Development and study of textile-based hydrogel wound dressing material

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ABSTRACT – REZUMAT

Development and study of textile-based hydrogel wound dressing material

In this article, the developed textile-based hydrogel dressing is seen to be effective in terms of its exudates management capacity, strength, elongation and water vapours permeability. The usage of active antimicrobial and antibiofilm agent i.e., Usnic acid, has given it a broad-spectrum antimicrobial activity and antibiofilm property. The combination of these properties makes this dressing a better choice for the management of wounds especially chronic wounds that are infected with biofilm. The design of the experiment (BBD) used in this study is able to point at the optimal combination of the materials for the construction of such hydrogel dressings and understand the contribution of each factor used in the study.

Keywords: *hydrogel, wound healing, knitted fabric, antimicrobial, biofilm, Usnic acid*

Dezvoltarea și studiul pansamentului textil pe bază de hidrogel

În acest articol, pansamentul textil pe bază de hidrogel este considerat a fi eficient în ceea ce privește capacitatea de gestionare a exsudatelor, rezistența, alungirea și permeabilitatea la vapori de apă. Utilizarea unui agent antimicrobian activ și antibiofilm, adică acidul usnic, i-a conferit o activitate antimicrobiană cu spectru larg și proprietate antibiofilm. Combinația acestor proprietăți face din acest pansament o alegere mai bună pentru gestionarea rănilor, în special a rănilor cronice care sunt infectate cu biofilm. Designul experimentului (BBD) utilizat în acest studiu este capabil să indice combinația optimă a materialelor pentru realizarea unor astfel de pansamente pe bază de hidrogel și să elucideze contribuția fiecărui factor utilizat în studiu.

Cuvinte-cheie*: hidrogel, vindecarea rănilor, tricot, antimicrobian, biofilm, acid usnic*

INTRODUCTION

Skin is the largest organ in the human body. It plays a crucial role as a protective barrier from the external environment; prevent external noxious agents such as all type of microbes from getting inside the body. It also maintains the internal environment by the regulation of water and electrolyte balance and thermoregulation. It is crucial to keep the skin intact so that the designated functions of the skin are uncompromised. When the skin is damaged, due to any cause (mechanical injuries, ulcers, burns, neoplasm or surgical trauma) [1], there are chances of it getting infected. In that case, the body resources are wasted in fighting them.

With the advanced progress of technology, the old understanding of dry wound healing has shifted to become a new one. The new approach emphasizes the need for moist wounds for better healing. However, the moist and warm dressing may act as favourable conditions for Microbial proliferation. Bacteria and other organisms in the exudates have been studied to have an adverse effect on the wound healing process [1]. Microbial proliferation may lead to a prolonged inflammatory stage and cause infection. It may even lead to acute wounds getting converted to hard-to-heal chronic wounds. Chronic wounds are those that do not progress through a normal, orderly, and timely sequence of repair [2].

Further, prolonged infection in the wound would lead to biofilm formation. These are complex microbial colonies where the microorganisms synthesize and secrete a protective, thick and slimy matrix around them [3]. The heterogeneous communities of microbes grow in the matrix and keep spreading all over the wound. They are responsible to cause delays in the healing of wounds.

Biofilm is a major contributor to diseases that are characterized by an underlying bacterial infection and chronic inflammation e.g., periodontal disease, cystic fibrosis, chronic acne and osteomyelitis [4, 5]. Electron microscopy of biopsies from chronic wounds found that 60% of the specimens contained biofilm structures in comparison with only 6% of biopsies from acute wounds [6].

Since biofilms are reported to be a major factor contributing to multiple chronic inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed.

The topical antiseptics that are effective on the colonies of microorganisms are not effective on the biofilms as the extra protective matrix does not allow the antiseptic to work. Thus, wound infected with biofilms needs very specific biofilm targeting substances to be able to take care of the infections.

In the present study, a wound dressing is developed that has intrinsic strength and stability of inert textile

material and it forms absorbent hydrogen dressing along with Usnic acid release which is a known antimicrobial and antibiofilm agent [7]. So, unlike the conventional hydrogel dressings, the reinforced textile dressing exhibits good strength and elongation properties that are conformable to the skin at the same time it is equally effective in exudates management of chronic wounds. The Box–Behnken design of experiment (BBD) [8] aims at understanding the best ratio of the raw materials in the construction of hydrogel dressing without limiting the antimicrobial and antibiofilm properties of the dressing.

