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Preparation and characterization of poly (vinylalcohol) / bentonite hydrogels for potential wound dressings

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ABSTRACT

During the last decade researchers have been working to find effective wound dressing materials. The materials have to be designed to hold moisture in the surface of the wound, providing the ideal environment for cleaning the wound, absorbing the exudates, eliminating the odour and promoting the healing process. In this scenario, hydrogels emerge as excellent options for that. However, due to poor mechanical and antimicrobial properties of many conventional hydrogels, composite hydrogels are now designed in order to improve mechanical stiffness and durability. For this purpose, nanocomposite based on poly(vinyl alcohol) (PVOH) and different concentration of bentonite (0-7 wt.%) were obtained by the freezing-thawing technique and characterized by means of morphological, physical, thermal, mechanical, barrier and antimicrobial properties. Herein it was developed a non-expensive, eco-friendly and a facile method to obtain nanocomposite hydrogels based on PVOH with reasonable mechanical properties (Young Modulus of 0.5-0.8 MPa), good microbial barrier properties, adequate water vapour transmission rates and excellent swelling behaviour (195-336%). Moreover, it was found that the porous sizes of the samples can be controlled by the addition of the clay. All obtained results indicate that the PVOH/ 3% bentonite hydrogels show potential to be used as wound healing. Copyright © 2016 VBRI Press.

Keywords: Wound dressing; nanocomposites; composite hydrogel; bentonite.

Introduction

Today, many sophisticated dressings made from a wide range of materials are available to the wound care field. Depending upon their structure and composition, some dressings may simply absorb exudates (traditional wound dressing such as gauze or cotton wool still possess the largest part of the wound dressing market), while others may inhibit the colonization of microorganisms or keep the moisture in the wound (advanced dressings such as: hydrofibers, alginates, foams, and superabsorbents), but the search for an ideal material still continues **[1-3]**. Avoiding wound infection and retaining an appropriate level of moisture around wound are major challenges in wound care management. Therefore, designing hydrogels with desired antibacterial performance and good water-maintaining ability is of particular significance to promote the development of wound dressing **[4]**.

Hydrogels are hydrophilic polymer networks with high water content, which have played an important role in medicine fields (as scaffolds, drug delivery devices, among others) **[5-6]** and in environment remediation field (as adsorbers and separators) **[7-9]**. During the last decade hydrogels have been considered to be very helpful as a wound dressing material **[10]**. Hydrogels for this purpose have been synthesized with poly(vinyl pyrrolidone) **[11]**, poly(vinyl alcohol) (PVOH or PVA) **[12-13]**, poly(ethylene oxide) **[14]** and polysaccharides among others. Hydrogel dressing is designed to hold moisture in the surface of the wound, providing the ideal environment for both cleaning the wound, and allowing the body to rid itself of necrotic tissue. The moisture in the wound is also essential in pain management for the patient, and these dressings are very soothing and cooling. They also help to prevent bacteria penetration providing a barrier for infections **[15]**.

Particularly, the PVOH polymer has biocompatibility and capability to form hydrogels. PVOH hydrogels can be obtained by different routes: γ-irradiation crosslinking, chemical method (by using a crosslinking agent, such as glutaraldehyde) and by physical method (carried out by freezing-thawing (F-T) cycles, resulting in cryogels) **[16]**. The F-T gelation process consist in freezing a PVOH solution at -20 °C and thawing it back to room temperature several times. Cryogels exhibit a porous structure, with pores filled by a polymer-poor phase. The micro-scale structural organization is originated from the presence of two separated phases: polymer-rich and polymer-poor zones **[17- 18]**. The polymer-rich phase is formed by small micellar crystalline aggregates of PVOH chains and amorphous domains; the amorphous domains connect the micelle-like crystals **[19-20]**. For all that, these hydrogels have better strength and stiffness than their chemically crosslinked counterparts as the mechanical load is distributed along the crystallites in the network **[21-22]**.

For all these features PVOH cryogel can be as the right option for dressing applications. However, synthetic hydrogels are markedly brittle and have poor mechanical stabilities. A strategy to improve its properties is the addition of nanoreinforcements such as clay **[23]**. Clays are layered silicates whose are formed by plateles with around 1 nanometer in thickness [**24**], and which have been used from the last 30 years to reinforce polymeric matrices [**25**]. Bentonite is an important mineral clay due to environmental and economic relevance and mechanical and chemical resistance, characterized for a moderate negative charge [**24, 26**].

