxation of polymer chains [34]. The optimum swelling was observed at a curing temperature 140°C. The curing time was varied from 5 to 15 min. The curing time of 5 min was found to be sufficient to form citric acid cross-linked hydrogel film while higher curing time led

to the formation of a brown film with insufficient swelling. As stated earlier, this may be exhibited due to the thermal degradation along with increased interpolymer cross-linking.

The esterification reaction is responsible for the formation of cross-links between citric acid and polysaccharides (CMC and TG). The possible reaction is given in Figure 1. When citric acid heated at high temperatures, there is the formation of intermediate cyclic anhydride which is the base mechanism responsible for the development of cross-links in between CMC and TG. The formed intermediate cyclic anhydride reacts with the OH functional groups of neighboring polysaccharide chains through esterification. The carboxylic acid formed as a result of this interaction can form new intramolecular anhydride moiety with neighboring carboxylic acid unit.[35,36] In esterification process, there may be involvement of primary –OH groups of polysaccharides as it is more reactive than the secondary –OH groups. The cross-linking is generally done under dry state and requires high temperatures for the reaction.[37]

The average thickness of citric acid cross-linked CMC/TG hydrogel dressing was found to be in the range of 155–193 µm. The variation in thickness of hydrogel dressings may be due to variation in polysaccharide concentration.

### Total carboxyl content of hydrogel dressing

The carboxyl content of hydrogel dressings was observed in the range of 408–259 mEq/100 g of hydrogel [Table 2]. The ratio of polysaccharide and concentration of citric acid had a significant effect on carboxyl content. When the TG was increased in the hydrogel dressings (HD1 to HD3), the carboxyl content was found to be increased. The possibility of an increase in carboxyl content may be due to the participation of the TG in the esterification reaction with citric acid. An increase in the concentration of citric acid from 10 to 20% (HD1, HD4, and HD5) increases the carboxyl content of hydrogel dressing. This may be because, when the film was heated, high amount of intermediate cyclic anhydride of citric acid was available for the cross-linking of polysaccharides leading to increased carboxyl content.

### Characterization of the polysaccharide-based hydrogel dressings

ATR-FTIR spectra of CMC, TG, citric acid, and hydrogel dressings are given in Figure 2. ATR-FTIR spectrum of TG exhibited strong broad peaks at 3500–3000 $cm^{-1}$ belonging to stretching vibration of -OH. A strong peak at 1039 $cm^{-1}$ and 1143 $cm^{-1}$ were attributed to the C-O stretching vibration of the alcoholic group. The medium peak at 2920 $cm^{-1}$ can be ascribed to asymmetric stretching of CH. The peaks at 1747 $cm^{-1}$ and 1689 $cm^{-1}$ were due to carbonyl (-HC=O) stretching.[38] The spectrum of citric acid showed a broad peak at 3279$cm^{-1}$ attributed to -OH stretching and a sharp peak at 1693 $cm^{-1}$ due to hydrogen bonded C=O stretch. The spectrum of CMC showed the broad peaks at 3500 $cm^{-1}$–3000 $cm^{-1}$ due to -OH stretching. The band at 2922 $cm^{-1}$ is due to C-H

