



Lignocellulosic membrane grafted with 4-vinylpyridine using radiation chemistry: antimicrobial activity of loaded vancomycin

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Abstract Controlled synthesis of biomaterials with pH-responsive materials by radiation chemistry allows the development of new materials with fine-tuned properties that is affordable for simple production schemes and without potentially harmful chemicals. This work develops new lignocellulose-based materials with antimicrobial properties for wound dressing. The proposal was the radiation-grafting-induced of 4-vinylpyridine (4VP) onto a lignocellulosic membrane from *Agave salmiana* (v. Maguey) to provide pH-response. The lignocellulose-based

materials were suitable for the load and release of an antimicrobial glycopeptide drug, improving the drug load and a prolonged release. Finally, the loaded pH-responsive materials exhibited excellent antimicrobial activity against Gram-positive bacteria at different pH. The new materials were characterized by several techniques such as thermogravimetric analyzes, contact angle, scanning electron microscopy, infrared spectroscopic, mechanical properties, nuclear magnetic resonance, X-rays photoelectron spectroscopy, and microbiological tests.

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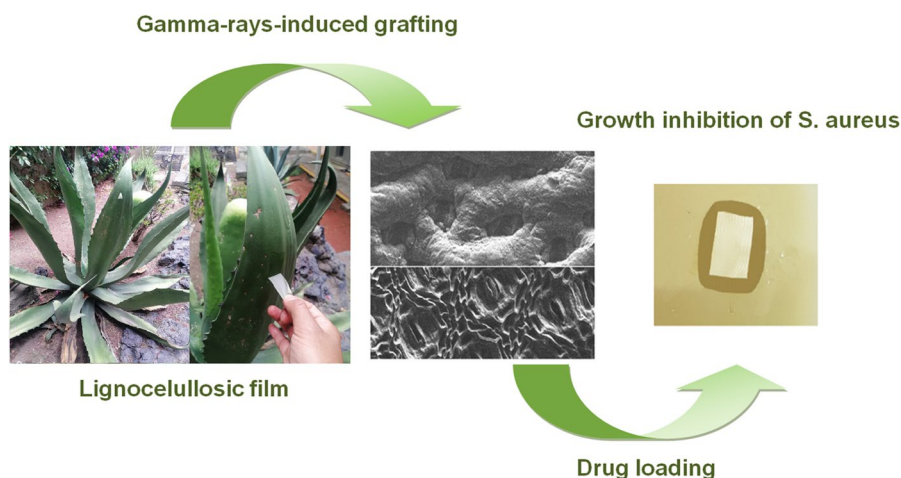
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Graphical abstract



Keywords Lignocellulose · Skin maguey · Biopolymer · Grafting · pH responsiveness · Gamma-rays · Supports · 4-Vinylpyridine · *Agave atrovirens*

Introduction

Natural cellulose-based materials have gained significant attention due to their promising properties, including biocompatibility, biodegradability, hydrophilicity, good mechanical properties, and low density (Tian et al. 2014; Wang et al. 2015, 2017). Said properties make them ideal for tissue engineering, regenerative medicine, and drug delivery (Thakur and Thakur 2014; Joshi et al. 2016; Vázquez et al. 2021). Controlled drug delivery is an essential topic in the medical area; since new methodologies for materials engineering are under development of more efficient smart materials, the future is promissory. The design of these new materials includes the modification by graft polymerization; this technique provides materials with properties such as the pH-response, which is very useful in the prolonged release of drugs from the polymeric system to the release medium, which can maximize its bio efficiency (Langer 1990). However, this simple process is affected by multiple factors, such as the physicochemical properties of the drug

and the release medium, the structural characteristics of the polymeric system, and derived interactions from these factors (Fu and Kao 2010).

In this study, natural lignocellulosic membranes from the skin of *Agave atrovirens* (commonly named maguey) were modified using gamma radiation-induced grafting of 4-vinylpyridine (4VP). The radiation chemistry method promotes the formation of free radicals that initiate the side-chain reaction of grafting polymerization (Ramos-Ballesteros et al. 2020). Compared to conventional chemical methods, there is no need for additional initiators or crosslinking agents in the radiation grafting method, so the products are free from toxic additives that can cause adverse side effects for the patient (Pino-Ramos et al. 2018; Zhuang et al. 2018). Therefore, gamma radiation allows obtaining new materials and nanomaterials (Flores-Rojas et al. 2020a) with a surface chemistry of tailored properties (Flores-Rojas et al. 2020b).

Poly(4VP) (here abbreviated as P4VP) is considered a safe material and exhibits a characteristic that allows modification of its pH responsiveness by controlling the molecular weight using polymer concentrations and copolymer composition (López-Saucedo et al. 2022). Therefore, grafting of 4VP onto lignocellulosic films should endow with a pH response enhancing controlled drug delivery to the treatment site, providing a potential application as an antimicrobial drug-releasing wound dressing (Kiziltas et al. 2014, 2016; Shrestha et al. 2016).

Firstly, this article presents the synthesis of 4VP-graft (-g-4VP) functionalized lignocellulosic membranes to create a pH-responsive material, followed by the load and release of the antimicrobial agent in the resulting material. The drug vancomycin, a glycopeptide, was selected due to its activity against most Gram-positive microorganisms (Szász et al. 2013; Fusco et al. 2018), including several pathogen bacteria for human beings, which cause severe or fatal infections. After, the new pH-sensitive lignocellulosic membranes were characterized by FTIR-ATR, ^{13}C -NMR, XPS analysis, and TGA. Also, the pH response of the modified membranes was studied at different pH values; the hydrophilicity was measured by contact angle with water droplet; the mechanical properties were determined by uniaxial tension; and finally, the microbiological activity of the vancomycin-loaded material was tested in vitro.

Materials and methods

Materials

Lignocellulosic membranes were obtained from the skin of *Agave salmiana* (MS), a digital Vernier was employed to perform measures, and found thickness was $104 \pm 5.4 \mu\text{m}$ and density 0.05 mg cm^{-3} . The membranes were rinsed in a basic solution (0.1 M NaOH) and subsequently soaked in a mixture of water/methanol (1/1 vol%) to remove biomass and other residues (arabinoses, hemicelluloses, etc.). Vancomycin was acquired from Fagron (Colombia); 4VP, NaOH, and methanol were purchased from Sigma Aldrich (St. Louis, MO, USA). The monomer 4VP was distilled at reduced pressure before its use.

Grafting of membranes

The lignocellulosic membrane (MS) was cut into $1.2 \times 5 \text{ cm}$ pieces and dried at room temperature under reduced pressure. The MS membranes were placed in an ampoule containing 7 mL of 4VP solution, varying the monomer concentration (0–13 vol%) in a mixture of water/methanol (67/33 vol%). Air was removed by argon bubbling (20 min) and sealed. Subsequently, the ampoules were exposed to a ^{60}Co gamma-rays source at different doses, up to 60 kGy. After gamma irradiation, the residual monomer and

homopolymer were removed by stirring in water/methanol (70/30 vol%), changing the solvent twice each 24 h, followed by vacuum drying at 40°C until constant weight.

The grafting yield (G%) was calculated using Eq. 1:

$$G(\%) = 100[(W_f - W_i)/W_i] \quad (1)$$

where W_f and W_i are the weights after and before graft, respectively.

Contact angle

Kruss DSA 100 drop shape analyzer was used to measure the contact angle on the surface of different samples.