The aim of this work was to improve the specific properties of PVOH hydrogels required for their use in wound dressings. For this purpose, composite hydrogels based on PVOH and different concentration of bentonite were synthesized and characterized by means of thermal and swelling analyses, scanning electron microscopy, X-Ray diffraction, mechanical tests, water vapor permeation measurements and bacterial proliferation assays.

Experimental

Materials details

Poly(vinylalchol) (PVOH) was supplied by Sigma-Aldrich (Molecular weight of 89000-98000 g/mol, hydrolysis degree of 98-99%). Bentonite, supplied by Minarmco S.A. (Neuquen, Argentina), was used as received to prepare the nanocomposites.

Material synthesis

10 g of PVOH were dissolved in 100 ml of distilled water, with constant stirring (with magnetic stirrer) at 85 °C for one hour. After stirring, 1, 3, 5 and 7 wt. % of bentonite (samples: 1B, 3B, 5B and 7B), previously swollen in water over night, was added and the stirring continued for three hours more in order to completely dissolve the polymer. After that, the solutions were placed in an ultrasonic bath (30 minutes). When the solutions reached the room temperature, PVOH composites were cast onto anti-adherent containers and frozen (-18 ºC, 1h) and then placed at room temperature (25 ºC, 1h, thawing process). This procedure was repeated 3 times to obtain hydrogels by F-T method. PVOH hydrogel (PVOH) samples were obtained in a similar way but without the addition of nanoclay. The selection of this technique was based on our experience [**27**].

Characterizations

Fourier transformed infrared spectroscopy (FTIR)

FTIR spectra of samples were acquired with a Nicolet 6700 Thermo Scientific instrument equipped with a diamond ATR probe, over the range 400–4000 cm^{-1} from 32 co-added scans with 4 cm^{-1} resolutions.

X-Ray diffraction (XRD)

Tests were performed to characterize the clay dispersion inside the polymer matrix using an X-Pert Pro diffractometer, operating at 40 kV and 40 mA, with CuKα radiation (λ = 0.154 nm). All samples were scanned in $2\theta = 2$ to 50º at a rate of 2º per minute.

Differential scanning calorimetry (DSC)

Samples of 5mg were scanned from room temperature to 250 ºC in a TA Instrument Q2000 at a heating rate of 10 ºC/minute in a N_2 atmosphere in aluminium pans. The degree of crystallinity (X_{cr}%) was calculated as

$$X_{cr}\% = \frac{\Delta H}{w_{PVOH} \times \Delta H_c} \times 100 \qquad (1)$$

where, ΔH is the area under the melting peak, w_{PVOH} was the PVOH weight fraction and ΔH_c was the maximum heat for melting a theoretical 100% crystalline PVOH, 138.6 J/g [**28**].

Gel fraction determinations (GF%)

A rectangular specimen of each sample was dried in an oven at 37 ºC for 2 days. Samples were immersed in distilled water at room temperature for 4 days. Subsequently, the immersed sample was removed from distilled water and dried at 37 ºC for 2 days. GF was determined as:

$$GF\% = \frac{W_1 - W_C}{W_0 - W_C} \times 100 \qquad (2)$$

where W_0 and W_1 are the weights of the dried hydrogels before and after immersion, respectively, and W_C is the weight of the clay (obtained from the TGA curves at 750°C).

Swelling degree measurements

Samples of approximately 100mg were immersed in distilled water at 25 ºC. All samples were dried before immersion at 37 ºC for 2 days. The equilibrium swelling degree (S_f %) was determined as:

$$Sf\% = \frac{M_1 - M_0}{M_0} \times 100 \qquad (3)$$

where, M_0 is the weight of the samples before immersion and M_1 is the weight of the sample at equilibrium water content.