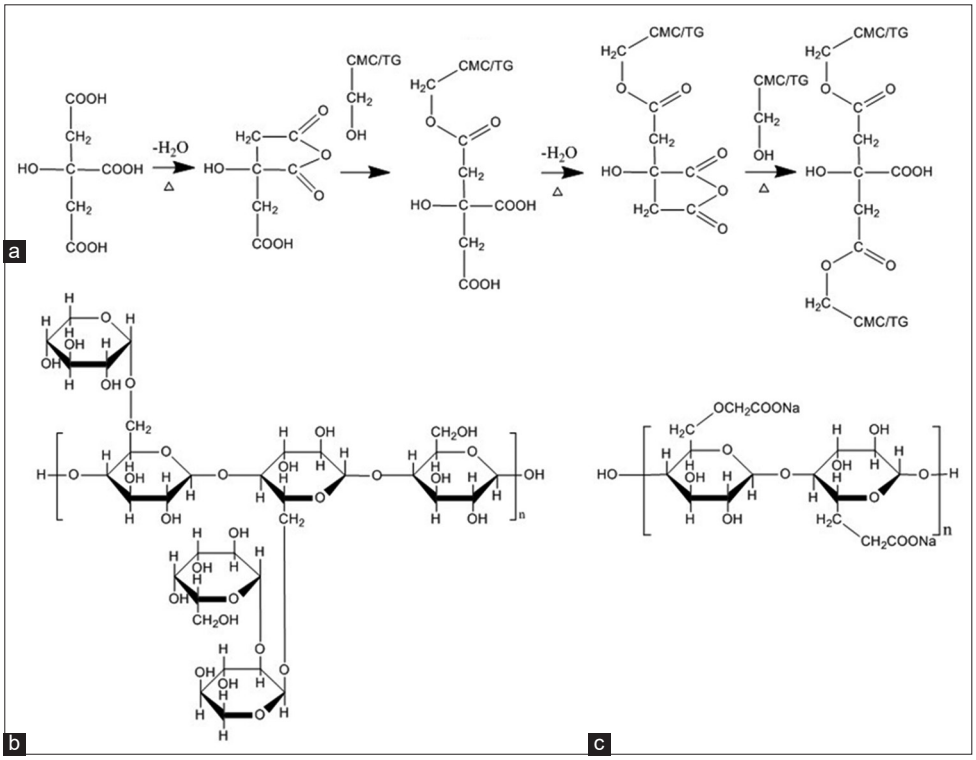


**Figure 1:** Possible cross-linking reaction between citric acid, tamarind gum (TG), and carboxymethylcellulose (CMC) (a) and structure of TG (b) and CMC (c)

**Table 2:** Evaluation of hydrogel films

| Batch | Total carboxyl content (mEq/100 g) | Drug loading* (mg/g) | SWF absorption capacity (%) | Hemolysis (%) |
|---|---|---|---|---|
| HD1 | 259.6±8.8 | 374.6±5.47[a] | 1775.4±25.7 | 1.56 |
| HD2 | 322.9±7.5 | 331.5±5.17[a] | 1124.9±23.6 | 1.14 |
| HD3 | 334.1±6.7 | 259.1±4.91[a] | 958.3±24.5 | 2.57 |
| HD4 | 338.6±8.3 | 294.6±2.51[a] | 762.8±21.9 | 1.79 |
| HD5 | 408.3±9.4 | 139.4±2.78[a] | 676.2±20.4 | 1.93 |

SWF: Simulated wound fluid, HD: Hydrogel dressing*, Mean ± standard deviation, [a]significantly different ($P$<0.05) than HD1