MATERIALS AND METHODS

Sodium Alginate (SA) (MW, 20–40 kDa, CAS No. 9005-38-3), Pectin (PC) (MW, 30,000–100,000, CAS No. 9000-69-5), Usnic acids (CAS No. 79902-63-9) and Glycerol, used as a plasticizer). Phosphate Buffer (PBS) all from Sigma-Aldrich (UK). Ethanol and calcium chloride dehydrate from Swastik chemicals India). Distilled water was used in all experiments. The Polyester fibre used in the production of reinforced textile was procured from Reliance Industries, India. Knitted fabric specifications are as listed below:

- Fabric: Weft Knitted Interlock;
- Yarn: 44 DTex, number of filaments: 14;
- Machine gauge: 20, GSM: 30.

Polyester being a widely used, economical and relatively very Inert material for medical usage is the preferred material of choice. Figure 1 shows high-resolution images taken by an optical magnifying lens with the digital scale of the textile material used.

Dressing preparation

A schematic diagram representing the dressing and the cross-linking process is shown as graphical abstract in figure 2. Sodium Alginate (SA)-Pectin(PC) (1:1) composite dressings were prepared by solvent-casting method. In brief, SA and PC (1:1) were dissolved in distilled wate and then 50% (w/w) of the total dry polymer weight, glycerol was added with continuous stirring at 1000 rpm for 3 h at 40°C. Thereafter, 4 when a uniform gel (free of any undissolved particles or bubbles) was obtained, 20 ml of ethanolic Usnic Acid solution was added

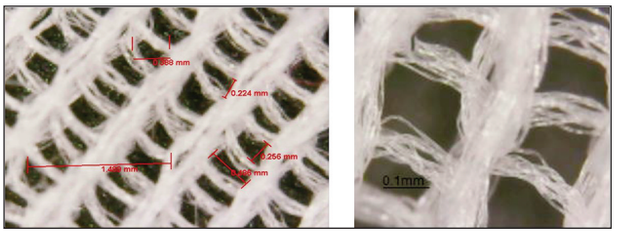


Fig. 1. Magnified image of the scaffolding fabric

to the gel. Next, 80 g of the gel was cast into a Specially designed tray along with the reinforcing fabric (figure 1). and dried in an oven at 45°C for 48 h. The dried films were uniform without any cracks or casting defects and had good flexibility required for a wound dressing application. The dressing did not curl at the edges due to the presence of reinforcing textile material in the structure. The obtained dried dressings on the reinforced fabric were labelled and stored in medical paper envelopes until further use. 15 different combinations were used to cast the dressings. Each combination was used in triplicate amounting to total of 45 dressings being formed [9].

It is only after the treatment of the dressing with calcium chloride the monovalent sodium ions are exchanged with divalent calcium ions whereby the calcium ion bounds two long chains together and forms a complex stable calcium alginate structure as schematically represented in figure 3. This figure shows a schematic diagram of the conversion of sodium alginate to calcium alginate.

