Water absorption and antimicrobial behavior of physically cross linked poly (vinyl alcohol)/carrageenan films loaded with minocycline

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ABSTRACT
In the present study, poly(vinyl alcohol) (PVA)/Carrageenan (Car) composite films have been prepared by ionic cross-linking of the two polymers with Borax (Bx) and K+ ions, respectively, in aqueous medium at room temperature. The films were characterized by FTIR, SEM, TGA, and AFM analysis. The equilibrium percent swelling (EPS) of PVA/Carr films, so produced, exhibited negative dependence on the amount of cross-linkers. A detailed investigation of their water absorption and moisture permeation behavior has been carried out. The films showed non-thrombogenic and non-cytotoxic behaviors. Finally, drug minocycline-loaded films showed excellent antimicrobial behavior.

1. Introduction
Wound healing refers to the process of replacement or re-generation of epidermal tissues by living tissues.[1] A wound dressing, usually consisting of a natural or synthetic or combination of both the polymer matrix, should be able to absorb exudates with simultaneous release of the entrapped bioactive agent at a pre-determined rate. [2] An ideal wound dressing film must have characteristic properties like fair moisture permeability, high-tensile strength and folding endurance, large water absorption capacity, and biocompatibility.[3,4] Recently, the research has focused on exploring possibilities to use environment friendly bio-polymers as wound dressing materials.[5] In recent past, a large number of wound dressings, comprising of natural polymers, has been reported. Some of the bio-polymers include cellulose,[6,7] alginites,[8] psyllium,[9] carrageenan,[10] collagen,[11,12] etc.

Carrageenans are linear sulfated polysaccharides and composed of D-galactose and 3, 6-anhydro-D-galactose units.[13] They are biodegradable, biocompatible, and abundant.[14] According to reports, kappa carrageenan-based films are said to have syneresis causing problems in the manufacturing of hard and soft films or coverings. [15] In addition, these films have poor vapor barrier property,[16] which may lead to drying of the wound, thus delaying the healing process. Although carrageenan is supposed to be a safe material for biomedical applications, the International Agency for Research on Cancer in 1982 identified sufficient evidence for the carcinogenicity of degraded carrageenan in animals to regard it as posing a carcinogenic risk to humans.[17] However, carrageenan is still used widely as a thickener, stabilizer, and texturizer in a variety of processed foods prevalent in the Western diet. The other polymer PVA has fair reputation as a nontoxic and biocompatible polymer.[18] It is also noteworthy that the proposed hydrogel film may have some risk factors like its improper mechanical strength, its adhesion on the skin, thus causing discomfort to the patient on removing its insufficient folding endurance (FE), etc. Indeed, there are several methods that could be used to test the suitability of the proposed wound dressing film.

Therefore, it appears that combination of carrageenan with some other synthetic polymer could be a better alternative to fabricate wound dressing films with desirable properties. We selected poly(vinyl alcohol) (PVA) as the other component due to its fair water solubility and excellent film-forming, emulsifying, and adhesive properties. It is also resistant to oils, grease, and solvents. PVA has been used on many biomaterial applications like artificial pancreas, hemodialysis and implantable biomaterials.[19,20] Although, PVA may conveniently be cross-linked with glutaraldehyde, but because of toxicity, glutaraldehyde is avoided in biomedical applications.[21]