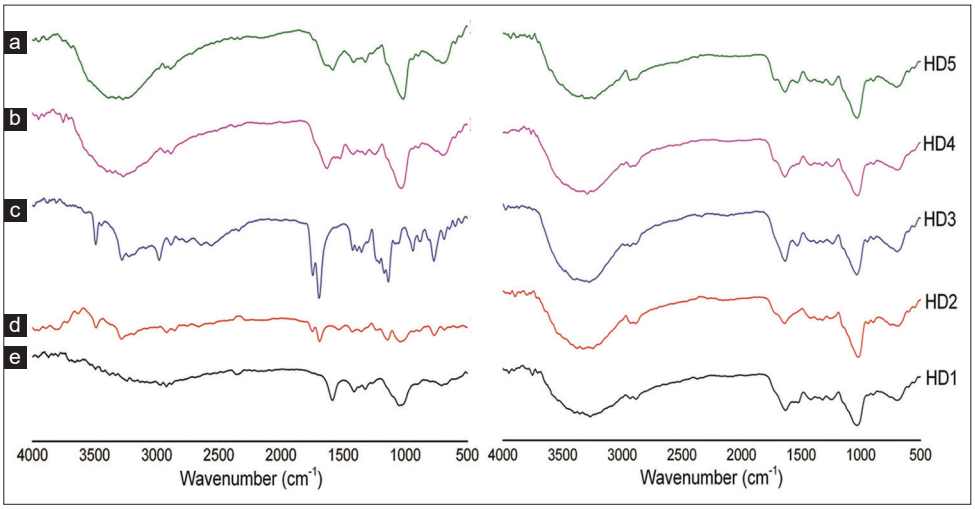


**Figure 2:** Attenuated total reflectance-Fourier transform infrared spectra of carboxymethyl cellulose (e), tamarind gum (d), citric acid (c), hydrogel dressing (b), and NaOH-treated hydrogel dressing (a) and hydrogel dressings (batch hydrogel dressings HD1-HD5)

stretching vibration. The presence of a strong absorption band at 1587 $cm^{-1}$ and 1411 $cm^{-1}$ were due to the asymmetric and symmetric stretching of $COO^-$ group, respectively. The peak at 1323 $cm^{-1}$ was due to CH-O-$CH_2$ stretching. The ATR-FTIR spectra of hydrogel showed an additional peak at 1710 $cm^{-1}$–1730 $cm^{-1}$ which can be attributed to the carbonyl band of free carboxylic acid groups and the carbonyl band of the ester formed during the CMC-TG, CMC-CMC, and TG-TG cross-linking. To confirm the formation of ester linkages, hydrogel was treated with methanolic 0.1N NaOH so as to separate ester carbonyl band from the acid carbonyl band by converting the carboxylic acid group to carboxylate anions. In case of NaOH-treated batch [Figure 1a], additional two bands were observed at 1742 $cm^{-1}$ (ester carbonyl) and 1589 $cm^{-1}$ (carboxylate).[29]

The TGA and DSC thermograms of hydrogel dressings are given in Figure 3. Thermal decomposition curve of TG showed two main stages of decomposition. The first stage begins at 35°C and ends at 100°C. This may be due to the removal of free and bound water from the polymer. The second stage of weight loss was observed at 228°C–300°C with 35% loss of weight. The CMC showed two main stages of decomposition. The first stage begins at 35°C and ends at 84°C occurring due to the removal of free and bound water from the polymer. The second stage of weight loss was observed from 225°C to 325°C with 38% loss of weight. The weight loss in both polymers in the second stage is attributed to the decomposition of the polymer backbone. The decomposition of anhydrous citric acid started at 160°C and ended at 349°C with 93% weight loss. In case of hydrogel dressing, thermal decomposition started at 199°C and ended at 389°C with 47.5% loss of weight. It indicates that the decomposition of citric acid cross-linked CMC/TG hydrogels is slower than individual polymers. The thermal stability of prepared hydrogel dressing was improved than individual polymer due to the formation of cross-links between CMC and TG by citric acid. The DSC thermogram of hydrogel dressing showed a considerable change in the heat flow indicating the formation of ester cross-links. Observations are in agreement with the earlier reports with CMC and hydroxyethyl cellulose.[25]

The solid-state $^{13}$C-nuclear magnetic resonance ($^{13}$C NMR) spectrum of TG shows three distinct peaks [Figure 4c]. The resonance peak at 105 ppm is assigned to an anomeric carbon atom (C1) and the peak at 74 ppm is assigned to the carbon atoms (C2 to C5) connected to –OH groups (i.e., the carbon atoms in the six-member ring except C1 carbon atom). The presence of a peak at 63 ppm is attributed to the C6 carbon atom of $CH_2OH$ group. The solid-state $^{13}$C NMR spectrum of CMC shows four distinct peaks [Figure 4b]. The resonance

peak at 105 ppm is attributed to anomeric carbon (C1) of glucose units and the peak at 75 ppm is due to the carbon atoms (C2 to C5) in the six-member ring and the C7 carbon atom in methyl group of the carboxylated side chain. The peak at 178 ppm is due to the carbonyl carbon of substituent groups $–CH_2COO^-$. The solid-state $^{13}C$ NMR of prepared citric acid cross-linked hydrogel dressing [Figure 4a] of TG-CMC shows broad resonance peak due to ester cross-links and free –COOH groups in the range of 174–182 ppm which confirms cross-linking. Thus, the results of ATR-FTIR, solid-state $^{13}C$ NMR, and thermal study of hydrogel dressings confirmed the formation of ester cross-links between CMC and TG.