Flat and dry films were placed inside the chamber. External angle of a drop of water was measured at 1 min using the native software; each experiment was performed in triplicate.

Study of swelling: pH-response

MS and MS-g-4VP membranes were immersed in 20 mL of a phosphate buffer at different pH values (2–11) at 25°C for 24 h. The membranes were extracted, removed excess buffer with filter paper, and weighed. The critical pH of the membranes was calculated by recording the degree of swelling (DS) at each pH value using the following equation:

$$DS(\text{wt}\%) = 100[(W_w - W_d)/W_d] \quad (2)$$

where W_d and W_w are the weights of the films before and after swelling, respectively.

Mechanical tests

Mechanical properties of the samples were studied by applying uniaxial tension test, firstly the samples were soaked in distilled water before cutting the dumb-bell shape, and subsequently the test specimens were dried at vacuum before carrying out the testing following the method described in ASTM D1708. All tests were conducted on an INSTRON 1125 (Instron Inc., MA, USA) universal tensile testing machine at a crosshead speed of 10 mm/min. All experiments were carried out in triplicate.

Load and release of vancomycin

Pieces of grafted membranes (MS-g-4VP) (1×1.2 cm) were placed in vials with 5 mL of a vancomycin aqueous solution (50 $\mu\text{g/mL}$) at buffer [0.01 M] pH=5, 7, and 9, and the load was monitored at room temperature and at pre-established time intervals. Samples were taken out from the medium, and measured the UV absorbance of the solution at 280 nm; the amount of loaded drug was calculated using Eq. 2, which was obtained from experimental values using a calibration curve and the membrane area. The experiment was carried out in triplicate.

$$\text{Loaded drug } (\mu\text{g/cm}^2) = (A_1 - A_2) / 0.0159084 \quad (3)$$

where A_1 and A_2 represent the initial and final absorbance of the loading medium.

Drug release was evaluated by transferring the loaded membranes to vials with 5 mL of buffer [0.01 M] at different pH=5, 7, and 9, and kept under stirring at 180 rpm and 25 °C. The released drug was monitored by UV, measuring the absorbance at 280 nm at different times, and calculated the drug concentration using a calibration curve. The tests were carried out in triplicate.

In addition, the drug release from MS-g-4VP membranes was assessed using representative mathematical models (Dash et al. 2010) as follows:

$$\text{Zero-order release model: } M_t = K_0 t \quad (4)$$

$$\text{First-order release model: } M_t = 1 - e^{-K_1 t} \quad (5)$$

$$\text{Higuchi release model: } M_t = K_h t^{1/2} \quad (6)$$

where M_t is the fraction of drug released at each time (t), and K_0 , K_1 , and K_h are the zero-order, first-order, and Higuchi kinetic constants, respectively. Furthermore, the drug release mechanism was studied using the semi-empirical Korsmeyer–Peppas model (Korsmeyer et al. 1983).

$$\text{Korsmeyer – Peppas model: } M_t = K t^n \quad (7)$$

K is a kinetic constant that considers the structural and geometric characteristics of the device; n is the release exponent, which is indicative of the release mechanism; if the value of n is $0.45 \leq n$ corresponds to a Fickian diffusion, $0.45 < n < 0.89$ to a non-Fickian

transport, $n = 0.89$ to Case II transport, and $n > 0.89$ to super Case II transport mechanism.

Antimicrobial tests

The capability of the drug-loaded membranes to inhibit bacterial growth was tested against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Petri plates containing Müller–Hinton agar were seeded in concentration for *S. aureus* $1.2 \cdot 10^9$ CFU/mL and *E. coli* $3.4 \cdot 10^9$ CFU/mL (where CFU is a colony-forming unit). Vancomycin-loaded MS-g-4VP and blanks of MS-g-4VP pieces ($\approx 1 \times 1.2$ cm and $\varnothing$ 0.5 cm diameter) were tested. Plates containing the sample were incubated at 37 °C for 24 h. The inhibition zone was measured using a scale. Each experiment was performed in triplicate.

Instrumental

Vacuum drying

Oven model Yamato ADP21 was employed to store the samples before its use (at least for 24 h) under conditions of 25 °C and 0.1 kPa.

Contact angle

Kruss DSA 100 drop shape analyzer (Matthews, North Carolina, USA) was used to measure the contact angle.

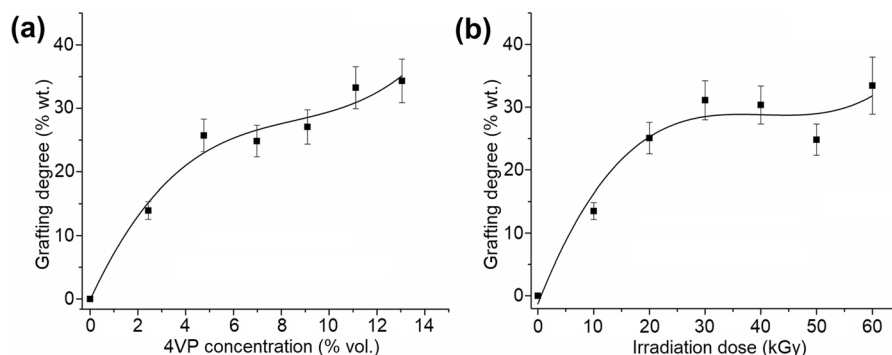
Fourier transform infrared attenuated total reflection (FTIR-ATR)

Dry samples (MS and MS-g-4VP films) were analyzed using a Perkin–Elmer Spectrum 100 spectrometer (Norwalk, Connecticut, USA) of 16 scans. TA Universal analysis software was used for data.

Thermogravimetric analysis (TGA)

Weight loss and decomposition of films were heated at a rate of 10 °C min^{-1} and run from 20 to 800 °C in a TGA instrument Q50 TA Instruments (New Castle, Delaware, USA).

Fig. 1 Graft degree vs. monomer concentration (a) with an irradiation dose of 20 kGy and (b) graft degree vs irradiation dose with a monomer concentration of 5 vol%



¹³C-CPMAS NMR

It was recorded in a Bruker Avance II 300 MHz spectrometer (75.48 MHz for ¹³C) at spinning rates of 4 kHz and 5 kHz, using 4 mm zirconia rotors and tetramethylsilane (TMS) as the external reference. Mnova 14 software was used for spectra data.

Scanning XPS

Microprobe PHI 5000 Versa Probe II (Chanhasen, Minnesota, USA), with an excitation source of Al K α monochromatic, energy 1486.6 eV, 100 μ m beam diameter, and with a Multi-Channel Detector (MCD). The energy scale was corrected using the C1s peak to 285.0 eV. Multipack software was used spectra data.

Mechanical test

INSTRON 1125 (Instron Inc., MA, USA) universal tensile testing machine at a crosshead speed of 10 mm/min.

SEM Images were acquired by the Zeiss Evo LS15 instrument (Jena, Germany). Small pieces of 1 cm in length were cut and coated with gold and analyzed under a high vacuum.

Ultraviolet–visible (UV–vis) Spectrophotometer model Agilent 8453 (Waldbronn, Germany), using quartz cuvettes of 1 cm length.