A thorough survey of the literature available through internet and other information sources reveals that there have been only a few reports presenting chemically
cross-linked hydrogels composed of carrageenan and PVA. For example, Hezaveh and co-worker [22] synthesized PVA/Car hydrogel films cross-linked with natural cross-linker genipin and investigated release of β-Carotene under controlled manner. It was found that using genipin can stop burst release in the hydrogels and control active material better than native films as a result of structural modification. In another report,[23] synthesis of hydrogels from aqueous solution of PVA and kappa-carrageenan (KC) has been performed with radiation processing technology using Co-60 gamma source. A detailed investigation on the influence of radiation dose and concentration of KC on gel content, swelling properties, and thermal behaviors of hydrogel has been performed. In our previous reports,[24,25] we have presented a detailed investigation of swelling behavior, moisture permeation, and drug releasing capacity of glutaraldehyde cross-linked Carr/PVA films. As there are no reports available on the preparation of ionically cross-linked Car/PVA hydrogels, we have reported the influence of radiation dose and concentration of KC on gel content, swelling properties, and thermal behaviors of hydrogel has been performed. In our previous reports,[24,25] we have presented a detailed investigation of swelling behavior, moisture permeation, and drug releasing capacity of glutaraldehyde cross-linked Carr/PVA films. As there are no reports available on the preparation of ionically cross-linked Car/PVA hydrogels, we have made a sincere attempt using K+ and ions as cross-linkers to cross-link carrageenan and PVA, respectively, to fabricate nontoxic Carr/PVA films with desirable physic-chemical properties. This study, for the first time, reports a totally physically cross-linked bi-polymeric hydrogel film, composed of carrageenan, and PVA. Borax, a salt of boric acid finds a number of applications such as pH buffer, food additives, anti-fungal agent for foot soak, fire retardant, etc.[26]

2. Experimental

2.1. Materials

Poly(vinyl alcohol) (PVA; Degree of hydrolysis (DH) 56%, molecular weight 80,000–88,000 g/mol), κ-carrageenan (Carr: molecular weight 1.5 × 10^5–2.2 × 10^6), were purchased from Research Laboratories, Mumbai, India. The cross-linkers borax and potassium chloride and other chemicals were purchased from Merck Chemical Industry, Mumbai, India and were analytical grade. Total protein and albumin assay kits were obtained from Hi Media Chemicals, Mumbai, India. The Dulbecco’s modified eagle medium (DMEM) was obtained from Hi Media, Mumbai, India. The double-distilled water was used throughout the investigations.

2.2. Method

2.2.1. Preparation of PVA/Carr films

The PVA/Carr films were prepared by in situ physical cross-linking of Carr and PVA by K+ and Borax, respectively, within the polymer matrix at room temperature. The two polymers, namely PVA and carrageen were dried at 40 °C in an electric oven (Tempstar, India) till constant weight. In a typical experiment, 1 g of PVA was dissolved in 20 mL of distilled water, pre-heated to 90 °C, under mild stirring and the solution was now cooled down to 60 °C. To this solution, 1 g of carrageenan was added under stirring till the solution became almost transparent. Now, the above solution was transferred into Petri plate and allowed to cool down to room temperature. The semi-solid film, thus formed was taken out carefully and immersed in 100 mL of aqueous solution containing 1 g and 0.750 g of cross-linkers borax and KCl, respectively, for a period of 6 h. Finally, the film formed was taken out carefully and kept in an air-tight plastic container for further use. The films were designated as PVA/Carr(x/y), where x/y denotes the ratio of weight percent of cross-linkers with respect to their respective polymers in the cross-linking solution. For example, the above film may be referred as PVA/Carr (100/75). In all, two such films with different ratios of wt. % of Carr and PVA were prepared and designated as PVA/Carr (100/75) and PVA/Carr (25/100). Here, it is worth mentioning that we prepared a number of film samples with varying amounts of cross-linkers, but the above two samples were found to maintain the structural integrity of the films during the preliminary water absorption studies. The film PVA/Carr (0/0), with no cross-linkers used was also used as control. The antibiotic drug minocycline-loaded films were prepared by the method of equilibration. The above-mentioned PVA/Carr(x/y) film was placed in aqueous solution of minocycline (MC) for a period of 12 h. Finally the films were taken out, washed superficially with distilled water to remove surface bound drug, and then dried in vacuum chamber till constant weight. The above two film samples, namely PVA/Carr (100/75) and PVA/Carr (25/100) were loaded with drug MC.