The surface morphology of the blank hydrogel dressings was studied using SEM [Figure 5]. The SEM image of the surface of hydrogel dressing HD1 showed a rough surface with pores and/or cracks. Furthermore, smooth particles <10 μ were observed in the dressing. These particles joined together to form the hydrogel film which consists of pores. This might be due to the low cross-linking density of hydrogel film. When the concentration of TG was increased in hydrogel film, roughness of film was found to be reduced and shows less porosity than HD1. This may be due to increase in the cross-linking density of film which suggests the participation of TG in cross-linking reaction. SEM of hydrogel films indicates the cross-linking has taken place between CMC and TG.

## Swelling and wound fluid absorption study

The swelling study was performed in phosphate buffer pH 7.4. The result of swelling study is given in Figure 6. The batch HD1 exhibited a maximum equilibrium swelling of 533%, whereas batch HD5 showed a minimum equilibrium swelling of 164%. When the hydrogel is in contact with medium, its three-dimensional hydrophilic network expansion is prevented by the cross-links and provides an elastic response which affects the swelling of hydrogel film.[39] All hydrogel batches showed steady equilibrium swelling after 1 h. The swelling ratio of the hydrogel at equilibrium is dependent on the ratio of polymer and the concentration of citric acid. As the polymer ratio was increased, the equilibrium swelling ratio was found to be decreased significantly (batch HD1 to HD3). This may be attributed to the high amount of TG and low amount of CMC in the hydrogel film which decreased ionic character and increased the hydrophilicity of hydrogel film.[40] In case of batch HD3, there might be the self-entanglement of TG chains by hydrogen bonding and decreased ionic contribution to the absorption of swelling medium, leading to decreased equilibrium swelling.[41] In case of batch HD1, there might be dissociation of a highly hydrophilic carboxylic group of CMC which helps to absorb large amount of water resulting in an increased equilibrium swelling.[42] It was noticed that when the concentration of citric acid was increased, the swelling ratio of hydrogel dressing was decreased significantly. This may be due to an increase in the degree of cross-linking which alters the mobility of polymer chains. Diffusion of the swelling medium into the

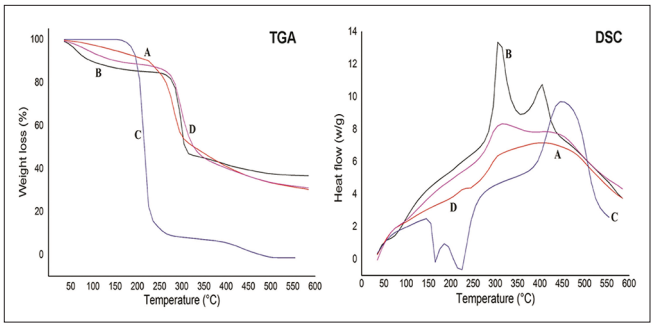


**Figure 3:** Thermogravimetric analysis and differential scanning calorimeter of tamarind gum (a), carboxymethyl cellulose (b), citric acid (c), and hydrogel dressing (d)