Results and discussion

Synthesis and characterization of MS-g-4VP membranes

4VP grafting was successfully carried out on MS membranes at a low monomer concentration (2.4% 4VP) with approximately $\approx$ 13% grafting, with similar results at the lowest irradiation dose of 10 kGy. However, the effect of concentration did not show a maximum of grafting, indicating that the degree of modification was increased by increasing the monomer concentration without requiring to increase in the radiation dose above 20 kGy, as shown in Fig. 1a. In the case of the irradiation dose kinetics with a fixed concentration of 5% (v/v) of monomer, it was not possible to increase the grafting degree because monomer homopolymerization is favored at an irradiation dose of 20 kGy, reaching an average maximum graft degree of $\approx$ 35% and remaining constant at higher doses. Therefore, irradiation doses above this value are an excess that can degrade the matrix and promote other reactions such as crosslinking of the grafted polymeric chains (Fig. 1b). In general, the grafting degree on MS membranes depends on these two variables, yield increasing as monomer concentration and irradiation dose increased.

Water contact angle and pH responsiveness

The hydrophilicity and pH responsiveness of the MS-g-4VP membranes were measured by contact angle and swelling in water at different pH values. The contact angles were obtained on both sides

of the membranes, showing a side more hydrophobic than the other one, as was observed in the non-grafted MS membranes (Fig. 2a). The behavior of hydrophobicity was explained by the surface structuring and composition of each side of the MS membrane, because the internal part has a higher content of nitrogenous and oxygenated groups from the possible biomass residues, which increased the hydrophilicity (Silva et al. 2016), in addition to a more complex surface structuring (“C-CPMAS NMR and XPS analysis” and “Scanning electron microscopy (SEM) study” sections). However, the contact angle on both sides tended to be similar as the graft yield was increased, indicating that despite the chemical composition and surface structure, the graft was uniformly made on both sides, reassigning an average contact angle for both sides of 91.2°. Despite the contact angle, the samples had an excellent swelling, and the pH responsiveness was also evaluated using different pH buffers at room temperature (Lina et al. 2009). The MS-g-4VP membranes exhibited a pH response reaching a swelling degree of up to 111% in an acid medium (pH=3) for the membranes with a graft of 25.7%, which decreased as the grafting degree decreased. In the case of the response to pH, the MS-g-4VP membranes exhibited a response to the change in pH, decreasing their degree of swelling compared to the MS membranes, which showed a degree of swelling without significant changes despite the content of nitrogenous groups present in the membrane, which could present an acid–base behavior. In both cases, the MS-g-4VP (13.9 and 25.7%) membranes showed a critical pH of 6.5 (Fig. 2b), considerably reducing the degree of swelling, even below the MS membrane.

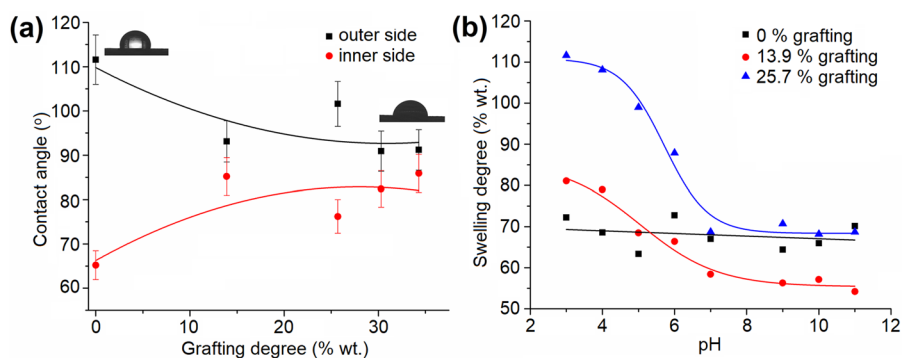
FTIR-ATR study

The infrared study of pristine MS membrane indicated the presence of several chemical functional groups such as $-\text{OH}$ (1461 and 3316 cm^{-1}), $-\text{NH}-$ (3372 cm^{-1}), $-\text{CH}-$ (2995 cm^{-1}), and $-\text{COC}-$ (1161 cm^{-1}) (Silverstein et al. 2005). However, each side of the MS membrane showed differences, such as less hydrophilic functional groups ($-\text{OH}$ and $-\text{NH}-$) on the outer part (Fig. 3a, b); this finding agreed with contact angle and later studies. On the other hand, the grafted MS membranes showed the bands corresponding to the -g-4VP, which were assigned to $-\text{C}=\text{CH}$ (3098 cm^{-1}), $-\text{C}=\text{C}-$ (1639 cm^{-1}), and harmonic bands from 1800 to 1982 cm^{-1} of the -g-4VP (Fig. 3c, d), confirming the unequivocal presence of the P4VP chains grafted on the MS membranes since similar bands are observed in the P4VP homopolymer spectrum as can be seen in Fig. 3e.

Thermal analysis

The results obtained from the thermogravimetric analysis (Table 1) showed that the MS membranes had a slight weight loss below a temperature of $100\text{ }^{\circ}\text{C}$, probably caused by volatile residues (i.e., water) and not by the decomposition of the sample, being stable at a temperature below $250\text{ }^{\circ}\text{C}$, and showing several decompositions corresponding to the sample above this (Fig. 4d). P4VP grafted chains provided the MS membrane with better thermal stability, which was according to the grafting degree (Fig. 4a–c), there was an improved thermal stability as the grafting increased and maximum decomposition temperatures

Fig. 2 (a) Dependence of water contact angle at $25\text{ }^{\circ}\text{C}$ vs the 4VP grafting percentage onto MS membranes and (b) pH-responsiveness of MS-g-4VP membranes in pH 2–11