2.3. Characterization of films

The Fourier Transform Infrared (FTIR) spectra were recorded with an FTIR spectrophotometer (Shimadzu, 8400, Japan) using KBr. The powdered sample was mixed with KBr. The scans recorded were the average of 100 scans and the selected spectral range between 400 and 4000 cm⁻¹. The X-ray diffraction (XRD) method was used to measure the crystalline nature films. These measurements were carried out on a RikaguDiffraXactometer (Cu radiation = 0.1546 nm) running at 40 kV and 40 mA. The diffractogram was recorded in the range of 2 from 3° to 50° at the speed rate of 2 degree/ min. In order to investigate the surface morphology KC/PVA film, SEM images were recorded with a Hitachi S-4700 (New Jersey, USA) operating at an acceleration voltage of 15 kV. All samples were dried in vacuum at room temperature and coated with gold before scanning. Surface morphologies were imaged at different magnifications. The surface topography of the
thin films was investigated using Tapping Mode – Atomic Force Microscopy (TM-AFM) Multi Mode Nano Scope IIIID Controller (Digital Instruments Veeco Metrology Group, Santa Barbara, CA, USA). Images were acquired at room temperature using a RTESP (Phosphorus (n) doped Si) cantilever with a spring constant of 20–80 N/m. Data acquisition and offline analysis was performed using AFM software v531r1.

2.4. Swelling studies

Swelling studies were carried out in the pseudo extracellular fluid (PEF) as described by Lin et al. [27]. This simulated wound fluid has following composition: 2.2 g of KCl, 6.8 g of NaCl, 25 g of sodium bicarbonate, and 3.5 g of sodium dihydrogen phosphate in 1 liter. The pH of this solution was found to be 7.36. The pre-weighed film sample was placed in 100 mL of PEF at 37 °C and taken out at different time intervals, wiped superficially with tissue paper to remove extra surface water, weighed accurately in an electronic balance (Denber, Germany), and then placed again in water. The Swelling Ratio (SR) was calculated using the following expression:

$$SR = \frac{M_t - M_0}{M_0}$$

(1)

where $M_0$ and $M_t$ are the initial mass and mass at different time intervals, respectively.

In order to determine Equilibrium Swelling Ratio (ESR), $M_t$ was replaced by $M_e$ which is the weight of the swollen film at equilibrium.

2.5. Water vapor permeation studies

A modified ASTM standard (inverted-cup, E96-90, procedure D) was employed to determine the water vapor transmission rate (WVTR) as described elsewhere. [28] In brief, disposable cup, id 40 mm, was filled with 5 g of deionized water and test film sample (pre-conditioned at a relative humidity environment of 40%) was placed on its mouth and fixed using a cello tape (Kores, India). The cup was now placed in a desiccator, filled with saturated solution of LiCl, to provide a relative humidity (RH) of 20%, pre-maintained at 305 K. The cup was taken out at regular time intervals, weighed accurately using a digital electronic balance and WVTR was calculated using the following expression:

$$WVTR = \frac{24m}{A \times \Delta t}$$

(2)

where, $m$ = water loss (g), $\Delta t$ = time period (day), $A$ = effective transfer area per m$^2$.

2.6. Expansion study

The expansion of wound dressing film on the surface of wound was mimicked by studying change in diameter of the circular film samples in a 4% gelatin solution as described elsewhere. [29] In brief, 4 g of gelatin powder was dissolved in 100 ml of distilled water at 85 °C under moderate stirring till a clear solution was obtained. Then, 30 ml of this solution was put into a Petri plate and allowed to cool over night at 25 °C. The film sample with known diameter was placed on the gelatin surface and change in its diameter was monitored periodically till the sample attained constant diameter. The expansion ratio (ER) was expressed as:

$$ER = \frac{D_t}{D_0}$$

(3)

2.7. Cytotoxic studies

The in vitro cytotoxic study of the representative sample Carr/PVA (100:75) was carried by ISO 10993-5, 2009 based ‘Test on Extract’ method using l-929 cell line. The film sample was conditioned with physiological saline prior to the extraction. The extract was prepared by incubating the film (area 3.0 cm$^2$) in 1-mL physiological saline at 50 °C for 72 h. The extract was mixed with MEM2X (1 part of extract and 1 part of MEM 2X) medium to get 50% extract. The positive control was prepared by diluting phenol stock solution (13 mg/mL) to 1.3 mg/mL with culture medium containing serum (dilute phenol) whereas negative control was prepared by incubating 1.25 cm$^2$ Ultra High Molecular Weight Poly(ethylene) with 1 mL of physiological saline at 50 °C for 72 h. The films were placed on subconfluent monolayer of L-929 cells. After incubation of cells with extracts of test sample and controls at 37 °C for 24 h, cell culture was examined microscopically for cellular response. Cells were examined microscopically and cellular responses were scored as 0, 1, 2, 3, and 4 based on the standard Table 1 of reference.