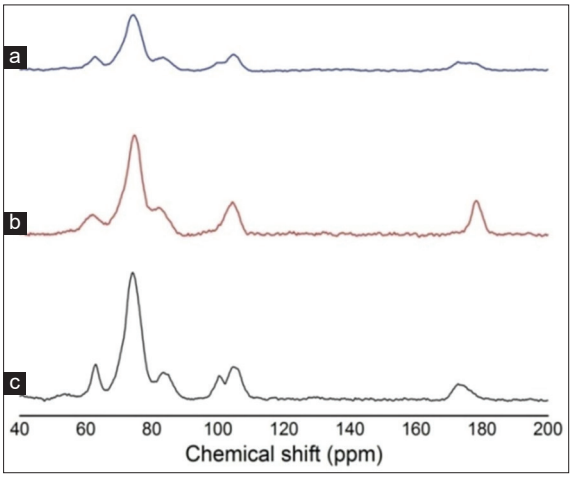


**Figure 4:** Solid-state $^{13}C$-nuclear magnetic resonance of tamarind gum (c), carboxymethyl cellulose (b), and hydrogel dressing (a)

polymer network also decreases, thereby giving rise to the more rigid structure of the polymer network.[34] As discussed earlier, the amount of carboxyl content in hydrogel increases with increase in the extent of cross-linking density.

To understand the effect of pH and salt on the swelling behavior of the hydrogel dressing, swelling study was also performed in 0.1N HCl [Figure 6b], 0.9% NaCl [Figure 6c], and water [Figure 6d]. All hydrogel dressing showed low equilibrium swelling in 0.1N HCl than buffer pH 7.4. The pH of 0.1N HCl is less than the pKa of the carboxyl group (4-5) present in CMC leading to decreased electrostatic repulsion due to protonation of carboxylic groups (COOH) and retarded swelling due to a reduction in solvent uptake capacity of hydrogel dressing.[25] When the concentration of TG was increased, the swelling was found to be decreased. This may be due to the hydrophobic nature of TG as well as high cross-linking. The swelling of hydrogel dressing noticeably decreased in salt solution than the buffer. This may be due to the screening effect of sodium ion which reduces electrostatic repulsion and ion-dipole interactions within the polymeric dressing. Such effect was observed when the system contains oppositely charged components and indicates CMC-TG cross-linked films are negatively charged.[43] The results of

swelling study in 0.1N HCl and phosphate buffer clearly indicates the pH-dependent swelling of hydrogel dressings. It suggests the suitability of polysaccharide-based hydrogel for oral drug delivery to avoid exposure of drug to the erratic gastric environment and release the drug in the intestine.

The high equilibrium swelling of hydrogel dressing in SWF is mandatory to absorb the exudates from wound site. The high amount of exudates in wound causes the growth of bacteria on wounded site and cause microbial infection. Hence, it is required to remove the exudates from the wound site to protect the wound from the mercerization.[8] The swelling of hydrogel dressing in SWF provides an idea about the capacity of hydrogel dressing for the encapsulation of wound exudates. The amount of SWF uptake by the hydrogel dressing was found to be high than the other solvents studied. The pH of SWF has exerted a strong effect on the absorption of wound fluid by the hydrogel dressing. At high pH, the ionization of the free carbonyl groups takes place and leads to electrostatic repulsion and expansion of the polymer matrix which promotes absorption of more wound fluid.[8] The result of wound fluid absorption study is given in Table 2. The hydrogel dressing HD1 showed the maximum capacity to absorb SWF (17.75 ml/g). In general, moderate-to-high exuding wounds produce 3–5 ml of exudates per 10 $cm^2$ in 24 h.[32] Thus, the results of the wound absorption fluid study indicated that these hydrogel dressings can absorb and retain a high volume of fluid. This may avoid the challenge of collection and leakage of excess exudates when applied to a wound.