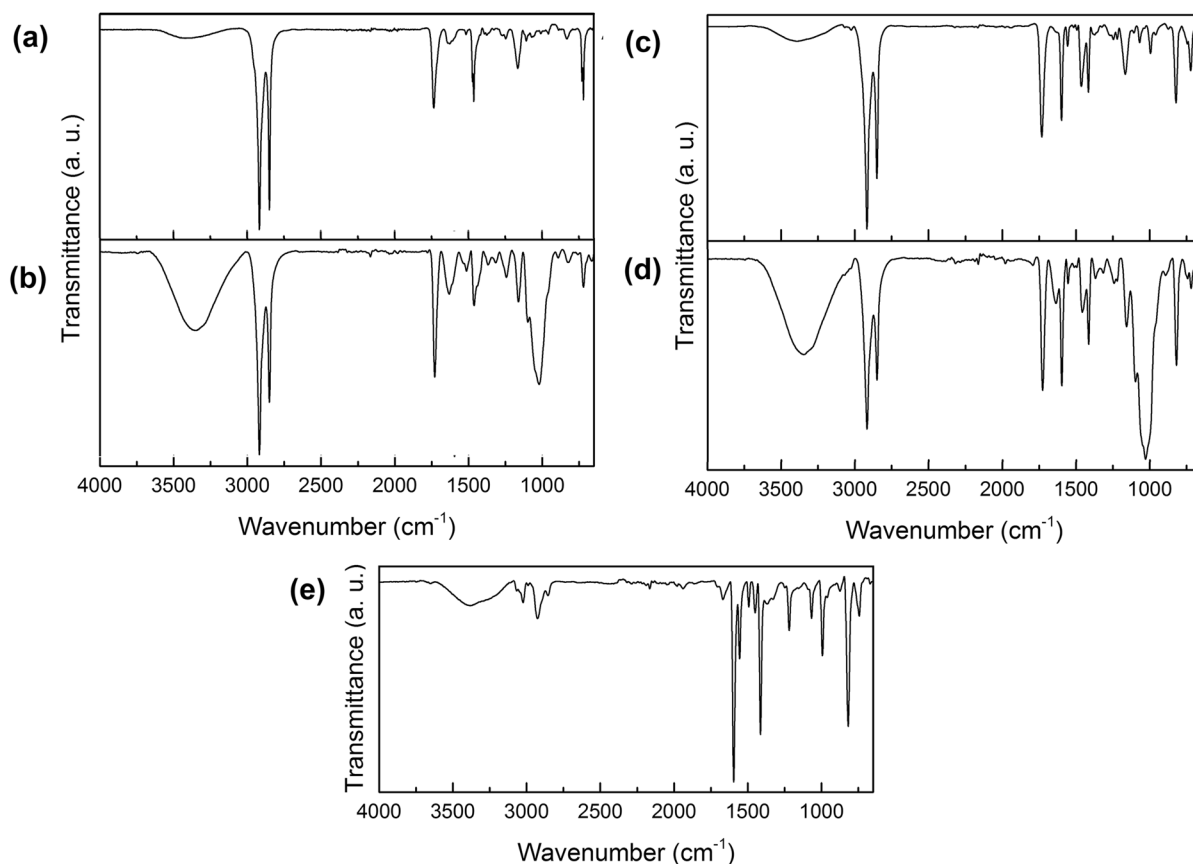


Fig. 3 FTIR-ATR spectra of both sides of the MS membrane modified with 4VP. (a) and (b) are the outside and inside of the pristine membrane, (c) and (d) are the outside and inside of MS-*g*-4VP (31.0%), and (e) P4VP obtained by gamma rays

Table 1 Decomposition temperatures of P4VP, MS, and MS-*g*-4VP membranes with different grafting degrees

Sample	Decomposition temperature (°C)
MS- <i>g</i> -4VP (31.0%)	354, 434.1
MS- <i>g</i> -4VP (25.7%)	264.2, 347, 388.1, 435.8
MS- <i>g</i> -4VP (13.2%)	260.8, 336, 386.6, 436.8
MS	252.4, 334, 412.2, 445
P4VP	388

at 354 and 434.1 °C, being this last decomposition attributed to -*g*-4VP (Fig. 4e).

C-CPMAS NMR and XPS analysis

¹³C-NMR spectra of the MS membrane indicated not only the signals of the cellulose chains but also the presence of probable aliphatic chains linked to amino

groups from the residues of biomass, as showed in the signals located at 32.2–35.0 and 59.1 ppm, respectively, being these findings in accordance with other studies such as infrared, contact angle, and XPS analysis. The signals at 65.0 and 67.1 ppm correspond to –CH₂OH, while the other signals were assigned to cellulose endocyclic carbons (from 59.1 to 107.4 ppm) (Flores-Rojas et al. 2020b), which are shown in Fig. 5. Meanwhile, the spectra of MS-*g*-4VP confirm the modification by means of grafting exhibiting the corresponding signal of P4VP, from these signals, the most characteristics are the correspond to pyridine moiety located at 102.2, 124.8–127.7, and 156.6–156.4 ppm, as well as the aliphatic chain from the polymerization of vinyl moieties at 42.4 ppm.

XPS analysis determined the chemical compositions of the MS and MS-*g*-4VP membranes, showing the corresponding peaks of carbon (C1s at 285.0 eV), oxygen (O1s at 531.0 eV), and nitrogen (N1s at

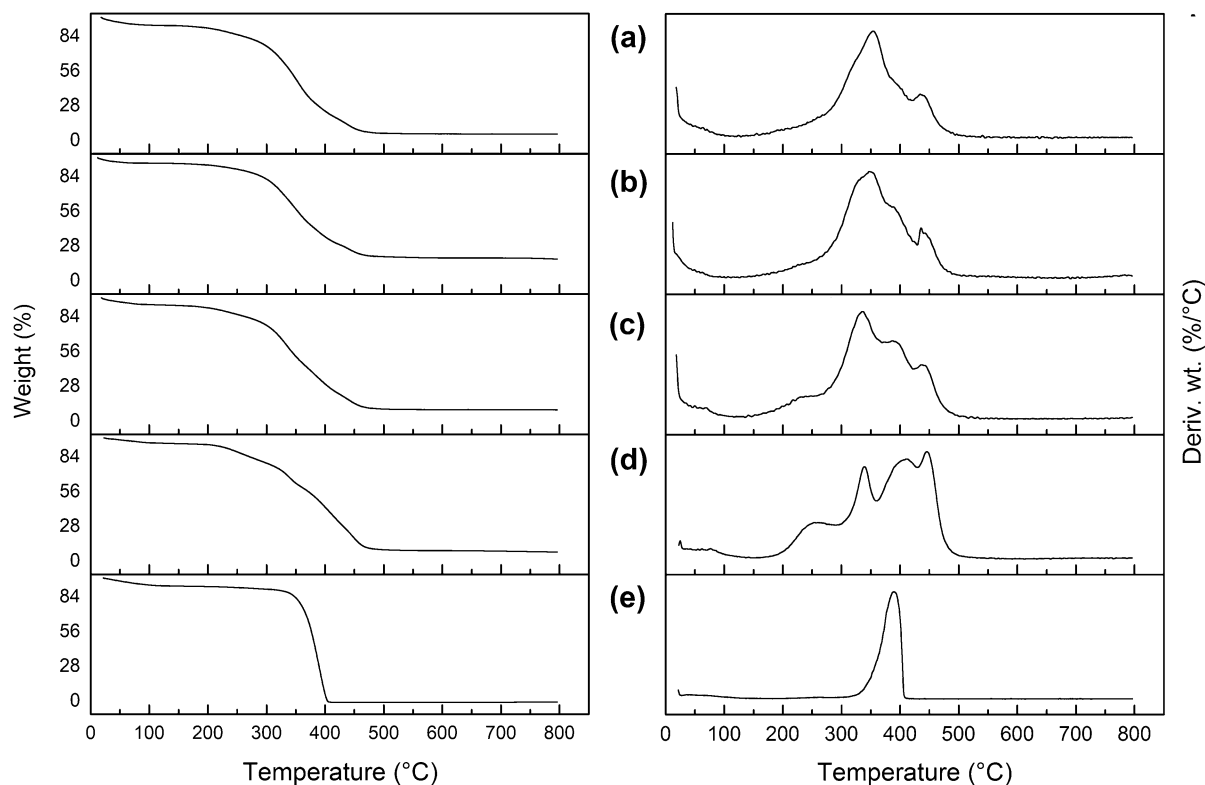


Fig. 4 TGA study of MS and MS-g-4VP membranes. (a) MS-g-4VP (31.0%), (b) MS-g-4VP (25.7%), (c) MS-g-4VP (13.2%), (d) MS, and (e) P4VP obtained by gamma-rays

399.4 eV) in MS and MS-g-4VP membranes and all the peaks were detected in the survey scan spectrum shown in Fig. 6. The atomic ratio of membranes was calculated from the areas of C1s, O1s, and N1s peaks in the XPS survey scan, multiplied by the appropriate sensitivity factors. The measured atomic composition of C, O, and N for each side are listed in Table 2. The results indicated an increase in the ratio of N1s on both sides of the membrane, which is consistent with the grafting of 4VP onto the MS membrane, and a decrease of the C1s and O1s on the surface of membranes, caused by the coating of the grafted P4VP chains. In addition, the results showed an increase of approximately 1.5 times the amount of nitrogen in the internal than in the outer part, suggesting that possibly there was a greater degree of grafting in the internal part of the membrane compared to the outer part, a behavior perhaps caused by due to the possible biomass residues present, which could form a greater number of graft sites.