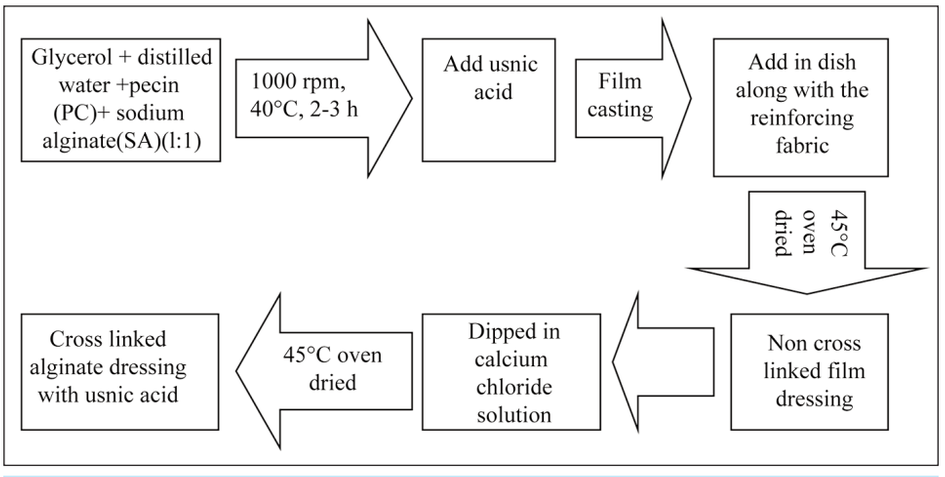


Fig. 2. Schematic representation of dressing preparation

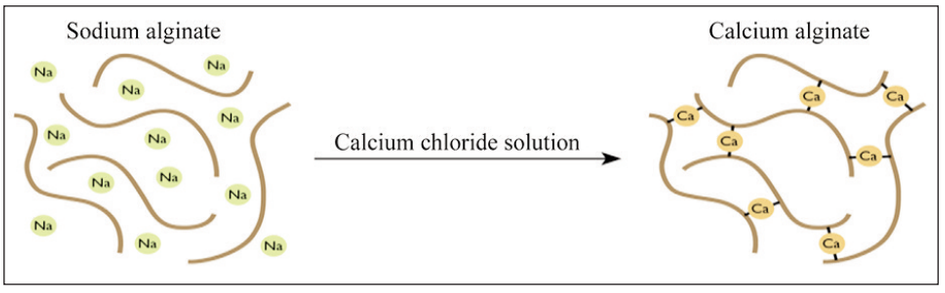


Fig. 3. Conversion of Sodium Alginate to Calcium Alginate

Preparation of cross-linked hydrogel dressing

The dressings were cross linked by immersing in 0.25% $CaCl_2$ for 1 min. Next, each dressing was rinsed by dipping in distilled water three times to remove the excess CaCl2 solution or cations that were not bound to the surface. The dressings were then immediately blot-dried using paper The dressings were then dried at room temperature for 12 h. Dried cross-linked dressings were then labelled and stored in medical grade paper envelops at room temperature until further use.

Experimental Design – Box and Behnken Design (BBD)

Design of experiment selected was Box and Behnken design for response surface curve. Here 3 factors at three different levels were studied for the various characteristics of the dressings. Thus 15 different combinations of the dressings work out for the study. The middle level combinations repeat for 3 times and thus effectively only 13 combinations need to be studied. Hence 13 combination of dressing material were used [8]. Table 1 shows the various levels of the three factors under study.

Table 1

BOX AND BEHNKEN DESIGN: FACTORS AND LEVELS			
Levels	**–1**	**0**	**+1**
X1(SA:PC) gram	16	20	24
X2(Glycerol) gram	5	10	15
X3 (Usnic Acid) gram	0.6	0.8	1.0

Methodology of evaluation

Appearance and thickness

Flexibility, colour, and transparency of the dressings were assessed visually. Thickness was evaluated using a digital calliper at 10 sites (Covering centre and edges).The mean was calculated along with its CV% (Coefficient of variation %). The sample specimens were conditioned at 25ºC and 50±5% RH (Relative Humidity) for 48 h prior to analysis.

Drug content uniformity test

To ensure the uniform distribution of drug (Usnic) within dressing, all drug-loaded samples of known mass (4 gm loaded with Usnic acid) were trimmed from random sites on different dressings marked in area as A, B, C, D and E respectively. The samples were placed in separate glass vials containing 40 ml of Phosphate buffer Solution (PBS) solvent. Vials were shaken at 50 rpm in a water bath for 4 h at 37°C, and then 10 ml of the sample solution was withdrawn. The sample solution was evaluated with Standard solutions of Usnic acid with by measuring transmittance at 290 nm by using a UV spectrophotometer (Agilent Technologies, Model-USA & G 6860 A). The transmittance of Usnic acid from the dressing samples was measured and the concentration of the Usnic acid was calculated based on the calibration curve.

In vitro release Kinetics

Extraction of dressing was done in PBS solution. PBS at pH 7.4 was used as the receptor medium. Dressings of 2.5 cm × 2.5 cm, were used, which works to ~0.2 g/ml, mass/volume extraction ratio. The contents were continuously agitated (50 rpm) during extraction using incubator shaker at 37°C +/– 2°C for 24 h [10]. The test item and solvent controls were subjected to extraction conditions as described in the table 2.

After the completion of the extraction period, extracts were decanted to a container and were tested for their leachability using the UV–Vis spectrophotometer (Agilent Technologies, Model-USA & G 6860) at a wavelength of 290 nm.

Mechanical properties of the dressing

Tensile strength (TS) and percentage elongation at break (%E) of the dressings were determined by Zwick Roell, Model UTM 10KN according to the ASTM D 882-02 standard. The sample specimens were conditioned at 25°C and 50±5% RH for 48 h prior to analysis. Dressings were cut to dumbbell-shaped strips that were 30 mm long and 5 mm wide. The mechanical properties of specimens were measured by stretching the dressings at a crosshead speed of 50 mm/min to their breaking point [11]. At least five replicates from each type of dressing were used for this analysis. Tensile strength (TS) in N/mm^2, tensile strain at break (E) and Young's modulus were calculated based on the following equations:

$$TS = \text{Maximum load at break} / \text{Transverse section area} \quad (1)$$

$$E = \text{Extension of length at rupture} / \text{Initial length} \times 100 \quad (2)$$

$$\text{Youngs modulus} = \text{Tensile stress (TS)} / \text{Tensile strain (E)} \quad (3)$$