2.8. Drug release studies

A pre-weighed film sample was placed in 25 mL of PEF at 37 °C and the amount of drug released was determined spectrophotometrically at 390 nm using Thermo Spectronic UV–vis spectrophotometer (Genesis, 10-S). The concentration of drug released was determined using Lambert–Beer’s plot obtained with a series of standard drug solutions.
The formation of Carr/PVA film through *in-situ* ionic gelation by K⁺/borax simultaneously involves following two electrostatic interactions: (1) K⁺ ions interact with negatively charged randomly oriented carrageenan coils and (2) borax molecules interact with hydroxyls of alcoholic groups via hydrogen-bonding interactions. The overall mechanism of Carr/PVA film formation may be explained as follows: initially, when the Carr is dissolved in distilled water at 80 °C, which is just above its melting temperature,[31] Carr macromolecular chains adopt random coil conformations. As the temperature decreases beyond melting point, there starts formation of helical dimers which aggregate to produce domains.[32] At this stage, K⁺ ion interact with two groups of anhydrous galactose, thus transforming random coil orientations in to helical structures, leading to gelation. During the whole process, one more cross-linking reaction of PVA by borax is also in progress. Actually, borax is an efficient cross-linker for polymers bearing hydroxyl groups, but the mechanism of cross-linking is still not very clear or is under dispute.[33] According to a most accepted view, pure chemical cross-links are formed between the polymeric chains and borax.[34] However, as per other opinion the borax ions hold together the polymeric chains by means of chemical/physical interactions.[35] Based on the above discussion, we propose that ions link with four –oH groups from PVA chains via hydrogen-bonding interactions. The two types of interactions, as shown in Figure 1(a) and (b), result in formation of PVA/Carr hydrogel. In this way it appears that the PVA/Carr film consists of physical interactions among cross-linking ions and their respective molecules.

The FTIR spectra of native carrageenan, PVA, and the cross-linked film are shown in Figure 2(a)–(c), respectively. A close look at the various peaks observed in the spectrum of cross-linked film and comparison with the peaks observed for the native polymers Carr and PVA reveals following facts. The spectrum of cross-linked Carr/PVA film contains some characteristic peaks of both Carr and PVA. For instance, a sharp peak at 846 cm⁻¹ corresponds to presence of galactose-4-sulfate group, ester sulfate at 1249 cm⁻¹, and hydroxyls in the range of 3200–3600 cm⁻¹. Similarly, a sharp peak at 2947 cm⁻¹ corresponds to C–H alkyl stretching of PVA. A close look at
Figure 2. (a) FTIR spectrum of native carageenan. Figure 2 (b) FTIR spectrum of native PVA. Figure 2(c) FTIR spectrum of PVA/Carr (100/75) film.
A 1991 times magnified image, as shown in Figure 3(a), reveals that the surface of the film is not very smooth. This may probably be due to the relatively low solubility of kappa carrageenan as compared to PVA. The film-forming solution obtained with Carr and PVA contains particulates of Carr which may get precipitated during the drying of the film. In addition, the cross-linker borax interacts simultaneously with four hydroxyls of PVA, while K\(^+\) ion binds electrostatically with one negatively charged sulfonic group.

This uneven cross-linking also contributes toward rough surface texture. The images (b) and (c), obtained with 2500 and 4997 times magnifications, also support our observation. The AFM image obtained for the film also shows uneven or little layered surface texture (see Figure 3(d)).

XRD analysis gives very useful information about the crystalline/amorphous nature of a material, size, and orientation of ordered regions in the material. The typical XRD pattern of KC/PVA film has been shown in Figure 4. The appearance of sharp reflections and diffused scattering is characteristic of crystalline and amorphous regions of typical semi-crystalline polymer. There is a characteristic broad amorphous peak at 2\(\theta\) value of around 20°.[36] This is characteristic of amorphous structure and has been reported in a number of polymers having amorphous region.[33] In addition, appearance of a sharp peak at 2\(\theta\) value of 28.4 was due to the reflection at (2 0 0) plane of KCl as identified using JCPDS file 41–1476. Similar observation has also been reported elsewhere.[37]

The thermal stability of the composite film PVA/Carr(100/75) was investigated by TG analysis, against
of multiphase degradation has also been observed in the case of glutaric acid cross-linked chitosan films.[38] Indeed, a slightly higher stability of the cross-linked film is attributable to the presence of potassium chloride and borax in the film matrix which contribute to a relatively less weight loss as compared to the un-cross-linked film. Finally, beyond 550 °C both of the samples show almost same percent weight loss attributable to the charring process occurring in both of the polymer samples.