Mechanical properties

Mechanical properties exhibited by the pristine membrane were excellent, and this was in accordance with their easy handling, but these suffered some considerable changes after the modification with the grafting polymerization using gamma-rays (Fig. 7 and Table 3). The most important modifications in the mechanical properties after the grafting was the increase of Young's modulus until 76.58 ± 3.82 MPa. Even though, Young's modulus of both grafted membranes was not considerably different. Similarly, tensile, tenacity, and elongation exhibited minor differences with pristine MS, where the tensile break increased, and elongation showed a decrease as graft degree increased, while tenacity had not an appreciable tendency. Finally, the maximum load did not differ considerably with a load of 3 N regardless of grafting degree (0, 13.2, and 31.0%).

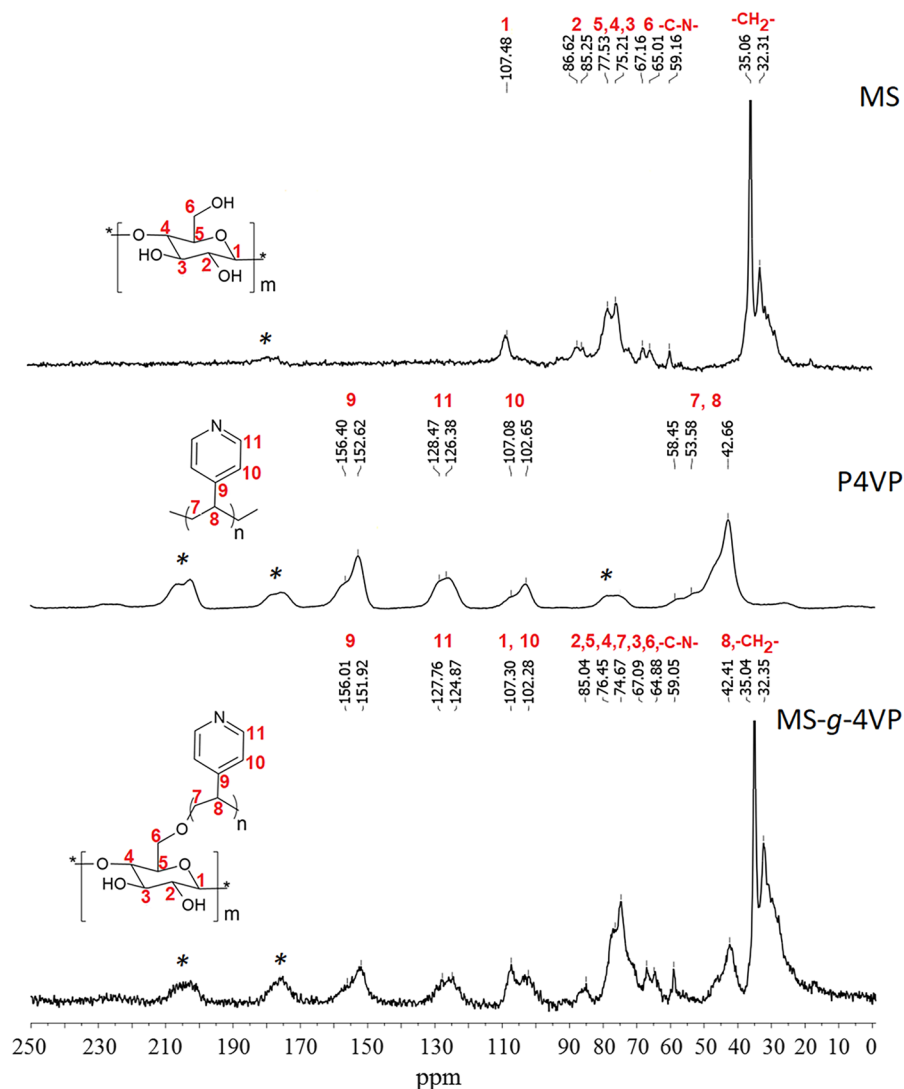


Fig. 5 CP-MAS ¹³C-NMR spectra of MS, P4VP, and MS-g-4VP (31.0%) membranes

Scanning electron microscopy (SEM) study

SEM images showed a complex morphology on the surface of the pristine MS membranes (Fig. 8a). The lignocellulosic material showed two completely different types of morphology depending on the outer and inner parts. The outer part had knitting fabric-like patterns with small channels, while in the inner part, a series of channels with a more complex structure was observed, which could be related

to the plant's water absorption system. The surface morphology after graft polymerization indicated a homogeneous modification of -g-4VP without generating cracks in the membrane and other damage caused by gamma radiation or graft polymerization. High magnification SEM images showed the formation of small aggregates on the grafted membranes, undoubtedly caused by the P4VP chains (Fig. 8b, c).

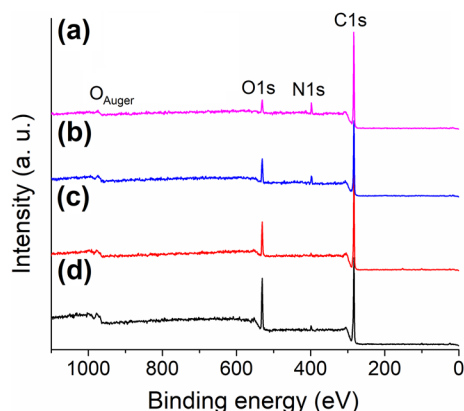


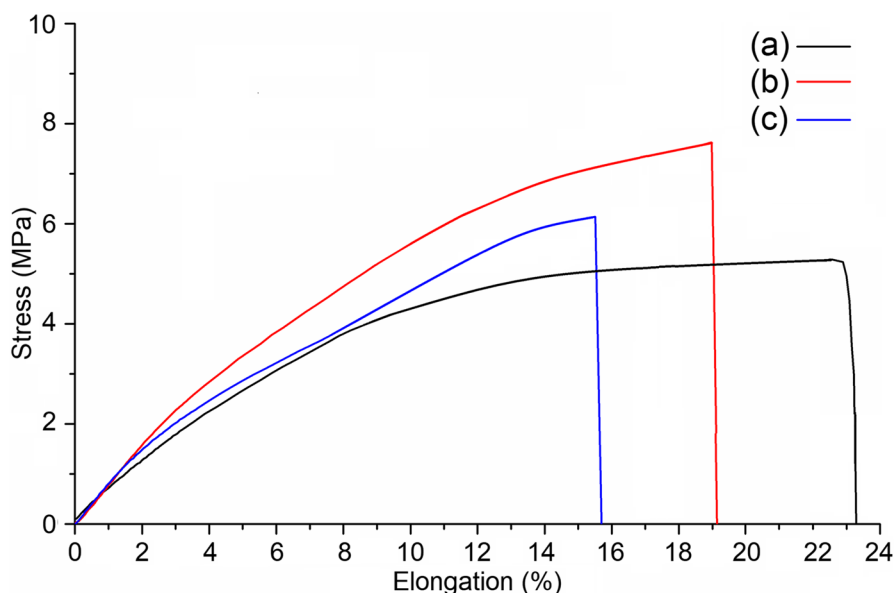
Fig. 6 XPS survey spectra of membranes, (a) and (b) correspond to MS-g-4VP (31.0% grafting) inside and outside part, (c) and (d) are de spectra of pristine MS membrane, assigned to inside part and outside part, respectively