Table 2

PARAMETERS – IN VITRO RELEASE KINEMATICS			
Type of extract	**Quantity of Test Item (g)**	**Volume of Solvent added (ml)**	**Extraction conditions**
PBS pH 7.4, Solvent Control with known amount of USNIC Acid as 0.3,0.4,0.5 and 0.6 gpl	-	60	37±2ºC for 24 h drawn equally at 4 h, 8 h, 12 h, 16 h, 20 h and 24 h
PBS pH 7.4 Plus Test Item Extract	4	60	37±2ºC for 24 drawn equally at 4 h, 8 h, 12 h, 16 h, 20 h and 24 h

Water vapour transmission

In this test, a fixed amount (around 70 ml) of distilled water was taken in the containers that were holding the water at a constant temperature of 37°C +/– 2°C. The face of the containers was sealed by using the dressing without leaving any gaps by proper clamping. A cross blowing fan ensured the air velocity above the containers at a constant speed of 50 cm/sec. The containers were kept at constant temperature cabinets and were weighted at equal intervals of 4 h, 8 h, 12 h, 16 h, 20 h and 24 h. The amount of moisture loss across the dressing was evaluated by calculating the difference in weight of the containers over the period of time.

Triplicate measurements were run, and mean values were obtained. Water vapours transmission (WVT) over each interval was determined using the following equation:

$$WVT = W/S \quad (4)$$

where W is the loss in weight of the container over the time interval and S is the exposed surface area of the dressing (m^2), WVT is expressed in g/m^2/time interval. The study was conducted for 24 h to evaluate WVT as g/m^2/24 h.

Exudate's absorptivity and absorption profile

The artificial gelatine wound model method along with simulated wound fluid was used in this study. The study uses a similar method as used by K.H. Matthew et al. [12]. In this study, gelatine is used as the material that is mimicking the role of exudates laden wound tissues.

Each dressing was trimmed into a square shape of defined size then placed at the centre of the gelatine gel laden with simulated biofluid (in the Petri dish). The change in the diameter, cross-section area and weight of the dressings was recorded at predetermined time intervals of 4 h, 8 h, 12 h, 16 h, 20 h and 24 h. The test was performed in triplicate for each formulation and the mean value was used to calculate the expansion behaviour using the following equation:

$$\text{Percentage Exudates absorption AI (\%)} = (Ws - Wd)/Wd \times 100 \quad (5)$$

where Ws and Wd are weights of the swollen wet dressing and dry dressing, respectively.

Evaluation of antimicrobial activity

The antimicrobial activity of the dressing was evaluated by using the quantitative method AATCC 100 (American Association of Textile Chemists and Colorists) [13]. The initial Inoculation chosen was 10^5 CFU/ml.

Here the test item pores with 1 ml +/– 0.1 ml of the inoculated microbial solution. Then the test item is transferred to 100 ml +/– 1 ml of 0.05% neutralizing, shacked vigorously and then made into serial dilutions with sterile water as per the timelines of the studies. This is then plated on a nutrient agar plate and incubated at 37°C for 24 hours. The material count is then taken and the number of bacterial count reduction is expressed as the reduction percentage over the prior load incubated on the test item.

The positive control used was sterile nonwoven fabric dipped in 0.4% Benzalkonium Chloride (BKC). Eight challenge microorganisms were used and the test outcomes are as shown in table 3. The study was conducted at the lowest level of Usnic acid (0.6 grams in 20 ml).

Biofilm inhibition assay

The methodology used here was adapted from Luciano Giardino et al. [14] with some modifications. Biofilms of *Enterococcus faecalis* strain ATCC 29212 were generated on cellulose nitrate membrane filters. An overnight culture of *E faecalis* grown in broth, adjusted to 0.5 Mc Farlandscale,1×10^8 CFU/ml, was used. An aliquot of 20 microlitre *E faecalis* was seeded onto 2 cm × 2 cm cellulose nitrate membrane filters (0.22-micron pore diameter. which were placed on the surfaces of BHI agar plates. 10 membranes were used for each plate. Plates containing membranes were then incubated for 48 hours at 37°C in an aerobic atmosphere. The efficiency of the method for biofilm generation was observed in a pilot study visually. After incubation, membrane filters were removed aseptically from the agar plate and transferred carefully to avoid any disruption of the biofilm. The study material was then placed on each of the biofilms without leaving any contact gap. There was one plate for control and one for positive reference. The Reference plate was with a blank dressing formed without any Usnic acid content in it. The Plates thus formed were then incubated for 48 hours at 37°C in an aerobic atmosphere. After 48 hours the specimen was leached with PBS (Phosphate buffer) solutions in aseptic conditions and aliquots of each leach content was incubated for 48 hours to see if there is any bacterial growth. The Plates were compared with the blank plate which did not have Usnic acid content in the dressing.