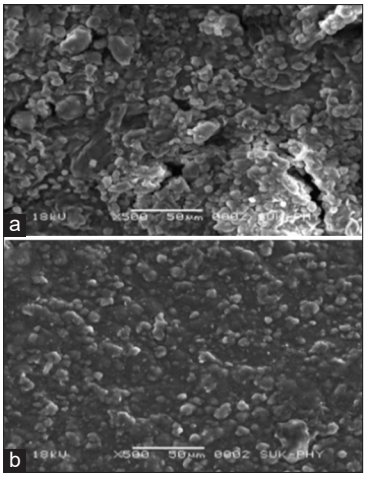


**Figure 5:** Scanning electron microscopy of hydrogel dressing hydrogel dressings (HD1) (a) and HD2 (b)

## Drug loading

The drug loading was done in an aqueous solution of VH through diffusion process in swollen networks. The result of drug loading is given in Table 2. The loading of VH in the hydrogel dressings was observed in the range of 139.4–374.6 mg/g of hydrogel dressing. As the equilibrium swelling degree increased, the VH loading was found to be increased. This may be due to faster diffusion of drug into swollen network. The drug loading was found to be decreased when

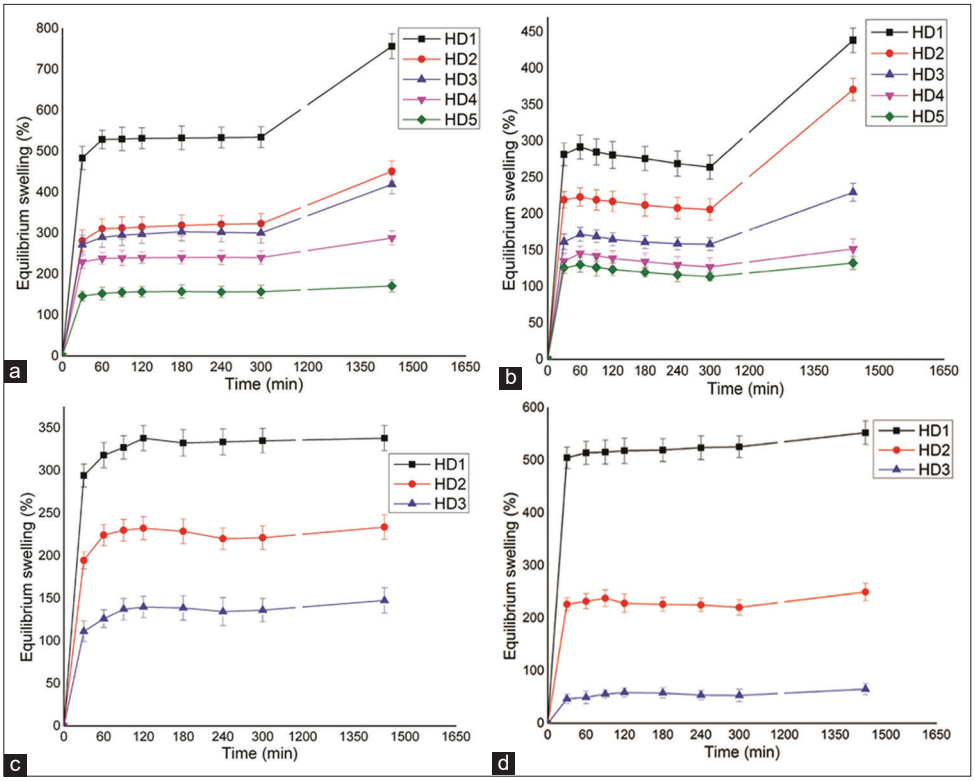


**Figure 6:** Equilibrium swelling of hydrogel dressing in phosphate buffer (a), 0.1N HCl (b), 0.9% w/v NaCl (c), and water (d)

the concentration of TG and cross-linker was increased. This is probably due to low equilibrium swelling degree of the hydrogel dressings.

### *In vitro* release

The *in vitro* drug release profiles of VH from the polysaccharide-based hydrogel dressings are shown in Figure 7. All hydrogel dressings exhibited ~10 to 40% of burst release of VH at the end of 15 min. This may be attributed to the surface-associated drug. The free drug molecule back diffused from the bulk of the hydrogel matrix at the surface along with solvent during drying of drug0loaded swollen hydrogel films. Hence, when the drug-loaded hydrogel film comes in contact with the dissolution medium, the surface associated free drug is released at a faster rate. The controlled release was observed after 30 min from all VH-loaded hydrogel batches. The retardation of drug release is associated with the swelling of the drug-loaded hydrogel dressings. The swelling of hydrogel film increased the thickness of the film from which drug gets diffused into the bulk of dissolution medium.