Table 2 Results of elemental composition by XPS of MS and MS-g-4VP membranes

	Atomic composition (%) [*]		
	C	N	O
MS outside part	86.9	0.7	12.7
MS inside part	80.9	1.9	17.2
MS-g-4VP (31.0%) outside part	83.0	4.7	12.2
MS-g-4VP (31.0%) inside part	76.7	8.1	15.2

^{*}ASF content in Multipack code estimated uncertain about 5%

Fig. 7 Tensile stress–strain plots of (a) MS and MS-g-4VP with different grafting percentage (b) 13.2% and (c) 31.0%



Loading and releasing of vancomycin

Vancomycin loading was performed using MS membranes with different percentages of 4VP grafted. Drug loading in all cases was prolonged and pH dependent, as indicated by the kinetic loading, with the maximum loading being obtained around 60 h at pH=7. Grafted P4VP chains modified these changes in the loading rate; being loading increased with grafting percentage, obtaining the highest vancomycin loading at pH=5 for all membranes, and decreasing loading at higher pH values showing the lowest vancomycin loading at pH=7.

In general terms, results indicated loading on all membranes, including MS membranes, even without modification. Nevertheless, -g-4VP membranes enhanced vancomycin loading at any pH value compared to pristine MS membranes, showing decreased vancomycin loading for 31.0% grafted membranes at pH values = 7 and 9, contrasting these values with those obtained at pH=5, where the higher the degree of grafting, the greater the drug load, with values from 28.76 to 8.62 and 12.8 $\mu\text{g mg}^{-1}$ at pH=7 and 9, respectively. These results indicated there is a greater affinity of the drug for the graft at pH=5, a behavior corroborated by the release of vancomycin because it presented the lowest vancomycin released with 28.95% compared to a higher release of 55.74% at

Table 3 Mechanical properties of membranes under uniaxial tension of MS and MS-g-4VP membranes

Sample	Young's modulus (MPa)	Tensile break (MPa)	Tenacity (MPa)	Elongation break (%)	Maximum load (N)
MS	45.25 ± 2.26	4.8 ± 0.24	0.722 ± 0.036	23.2 ± 1.16	3 ± 0.15
MS-g-4VP (13.2%)	76.58 ± 3.82	7.5 ± 0.37	0.819 ± 0.040	19.3 ± 0.96	3 ± 0.18
MS-g-4VP (31.0%)	74.90 ± 3.74	5.7 ± 0.28	0.557 ± 0.027	15.7 ± 0.78	3 ± 0.12

higher pH (Fig. 9a, Table 4). Indicating weaker interactions between the drug and -g-4VP-modified MS membranes, thus a graft above pH=5 did not benefit from vancomycin loading via N–H and O–H interactions between the P4VP chains and the drug, but through ionic and dipole–dipole forces between the drug and the -g-P4VP chains (Dancy et al. 2016). Therefore, the study suggests that the optimal graft found is around 31.0% and a pH=5.

Vancomycin release from MS-g-4VP membranes was tested at 25 °C in phosphate buffer at different pH values (Fig. 9b), showing a sustained release of

vancomycin for 30 h at pH=5 and considerably decreasing at pH=7 and 9, the shortest release time being for pH=9 with a release time of approximately 10 h. The prolonged release of vancomycin is consistent with the marked increase in affinity conferred by -g-4VP. This finding became even more evident when the percentages of drug released after 7 h were compared. As shown in Table 4, where the release of vancomycin was almost complete in 7 h in the case of the MS membrane at pH=7 and 9. However, a delay was noted for the membranes at pH=5, observing a release of less than 30%.

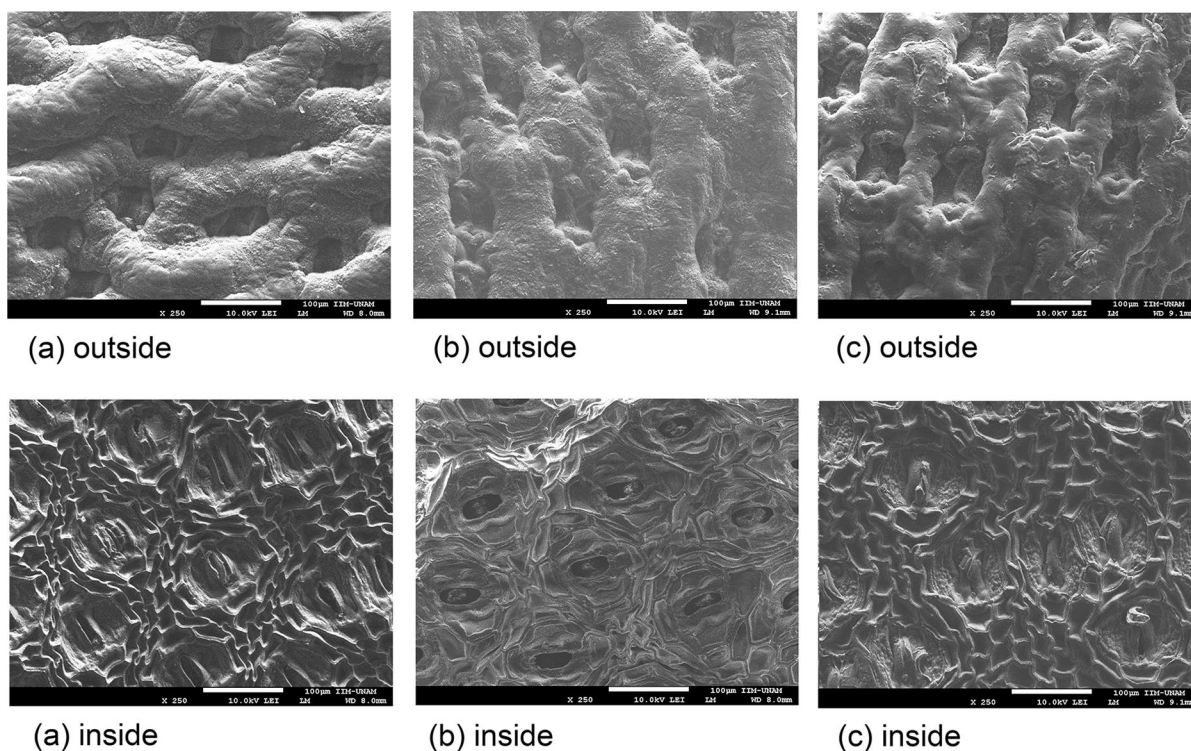


Fig. 8 SEM images of membranes at different magnifications, outside (top row) and inside part (bottom row); pristine MS (a), MS-g-4VP membrane with 13.2% grafting (b), and MS-g-4VP membrane with 31.0% grafting (c)

Fig. 9 (a) Loading and release of vancomycin at different pH values and (b) Loading and release kinetics of vancomycin at different pH