RESULTS AND DISCUSSIONS

Appearance and thickness

Appearance and thickness results showed that the visual appearance of dressing was consistent over all the places. Measurement of thickness showed no major change in thickness within the combination. It was seen that proper stirring and settling of the material was vital to avoid any defects in the reinforcement of the textiles.

Drug content uniformity test

This study was done to demonstrate that the procedure and methods followed in the preparation of dressing were capable of ensuing uniform content of Usnic acid in the film. Any variation in uniformity of Usnic acid in the dressing may cause variation in the dosage of it in the wound.

Results of this study showed that the drug (Usnic acid) was uniformly distributed in the dressing at different areas. The effect of the variables like SA:PC

concentration or Glycerol concentration was not seen to affect the uniformity of Usnic acid in the dressing. It was fairly consistent among dressings of specified combinations.

In vitro release Kinetics

The in vitro release kinetics study done on the dressing for different time durations showed that the Usnic acid release in the phosphate buffer solution kept on increasing with time for all dressing combinations. The release rate was higher upto16 h which flattened by around 24 h for the majority of the dressings as shown in figure 4. This figure shows in vitro release kinetics of each experimental run that is indicated by experiment number as per the BBD experimental design explained earlier.

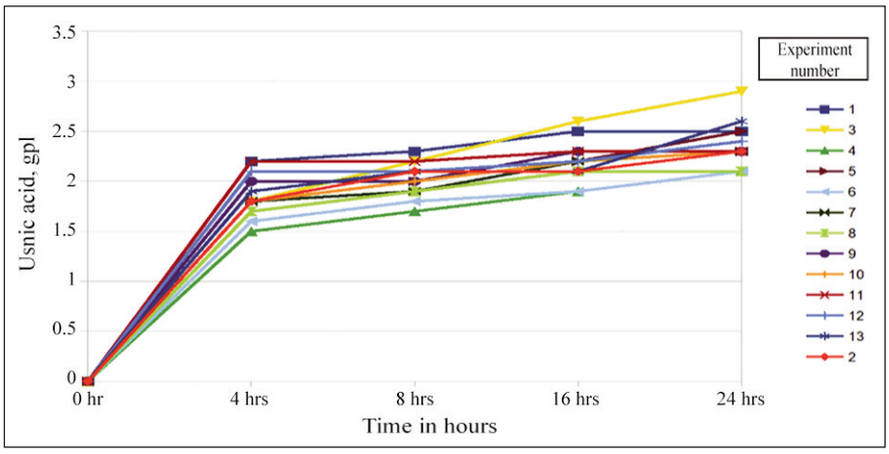


Fig. 4. In vitro release of Usnic acid from the dressing over a period of time

The results show that the dressings show full swelling in around 4 hours of time and after that, the entrapped Usnic acid is released from the structure. The release proceeds fairly for around 24 hours of time. Figure 5 shows the In vitro release of Usnic acid compared over each time zone of the study.

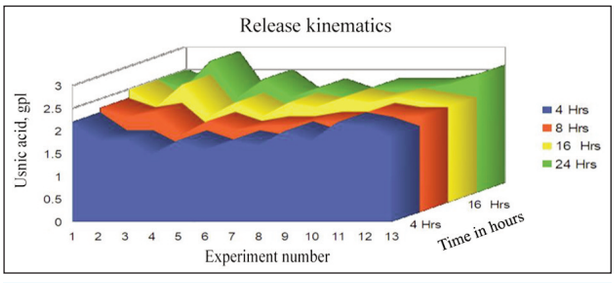


Fig. 5. In vitro Release of Usnic acid compared over each time zone

From figure 6, it is clear that for a given concentration of Usnic acid the release of Usnic acid increases with the amount of glycerol concentration and decreases with the amount of SA:Pectin concentration. This is seen to be true for all levels of Usnic acid concentrations. In other words, a lower level of SA: Pectin gives good release characteristics to the dressing especially at higher concentrations of glycerol.

This can be explained by the fact that glycerol being a plasticizer helps in reducing the compact packing of the molecular chains of the Alginate and Pectin and hence release of the entrapped Usnic acid is easier. The lower levels of SA:Pectin also makes the dressings thinner and hence more prone to leaching of Usnic acid in extraction solvent like PBS.