Thermogravimetric analysis (TGA)

Measurements were carried out using a Shimadzu TGA-50 instrument at a heating rate of 10 °C/minute from 30 to 850 °C in air flow. The specimen weight was in the range of 7–15 mg. Degradation temperatures (T_p) were obtained from the maximum of each event at the derivative curves of TGA (DTG). Bentonite contents (C%) were obtained from TGA curves as the residual mass at 750°C where the whole polymer was degraded and lost.

Field Emission Scanning Electron Microscopy (FESEM)

To analyze the pore sizes of hydrogels, FESEM micrographs with a FE-SEM Zeiss Supra were taken. Samples were swollen, frozen, lyophilized and then cryofractured and coated with gold before testing.

Water vapor transmission rate (WVTR)

Firstly, composite hydrogel samples with circular shape (radius of 14 ± 1mm) and a thickness (t) of 0.16 ± 0.04 mm were put as a cap with an adhesive on the mouth of a bottle containing 20 ml of distilled water (based on ASTM E96. Standard Test Methods for Water Vapor Transmission of Materials). Then the system (bottle + hydrogel sample) was maintaining for 3 days in a constant temperature-humidity homemade chamber (37 ºC at 75% RH).

The WVTR of each nanocomposites sample was calculated by using the following equation [**29**]:

$$WVTR\left(\frac{g}{m^2 \times h}\right) = \frac{M_0 - M_1}{72 \times A} \times 10^6 \qquad (4)$$

where, A is the area of bottle mouth (mm^2), M_0 and M_1 are the mass of the system before and after placing it in the chamber, respectively. The WVTR was normalized to sample thickness to obtain the specific water vapor transmission rate $(\Re = WVTR \times t)$ in $\left(\frac{g \times mm}{m^2 \times h}\right)$ [**30**].

Tensile tests

Rectangular specimens 12 x 0.15 x 50 mm in size were cut from as-prepared products. Tests were performed in a universal testing machine Instron 3369 at room temperature at a crosshead speed of 50 mm/minutes (ASTM D412) and a load cell of 100 N. The samples were in swollen state.

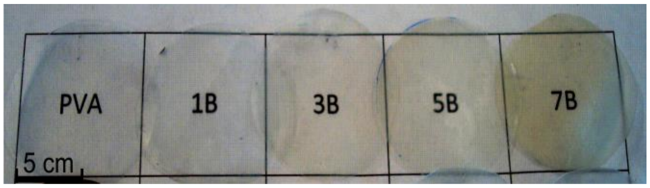

Fig. 1. Photograph of neat PVOH and PVOH/bentonite hydrogels. Where 1B is a hydrogel of PVOH with 1% of bentonite, 3B is a hydrogel of PVOH with 3% of bentonite, 5B is a hydrogel of PVOH with 5% of bentonite and 7B is a hydrogel of PVOH with 7% of bentonite.

Bacterial penetration assays

To estimate the resistance of PVOH/ bentonite hydrogel wound dressings against bacterial transmission from environment to the top surface of the wound the bacterial penetration test was performed. Circular specimen of each hydrogel (diameter of 5 cm and a thickness of 3 mm) was washed and sterilized for 2 minutes in a autoclave chamber. The specimen was put on the Tryptone Soy Agar that already incubated for 20 h at 30 °C. On the upper surface of hydrogel a suspension of Pseudomonas auroginosa ATCC 27853, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 with a concentration of 10^8 UFC/ml was dropped, then the system was incubated at 33 °C. Bacteria transport through hydrogel was monitored daily for a week via observing the growth of bacteria zone in the medium.

The statistical method used in all the showed results is Student´s T-test with a sample size of 3 and obtained the confidence interval for these samples.

Results and discussion

Pictures of PVOH/bentonite hydrogels can be observed in **Fig. 1**. Nanocomposites were transparent for all the bentonite contents due to nanometric size of the filler added [**31**]. Apparent differences in color appear in samples with highest concentration of bentonite (5B and 7B samples show a light brown color).