### 3.2. Swelling behavior analysis

The cross-linking of Carr and PVA by K⁺ and B⁻(OH)₄ ions, respectively, is expected to bring a change in the water absorption capacity of resulting hydrogel films.[39] In order to investigate this, dynamics of swelling ratio (RS) of film samples PVA/Carr (100/75), PVA/Carr (25/100), and PVA/Carr (0/0), was investigated in the physiological fluid at 37 °C. The results, as shown in Figure 6, reveal that the un-cross-linked film PVA/Carr (0/0) is not stable and it disintegrates soon after achieving maximum SR of 3.35 g/g.
more controlled by the amount of K\(^+\) ions present within the film matrix (or in the cross-linking solution). Thus, we see that the relative concentrations of the two cross-linkers influence the swelling ratio of the resulting hydrogels. However, in the case of covalently cross-linked hydrogels, cross-linked with the common cross-linking agent, there is simple inverse relationship between the SR and cross-linker concentration. As reported in our previous work,[24] the glutaraldehyde (GTA) cross-linked Car/PVA hydrogels exhibited SR of 1.0 to 2.5 as the GTA concentration decreased from 5 to 1%(v/v) of the total reaction mixture.

The kinetic water uptake data obtained for polymeric hydrogels have been interpreted in terms of some models such as power function model,[40] first-order kinetic model and Schott model.[41]

The ‘power function law’ describes fractional water uptake as a time-dependent phenomenon.

\[
\frac{M_t}{M_\infty} = kt^n
\]  

where, \(M_t\) and \(M_\infty\) are the masses of the swollen hydrogel at time \(t\) and in the initial dry state, respectively. Moreover, \(n\) and \(k\) are swelling exponent and gel characteristic constant, respectively. In order to determine the above parameters, logarithmic form of Equation (2) is us

\[
\ln F \left( \frac{M_t}{M_\infty} \right) = \ln k + n \ln t
\]  

The slope and intercept, obtained from the linear plots between \(\ln F \left( \frac{M_t}{M_\infty} \right)\) and \(\ln t\), enabled us to calculate \(n\) and \(k\). The values of swelling exponent \(n\) determine the mode of water transport based on the relative rates of diffusion of solvent into hydrogels and relaxation of polymeric chains: (1) Fickian-diffusion or case-I diffusion involves invasion of solvent molecules through the polymer matrix without any specific interactions between them, with \(n \leq 0.5\); (2) case-II diffusion involves interactions between solvent molecules and polymer matrix, with \(n = 1.0,[42]\) and finally (3) anomalous transport in which the two rates are comparable with \(0.5 < n < 1.0\).

The kinetic water uptake data, given in the Figure 6 were used to obtain linear plots between \(\ln F \left( \frac{M_t}{M_\infty} \right)\) and \(\ln t\) (see Figure 7) and their slopes and intercepts were used to evaluate swelling exponent \(n\) and gel characteristic constant \(k\). The results are given in the Table 2.

A close look at the values given in Table 2 reveals that for the samples PVA/Carr (100/75) and PVA/Carr (25/100), the swelling exponent \(n\) is 0.58 and 0.34, respectively, thus indicating a non-Fickian and ‘less-Fickian’ behaviors, respectively. The sample PVA/Carr (100/75) contains less concentration of K\(^+\) ions, and therefore the carrageenan chains are cross-linked to a relatively smaller