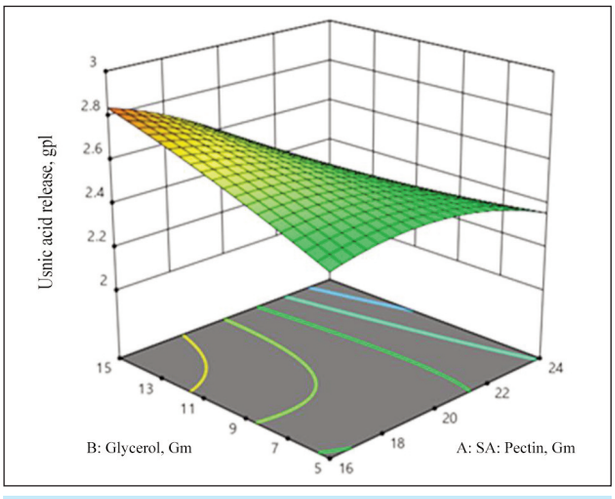


Fig. 6. Effect of SA:PC and glycerol levels on the In vitro release of Usnic acid

Mechanical properties of the dressing

An ideal wound dressing is expected to have sufficient strength, elongation and flexural rigidity that it may encounter when used on the wound as a primary or secondary dressing.

The tensile properties of the dressing show that the dressings were having varying levels of elongation at break (figure 7). Significant variation was seen in values of tensile modulus and stress at 6% strain (which represents low levels of stretching that the dressing is eventually expected to experience when used on the skin as a wound dressing) This can be attributed to varying composition levels of the SA:PC ratio and Glycerol. The amount of Usnic acid was not seen to have any significant impact on the tensile properties of the dressing.

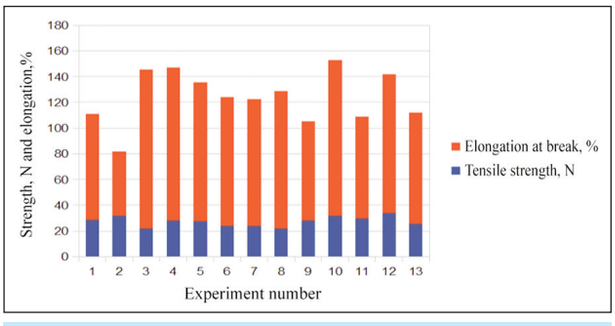


Fig. 7. Strength and elongation of dressing materials

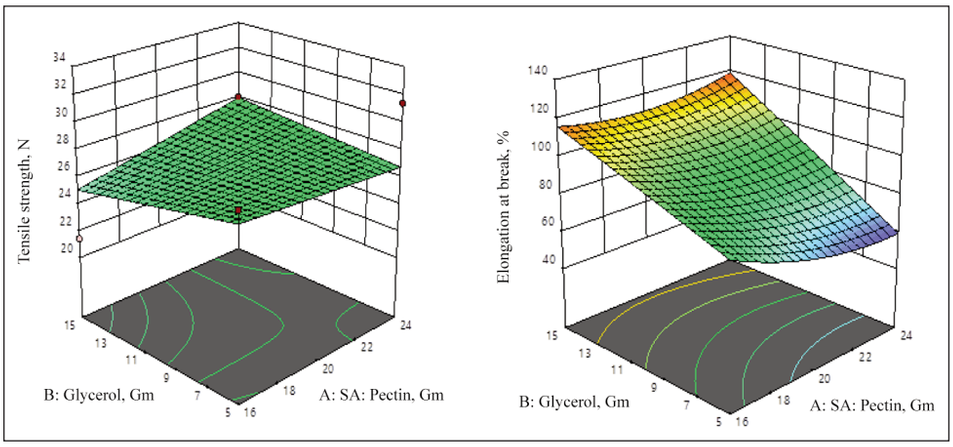


Fig. 8. Effect of SA:PC and glycerol levels on the tensile strength and elongation at break

It was seen that the tensile strength of the dressings drops slightly with the increasing amount of glycerol. A higher amount of SA:Pectin combination gives slightly better strength especially at lower glycerol concentrations. The effect is found to be fairly the same with different levels of Usnic acid. The reason for the lower strength at a higher concentration of glycerol is due to the increased flexibility of the dressings. Stiffer dressings are seen to break at lower strength compared to the more flexible dressings.

Figure 8 shows that the elongation at break for the dressings is sensitive to the amount of glycerol content. The higher the content of glycerol, the higher is the elongation, especially so when the SA:Pectin content is lower. This can be attributed to the fact that plasticizing effect is achieved by the presence of more glycerol in the dressing.

Water vapour transmission

Moisture vapours transmission (WVT) across the dressings under the experimental conditions show that moisture vapours transmissions across the dressings for all combinations were good and fairly consistent over each time zone of the study. This is a very important characteristic of a wound dressing. A good wound dressing material should not lock vapours from the wound and create a condition of exudates locking in the wound (figure 9).

The experimental analysis shows that the dressings had 350 grams per square meter per 24 h or more of the WVT levels. And none of the variable factors in the study considerably impacted the performance of the dressing for this property. This can be attributed to the fact that the thickness of the dressings across all combinations was almost the same with no significant difference and MVT rate is more linked with the path the vapour takes to pass through the structure (figure 10).