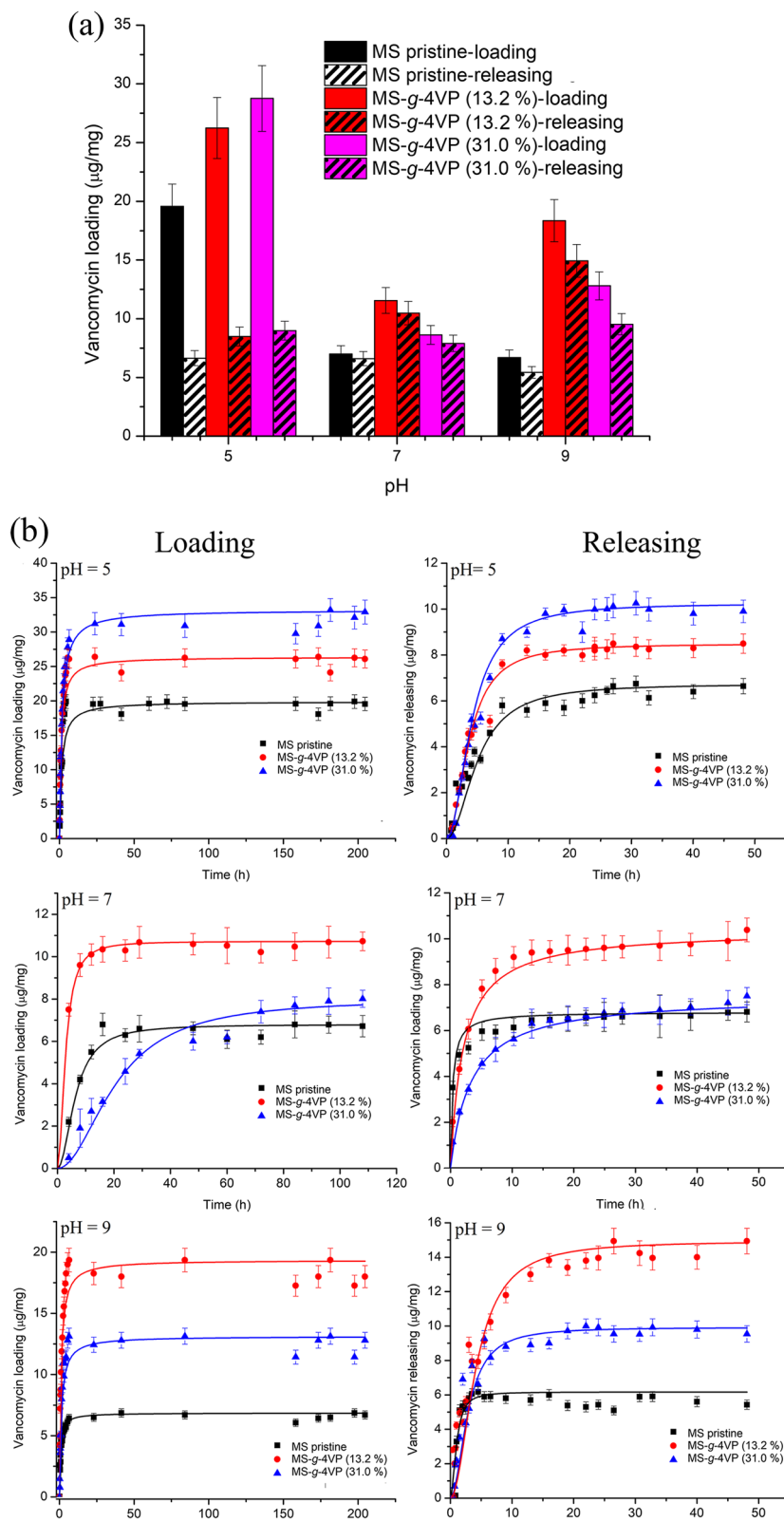


Table 4 Percentage of cumulative releasing of vancomycin at 7 h, time estimated to change a wound dressing

	Grafting degree (wt%)	cumulative vancomycin releasing at 7 h ($\mu\text{g}\%$)
pH=5	0	23.50
	13.2	28.95
	31.0	24.33
pH=7	0	85.13
	13.2	81.62
	31.0	74.33
pH=9	0	89.39
	13.2	55.74
	31.0	68.75

The vancomycin release profiles at 25 °C at the sampling points were sufficient to investigate the fit of the different mathematical models to the first 60% release interval. As shown in Table 5, the pH value modified the release mechanism showing a good release fit for the zero and first-order models as well as Korsmeyer–Peppas with a super Case II transport mechanism at pH=5, regardless of the degree of engraftment, revealing a possible drug release mechanism mix. However, at pH=7 and 9, the best fit was recorded for the Korsmeyer–Peppas model and indicated that vancomycin release followed the quasi-Fickian diffusion mechanism at pH=7 and non-Fickian at pH=9 indicated by the values of n (quasi-Fickian transport ≤ 0.45 and non-Fickian transport $0.45 \leq n \leq 0.89$). This behavior is because, in these membranes, the release of vancomycin occurs through the channels of the membranes, as well as the

relaxation of the polymer graft chains, which occurs once the material is in contact with the medium. Therefore, these properties are important for the controlled loading and release of vancomycin (Xu et al. 2020).

Microbiological tests

Finally, the antimicrobial performance of vancomycin-loaded membranes was evaluated against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). The antimicrobial activity of vancomycin against *S. aureus* is well known (Chakraborty et al. 2012), so the aim of study MS and MS-g-4VP membranes loaded with vancomycin is to determine possible differences in their antibiotic capability. Non-loaded MS membranes did not inhibit the growth of the bacteria (Fig. 10a). Differently, the same samples loaded with vancomycin showed inhibition zones in the *S. aureus* plate (Fig. 10b). Interestingly, the inhibition zone caused by vancomycin-loaded MS-g-4VP membrane was significantly greater (2.0 vs. 1.5 cm) than that observed for vancomycin-loaded MS membrane, which can be related to the fact that MS-g-4VP membrane can load more vancomycin. Overall, these results highlight that the amounts loaded are sufficient to inhibit the growth of *S. aureus* (Moses et al. 2020), a common pathogen present in chronic wounds.

Subsequent antimicrobial activity tests at different pH values showed a greater zone of inhibition at pH=5 for *S. aureus*, but release kinetics studies showed less vancomycin release, indicating the

Table 5 Fitting R parameters of different kinetic models (zero-order, first-order, and Higuchi) and the Korsmeyer–Peppas models on vancomycin release profile

Graft (wt%)		Zero-order	First-Order	Higuchi		Korsmeyer-Peppas	
		R ²	R ²	R ²	K _n ^a	R ²	n ^b
pH 5	0	0.9160	0.9169	0.7882	1.430	0.9105	0.942
	13.2	0.9475	0.9480	0.7721	1.887	0.9443	1.045
	31.0	0.9270	0.9258	0.7100	1.885	0.9443	1.045
pH 7	0	0.3246	0.3424	0.8294	4.339	1	0.072
	13.2	0.6265	0.6553	0.9730	3.845	0.9927	0.342
	31.0	0.7001	0.7152	0.9877	2.285	0.9967	0.389
pH 9	0	0.3795	0.3985	0.6234	2.985	0.8684	0.733
	13.2	0.7348	0.7560	0.8825	3.797	0.8861	0.588
	31.0	0.8299	0.8405	0.8197	3.241	0.8674	0.733