This is simply attributable to the fact that due to absence of any cross-linking there are no binding forces that could have kept the film matrix intact. Instead, the components Carr and PVA begin to disintegrate/dissolve and finally the film loses its existence. However, the film samples PVA/Carr (100/75), and PVA/Carr (25/100), exhibit a swelling ratio of 3.89 and 3.17 in 150 min, respectively, maintaining their structural integrity for more than 96 h. The equilibrium percent swelling was found to be 5.50 and 3.92 as observed after 24 h. The higher SR exhibited by the sample PVA/Carr (100/75) may be attributable to the fact that this film contains less quantity of K\(^+\) ions as compared to the sample PVA/Carr (25/100). This causes relatively weaker ionic interactions between K\(^+\) and ions in the former film sample. Here, it is to be noted that since PVA chains are non-ionic in nature, they do not have tendency to undergo relaxation like carrageenan chains which have negatively charged –SO\(^-3\) groups. Therefore, chain relaxation-induced contribution of PVA is less than that of carrageenan toward swelling in water. Hence, overall swelling tendency may be

![Figure 6. Dynamic water uptake of the samples in PF at 37 °C.](image)

![Figure 7. ln t vs. ln \(M_t/M_\infty\) plots for evaluation of \(n\) and \(k\).](image)
Thus, \( \ln(1-M_t/M_\infty) \) vs. \( t \) plots should be linear with a slope equal to \( k_1 \) (see Figure 8). The slope of the plots yielded constants \( k_1 \) as given in Table 1.

According to Schott model, swelling rate at any time is directly proportional to the quadratic of the swelling capacity before the attainment of equilibrium state.[47] Mathematically,

\[
dM_t/dt = k_2 (M_\infty - M_t)^2
\]

where, \( M_t \) is the water uptake at time \( t \) and \( k_2 \) is the second-order rate constant for swelling. Integration of above equation within the limits \( t = 0 \) \( M_t = 0 \) and \( t = \infty \) \( M = M_\infty \) yields:

\[
t/M_t = 1/k_2 M_\infty^2 + t/M_\infty
\]

Or

\[
t/M_t = A + B \times t
\]

where, \( A \) and \( B \) are two coefficients whose physical meaning is interpreted as follows: at a long retention time \( B \times t \gg A \) and therefore \( B = 1/M_\infty \). On the contrast, at a very short time interval \( B \times t << A \) and so,

\[
Lt_{t=0} (dM_t/dt) = 1/A
\]

Therefore, the intercept \( A \) is reciprocal of initial swelling rate. Finally, the Schott kinetic rate constant \( k_2 \) is calculated as:

\[
k_2 = \text{slope}^2/\text{intercept} = B^2/A
\]

The \( t/M_t \) vs. \( t \) plots for the samples PVA/Carr (100/75) and PVA/Carr (25/100) are shown in Figure 9.

The various related parameters of Schott model, along with respective regressions are given in Table 2. It can be seen that for both of the samples, namely PVA/Carr (100/75) and PVA/Carr (25/100), the theoretical vs. experimental values of \( M_\infty \) (ESR) are 3.17 vs. 3.05 and 3.89 vs. 3.78, respectively, thus showing a fair agreement. Similarly, in a study,[48] \( E_{\text{init}} \) and \( E_{\text{exp}} \) for carboxymethyl cellulose-g-poly (acrylic acid-co-2-acrylamido-2-methyl propane sulfonic acid) hydrogels were almost same, i.e. 4.65 vs. 4.62, 4.55 vs. 4.52, 3.86 vs. 3.90, etc. for different hydrogel samples. Here, it is also noticeable that values of the first- and the second-order rate constants \( k_1 \) and \( k_2 \), respectively, lie in the range of \( 0.023 \times 10^{-3} \) to \( 0.40 \times 10^{-3} \) min\(^{-1}\).
Contrary to this, if the MVTR is fairly low then there may be leakage of exudate from the edges of film, which may promote wound dehydration and bacterial infection.

The results of permeation test for the samples PVA/Carr (25/100) and PVA/Carr (100/75) are shown in Figure 10. It is clear that sample PVA/Carr (25/100) exhibits faster moisture transmission rate as compared to sample PVA/Carr (100/75). This is due to the fact that the sample PVA/Carr (25/100) has less content of cross-linker borax and therefore the sample possesses relatively less degree of cross-linking as compared to the sample PVA/Carr (100/75).