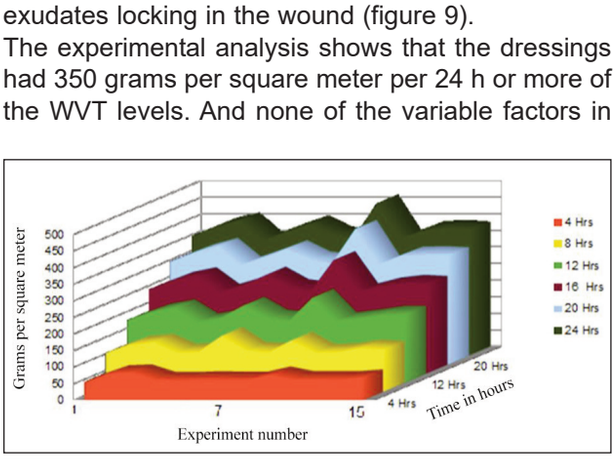


Fig. 9. Water vapour transmission studied over each time zone

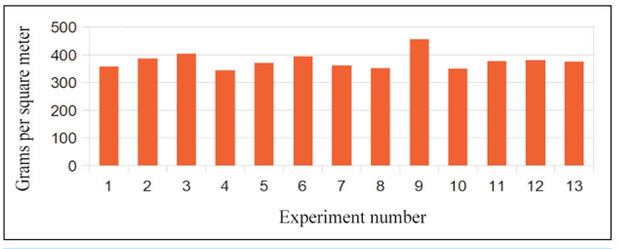


Fig. 10. Water vapour transmission per 24 hours for the dressing materials

Exudate's absorptivity and absorption profile

The exudates absorption property of dressing was seen to be rapidly increasing from 4 to 8 hours of the study and thereafter the absorption stabilizes (figure 11). This is seen more so in combinations containing higher glycerol contents. This is due to the saturation of the hydrogel in the dressing. Experimental analysis shows that exudates absorption is higher when SA:PC is Low and Glycerol is higher and also at a higher level of SA: PC level when Glycerol is low (figure 12).

The reason for the former effect can be attributed to the more porous content of the dressing with higher glycerol content helping for exudates absorption and retention. The latter effect can be attributed to the content of a more solid mass of SA:PC causing more exudates absorption, though a bit slower than the one containing more Glycerol.

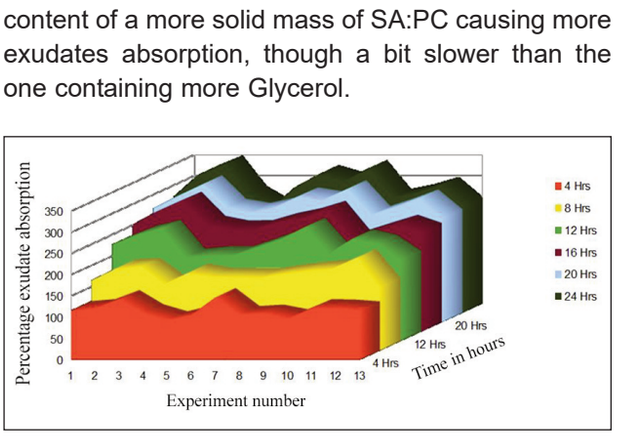


Fig. 11. Percentage exudate's absorption study over each time zone

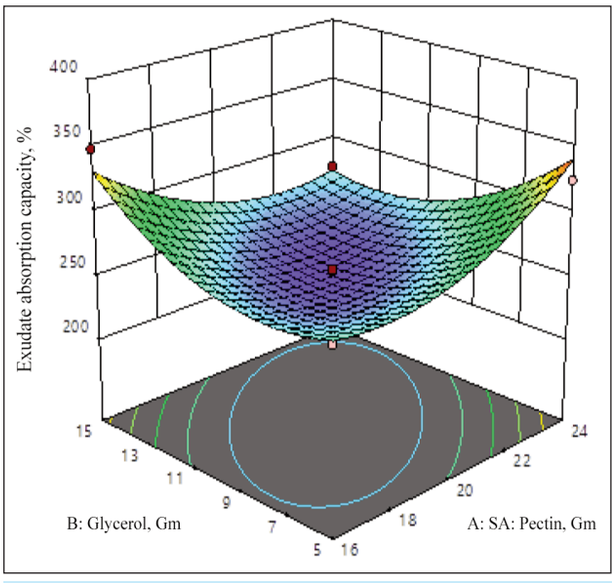


Fig. 12. Effect of SA:PC and glycerol levels on the exudate's absorption of dressing

Evaluation of antimicrobial activity

Results in table 3 show that the dressing was effective against a broad range of microbes including gram-positive, gram-negative bacteria and fungi/yeast. Table 3 summarizes the reduction in microbial count in 8 hours and 24 hours.