^aHiguchi release kinetic constant

^bRelease exponent

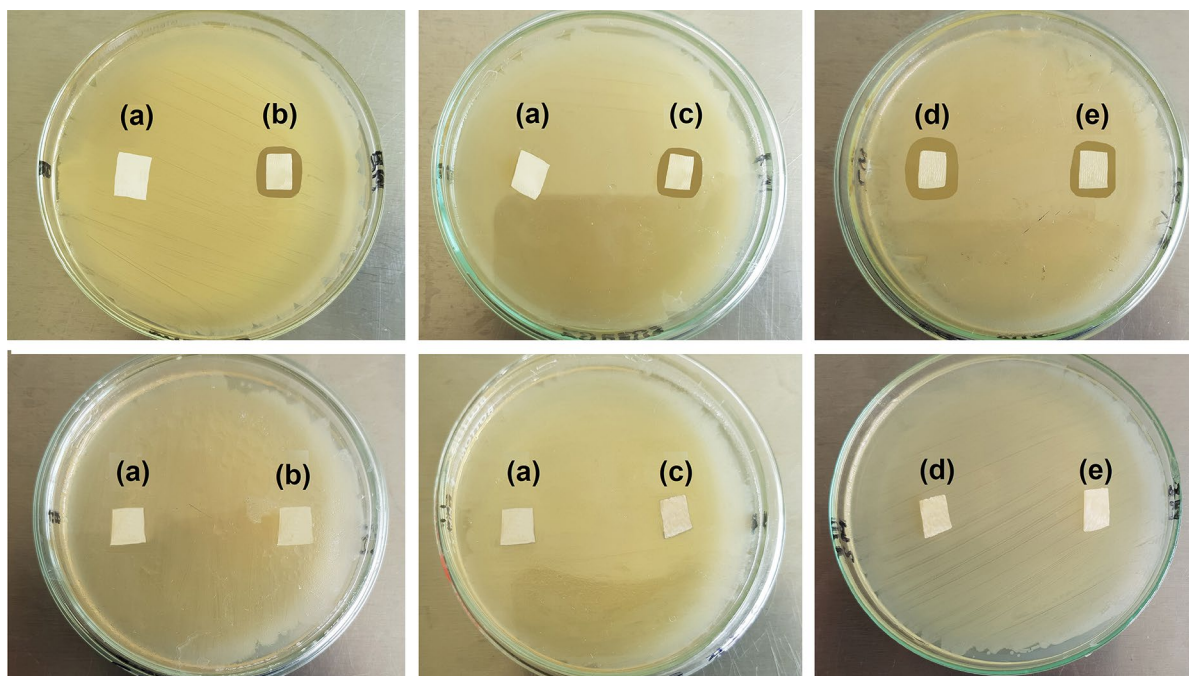


Fig. 10 Inhibition zones recorded for *S. aureus* (top row) and *E. coli* (bottom row) in contact after 24 h with vancomycin loaded MS and MS-g-4VP (13.2, 25.7, and 31.0% grafting) membranes (1x1.2 cm). (a) Pristine MS without vancomycin,

(b) pristine MS with vancomycin, (c) MS-g-4VP (13.2% grafting) with vancomycin, (d) MS-g-4VP (25.7% grafting) with vancomycin, and (e) MS-g-4VP (31.0% grafting) with vancomycin

drug has a better activity at acidic pH values. While *E. coli* strain basically showed resistance at all pH values (Fig. 11a, b).

Conclusion

The synthesis of MS-g-4VP membrane by 4VP graft on lignocellulosic membranes using gamma-rays was performed successfully with low monomer concentrations and absorbed doses. The grafting provides to membrane pH responsiveness with a critical pH of 6.5 and producing a similar contact angle for both sides of the membrane; this indicates that the graft of 4VP was carried out homogeneously on the lignocellulosic material, although its complex surface structure, as was observed in SEM. The XPS, NMR, and FTIR-ATR studies unequivocally confirmed

the modification of the lignocellulosic membrane with -g-4VP promoted by gamma radiation, showing the signals corresponding to the aromatic ring in FTIR-ATR and NMR, while in XPS was observed an increase in the percentage of N atoms from the P4VP grafted chains. In the case of the mechanical properties, these were considerably affected, mainly in Young's modulus and elongation at break caused by the grafting. Nevertheless, in the MS-g-4VP, there was an improved load and release of vancomycin at different pH values, allowing a sustained releasing for several hours and providing antimicrobial activity, as shown in the studies that indicated an excellent activity against Gram-positive bacteria at pH=5. In conclusion, MS-g-4VP may provide therapeutic levels of antimicrobial drugs, which may be of interest to designing advanced therapeutic materials for the treatment of chronic wounds or as a postoperative.

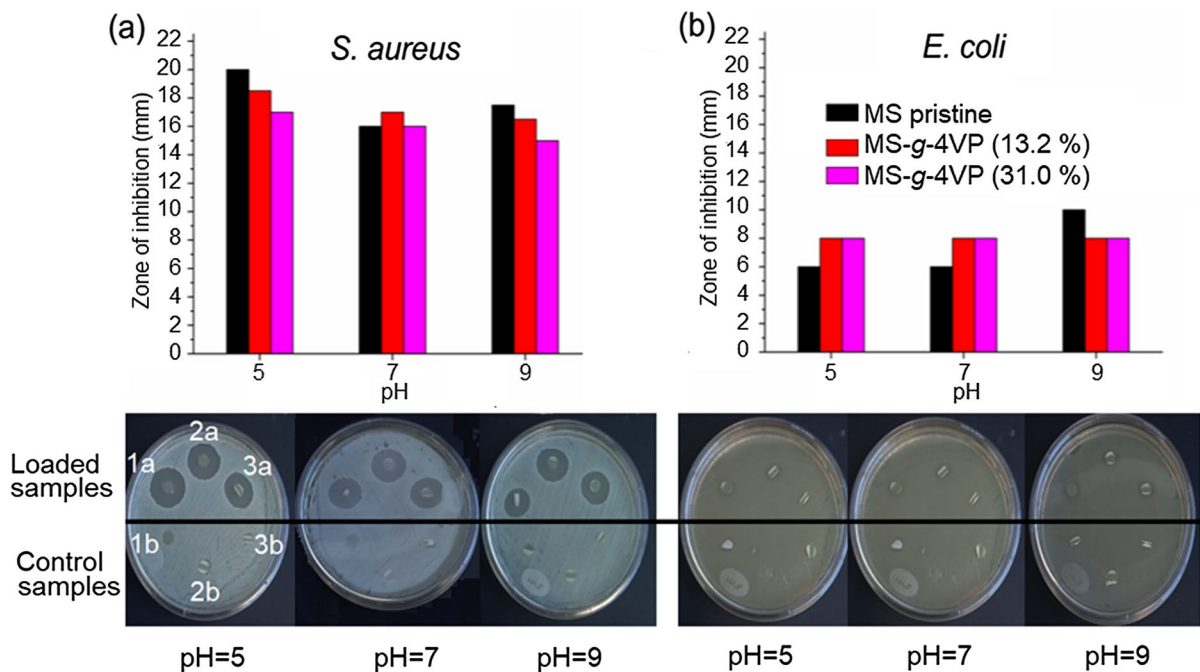


Fig. 11 Inhibition zones recorded for *S. aureus* (top row) and *E. coli* (bottom row) in contact after 24 h with vancomycin loaded at different pH tested in MS and MS-g-4VP (13.2, and 31.0% grafting) membranes (0.5 cm diameter). Loaded sam-

ples with vancomycin are (1a) Pristine MS, (2a) MS-g-4VP (13.2% grafting), and (3a) MS-g-4VP (31.0% grafting). Control samples are (1b) Pristine MS, (2b) MS-g-4VP (13.2% grafting), and (3b) MS-g-4VP (31.0% grafting)

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Declarations

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