The release study was performed in 0.1N HCl [Figure 7a] and phosphate buffer pH 7.4 [Figure 7b]. High drug release was noticed in phosphate buffer pH 7.4 than the 0.1N HCl. It indicates pH-dependent release of VH from the hydrogel dressings. TG is non-ionic in nature, while CMC is anionic in nature. Anionic nature of CMC, as well as the free carbonyl groups present in hydrogel dressings after cross-linking, alters the swelling of hydrogel dressing leading to changes in release behavior of VH. The pH of 0.1N HCl solution is less than the pKa of the carboxyl group (4-5) present in CMC leading to decreased electrostatic repulsion due to protonation of carboxylic groups (COOH) and retards the drug release due to a reduction in solvent uptake capacity of hydrogel film.[25] Irrespective of the path length, VH was released at a faster rate in phosphate buffer pH 7.4. This may be due to the ionic contribution of the CMC at buffer pH 7.4. When the amount of TG was increased in HD1-HD3, the drug release was found to be decreased. This may be due to low swelling of hydrogels due to a high cross-linking density which increases hydrophobic character of hydrogel dressing. The drug release was found to be decreased in the batch HD1, HD4, and HD5 when the concentration of cross-linking agent was increased. As the concentration of citric acid was increased, the cross-linking density was increased and, in turn, decreased the swelling of the polymer matrix which resulted in retardation of the drug release.

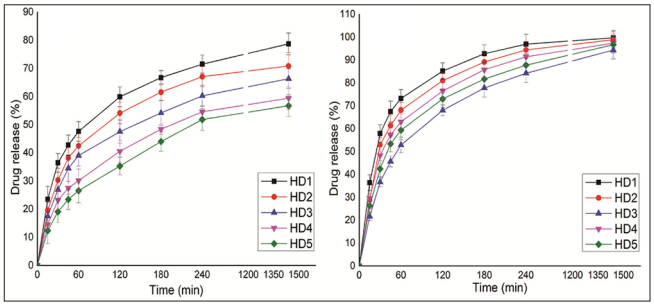


**Figure 7:** Release of vancomycin hydrochloride in 0.1N HCl (a) and phosphate buffer (b)

The release data up to 60% of total drug released in buffer was fitted into the Korsmeyer–Peppas equation to determine release mechanism.[44]

$$\frac{M_t}{M_\infty} = at^n \quad (5)$$

where $M_t/M_\infty$ is the fraction of drug released in time "t" (min), "a" is the kinetic constant, and "n" is the diffusional coefficient which depends on the interaction in between drug and the components of hydrogel matrix. The result of diffusion coefficient (n) and release mechanism is given in Table 3. All hydrogel dressings exhibited non-Fickian release behavior. The value of diffusion coefficient was found to be >0.5 indicating a high interaction between drug and hydrogel and the drug was released by diffusion with polymer chain relaxation. An increase in "n" value from HD1 to HD3 suggests an increase in drug hydrogel interaction due to TG.[22] Furthermore, an increase in the concentration of cross-linker (HD1, HD4, and HD5) increases the "n" value which indicates increase in the interaction of drug and hydrogel dressing.