Table 3

ANTIMICROBIAL ANALYSIS OF DRESSING WITH LOWEST USNIC ACID CONTENT

Sr. no.	Test organisms	% Reduction in	
		8 hours	24 hours
1	*Staphylococcus aureus ATCC 6538*	99.90	99.99
2	*Listeria monocytogenes ATCC 19115*	99.90	99.99
3	*Enterococcus faecalis ATCC 29212*	99.90	99.99
4	*Escherichia coli ATCC 25922*	99.90	99.99
5	*Pseudomonas aeruginosa ATCC 15442*	99.90	99.99
6	*Klebsiella pneumoniae ATCC 4352*	99.90	99.99
7	*Candida albicans ATCC 10231*	90.00	99.90
8	*Aspergillus niger ATCC 6275*	90.00	99.90
9	*Positive Control*	100.00	100.00

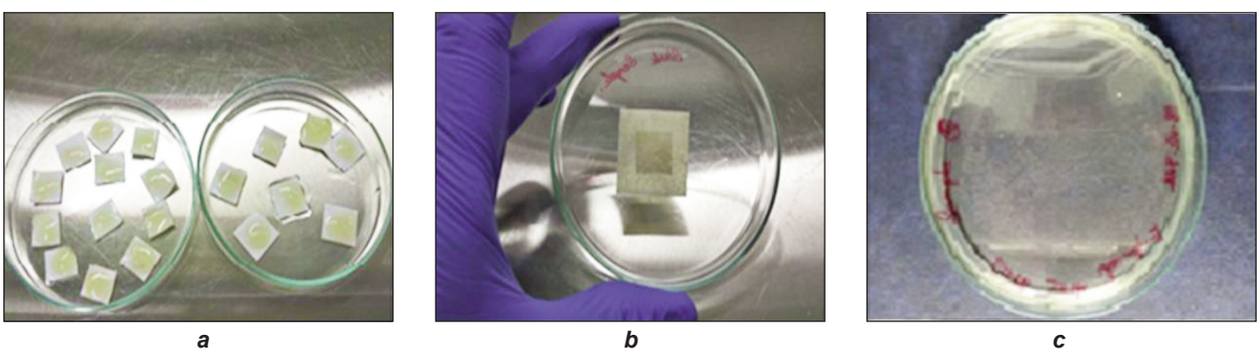
With these efficacy tests, it can be inferred that the developed dressing material can be used for infected wounds that have polymicrobial co-infections.

Biofilm inhibition assay

In this study, it was found that the plate with no Usnic acid dressing had bacterial growth on the plates, whereas all the other study plates demonstrated excellent biofilm inhibition. In this study, it can be seen that the presence of Usnic acid is able to nullify the bacteria even in the biofilm and hence after incubation none of the plates containing Usnic acid showed bacterial growth. Thus, it can be concluded that the lowest amount of Usnic acid used in the experimental study was sufficient enough to take care of bacterial growth in the form of biofilm and hence the formulation can be used very effectively in the development of wound care dressing that can be used in managing chronic wound conditions (figure 13). The drug release studies have shown that the Usnic acid is released significantly in the first 4 hours during swelling and there is the presence of drug for 24 hours in the swelling atmosphere. This will ensure that the biofilms are targeted by the presence of Usnic acid and its residual antimicrobial action will help in reducing the microbial load on the wound significantly.

CONCLUSION

The developed textile-based hydrogel dressing is seen to be effective in terms of its exudates management capacity, Strength, elongation and Water vapours permeability. The usage of active antimicrobial and antibiofilm agent, Usnic acid, has given it a broad-spectrum antimicrobial activity and antibiofilm property. The combination of these properties makes this dressing a better choice for the management of chronic wounds. The design of the experiment used in this study is able to point at the optimal combination of the materials for the construction of such hydrogel dressings and understand the contribution of each factor used in the study.



a *b* *c*

Fig. 3. Stages in biofilm inhibition assay study: *a* – biofilm on Nitrocellulose base; *b* – dressing placed on the biofilm; *c* – incubated plate for bacterial count

The disadvantage associated with the current hydrogel dressings is that the gels after swelling are too weaker to be removed and disintegrates, whereas the current approach makes it possible to be handled easily. The dressing developed can be cut into rope form also and used as wound well-filling material which later can be easily removed once laden with exudates. This will ensure good debridement of chronic wounds, which is essential in the management of chronic wounds.

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