Normally, the MVTR of the normal skin is about 204 g/m²/day. However, the WVP of the injured skin can range from 260 to 5400 g/m²/day. A wound dressing should have a suitable MVTR to prevent excessive dehydration as well as buildup of exudates. According to reports available regarding the suitability of wound dressing film on the basis of their MVTR, it has been recommended that normal MVTR for dressings range from 70 to 9400 g/m²/day. This range has been set up on the basis of condition of wounds such as low injured skin, first degree burns, granulating wounds, etc.

In this work, the MVTR of the samples PVA/Carr (25/100) and PVA/Carr (100/75) was found to be 11,624 g⁻¹/m²/day and 8825 g⁻¹/m²/day, respectively, which is the desirable range for most of the wound dressings. It is clear that the sample with higher borax content exhibits less water vapor transmission rate. This is attributable to the fact that a highly cross-linked network puts hindrance in transmission of water vapors through the network. In a work by Dias et al., the carboxybutyl chitosan and agarose-based films and foam-like structures had WVTR in the range of 3597 to 4352 g/m²/day. However, there may be variations depending upon the conditions like humidity of environment, temperature, etc. Based on the above discussion, it may be claimed that MVTR of all the samples fall within the prescribed range of MVTR while other samples show higher transmission rate and may be useful in the case of wounds with abnormally high exudates.

### 3.4. Expansion study

The gelatin solution has already been used to mimic a supporting wound. The study of expansion behavior of a film on the surface of gelatin medium provides useful information regarding suitability of the film in high exuding wounds. The results of expansion study are shown in Figure 11 for the film samples PVA/Carr (25/100) and PVA/Carr (100/75). It can be observed that the sample with lower borax content shows a little more expansion in the dimension of film in the gelatin medium.
3.5. Cytotoxic studies

The results of cytotoxic studies are shown in Figure 12. As per ISO 10993-5, the achievement of numerical grade more than 2 is considered as toxic. It can be seen that there are discrete intra-cytoplasmatic granules, no cell lysis, and there is no reduction in cell growth. This indicates that the film sample is non-cytotoxic.

3.6. Drug release studies

The results of drug minocyclin release from the film samples PVA/Carr (100/75) and PVA/Carr (25/100) are shown in Figure 13. It may be seen that the sample PVA/Carr (25/100) exhibits a faster release as compared to the sample PVA/Carr (100/75). This is attributable to the fact that the sample PVA/Carr (25/100) is having relatively lower degree of cross-linking as the cross-linking solution had low borax content. In a duration of 5 h, the samples PVA/Carr (100/75) and PVA/Carr (25/100) demonstrate almost 48 and 55% release.

3.7. Antibacterial study

The results of antibacterial study, carried out using ‘zone of inhibition’ method are shown in the Figure 14. The Petri plate, supplemented with the circular film sample PVA/Carr (25/100) (without drug) exhibits a dense population of bacterial colonies (See Figure 14(a)), whereas the Petri plates containing the samples PVA/Carr (100/75) and PVA/Carr (25/100) show clear ‘zones of inhibition’ with respective diameters of and mm, respectively (see Figure 14(b) and (c)). It may be inferred that film showing higher release exhibits bigger zone as compared to the other sample.

It is noteworthy that both the samples retained their shape stability even after 36 h. In a study by Thu et al. [58], the alginate-based single-layer film was found to exhibit a faster expansion and it transformed into gel form while the double-layer film showed slower increase in its dimension and maintained its stability even after 24 h. It is reported by same authors that early transformation of a film texture into gel may leave the film with gummy residue deposited around the wound and hence it may be painful to the patient to replace the dressing. However, no such gummy appearance was observed in these films.
4. Conclusion

From the above study, it may be concluded that PVA and Carr can be cross-linked physically to give composite film with excellent water and moisture absorption properties. The amounts of cross-linkers, namely borax and K+ ions control the water absorption of hydrogels. The dynamic water uptake data were well interpreted by Schott kinetic model. These films have a higher degree of water vapor transmission rate, and possess fair non-cytotoxicity. The release of antibacterial drug minocycline is affected by the variation in relative amounts of cross-linkers. The films show fair antibacterial action against model bacteria E. Coli.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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