### Hemolysis assay

The biocompatibility of the hydrogel dressings was determined by hemocompatibility study. The test is based on the determination of the lysis of red blood cell (RBC) in the presence of the hydrogel films. The released hemoglobin gets dissolved

**Table 2:** Evaluation of hydrogel films

| Batch | Total carboxyl content (mEq/100 g) | Drug loading* (mg/g) | SWF absorption capacity (%) | Hemolysis (%) |
|---|---|---|---|---|
| HD1 | 259.6±8.8 | 374.6±5.47[a] | 1775.4±25.7 | 1.56 |
| HD2 | 322.9±7.5 | 331.5±5.17[a] | 1124.9±23.6 | 1.14 |
| HD3 | 334.1±6.7 | 259.1±4.91[a] | 958.3±24.5 | 2.57 |
| HD4 | 338.6±8.3 | 294.6±2.51[a] | 762.8±21.9 | 1.79 |
| HD5 | 408.3±9.4 | 139.4±2.78[a] | 676.2±20.4 | 1.93 |

SWF: Simulated wound fluid, HD: Hydrogel dressing*, Mean ± standard deviation, [a]significantly different ($P$<0.05) than HD1

**Table 3:** Diffusion coefficient (n) and release mechanism for hydrogel dressings in buffer pH 7.4

| Batch | n | $r^2$ | Order of release |
|---|---|---|---|
| HD1 | 0.57 | 0.982 | Non-Fickian |
| HD2 | 0.62 | 0.952 | Non-Fickian |
| HD3 | 0.65 | 0.990 | Non-Fickian |
| HD4 | 0.61 | 0.985 | Non-Fickian |
| HD5 | 0.65 | 0.997 | Non-Fickian |

n: Release exponent; $r^2$: correlation coefficient

**Table 4:** *In vivo* percent wound contraction

| Day | Wound contraction (%), *n*=6 | | |
|---|---|---|---|
| | NC | BHD | VLHD |
| 4th | 16.66±6.45 | 20.83±6.45 | 45.83±6.45 |
| 8th | 31.25±6.84 | 43.75±6.84 | 72.91±5.10 |
| 12th | 47.51±5.10 | 73.95±6.14 | 96.87±5.22 |

Data are expressed in mean±standard deviation, NC: Negative control, BHD: Blank hydrogel dressing, VLHD: Vancomycin-loaded hydrogel dressing, *n*: Number of animals

in external fluid forms yellowish color which can be measured spectrophotometrically. The higher the optical density of the supernatant greater the cell damage.[24] The result of hemolysis assay of hydrogel dressings is given in Table 2. The percentage hemolysis for all hydrogel films was found to be in the range of 1.14–2.79%. The low value of percentage hemolysis for hydrogel film can be attributed to the higher hydrophilicity of polymer matrix which decreases polymer-RBC interactions and lowers disruption of RBCs.[32] The observed percentage hemolysis was found to be less than the permissible limit 5%[20] indicating hemocompatibility of hydrogel dressings.

### *In vivo* wound healing activity of hydrogel dressing

Table 4 illustrates the percent wound contraction of negative control, blank hydrogel dressing, and VH-loaded batch HD1 in mice. The wound contraction was faster in the case of VH-loaded hydrogel dressing than the negative control group. Results revealed that the wound healing of all groups of mice was decreased with the time intervals of the 4th, 8th, and 12th day. The healing rate was significantly ($P < 0.05$) high in case of drug-loaded hydrogel dressing group than the negative control and blank hydrogel dressing group. It indicates that the VH-loaded hydrogel dressing exhibited good wound healing activity. It was observed that blank hydrogel dressings also showed significant wound contraction than the negative control. This can be ascribed to the prohibition of bacterial infection for accelerating tissue regeneration by hydrogel film.[4]

## CONCLUSION

Polysaccharide-based hydrogel dressings were successfully developed using citric acid as a cross-linking agent. The instrumental analysis helped to confirm the formation of ester cross-links. The hydrogel dressing prepared using CMC: TG in ratio 1:1 showed maximum swellability under varying pH conditions and controlled the release till 24 h. The hydrogel dressings were found to be hemocompatible. The VH-loaded hydrogel dressing exhibited significant wound healing activity in mice. Thus, VH-loaded CMC/TG hydrogel dressings showed good potential for the treatment of wounds.

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## CONFLICT OF INTERESTS

All authors approve the final manuscript and declare that there are no conflicts of interests.

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