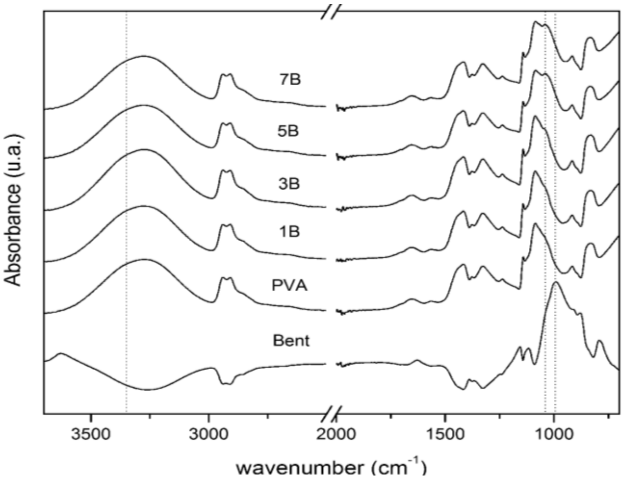

Fig. 2. FTIR spectra of PVOH matrix, bentonite and and PVOH/bentonite nanocomposites with different clay contents.

FTIR analysis

The interaction between PVOH matrix and bentonite can be studied by means of FTIR spectra (**Fig. 2**). The O-Si bonds peak appears at 995 cm^{-1} for the bentonite whereas the same peak appears at 1040 cm^{-1} in the case of nanocomposite gels [**25**]. In addition, the wide peak corresponding to the OH vibrations of the PVOH [**26**] (around 3269 cm^{-1}) was shifted into a higher wavelength (i.e. 3287 cm-1) [**34**]. These both shifts can be attributed to the presence of hydrogen bonding between –OH groups of PVOH and Si-O groups of bentonite.

There are two possible reasons that could explain the interactions between clay and PVOH resulting in a good composite. The former is that the PVOH is a water soluble polymer (due to its OH groups), so the clay nanoparticles suspended in water are wrapped by chains polymer forming a homogeneous suspension [**35**]. The latter is that the C-OH groups of PVOH may associated with the OH groups of the surface of the clay to form hydrogen bonds that may prevent the formation of aggregates during water/polymer phase separation process in the freezing-thawing cycles [**35**].

XRD analysis

In the case of the nanocomposites with lower clay contents (1 and 3wt. %) a good dispersion of the filler in the PVOH matrix can be observed, based on the fact that no diffraction peak of the clay appears in the XRD spectra **(Fig. 3)** for these samples. This could be explained due to the mechanism of polymer chain entrance in the interlamellar space of the clay. In the case of 5B and 7B samples the consecutive peaks are present due to the increase in the interlamellar space in the clay associated with the polymer entrance in clay galleries [**36**].

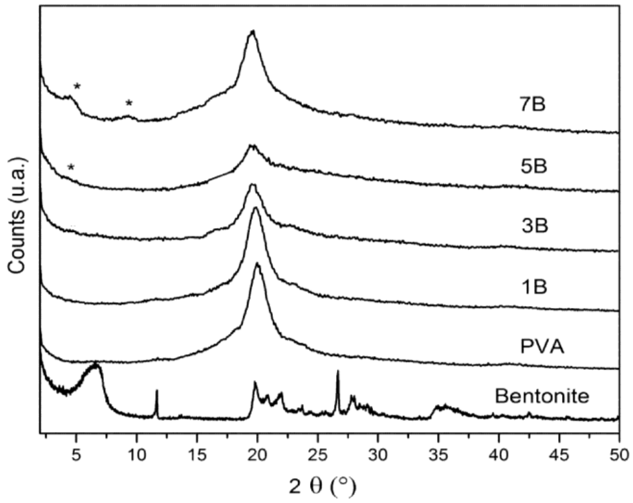

Fig. 3. XRD spectra of PVOH matrix, bentonite and and PVOH/bentonite nanocomposites with different clay contents.

Thermal and physical properties

The influence of the bentonite content over the structural properties such as gel fraction and equilibrium water content are summarized in **Table 1**. It is possible to observe that water uptake (S_f) decreased with clay incorporation as the filler content was increased as other authors have been reported on similar polymer/clay systems [**37**]. It is known that the clay itself is hydrophilic, and so absorbs more water than most of the thermoplastic polymers used as matrix, leading to an increase in the equilibrium water absorption with clay incorporation. On the other hand, clay layers dispersed in the nanometer scale in a matrix can decrease the mean free path of water molecules to pass through the nanocomposite network compared the pure matrix which leads to lower water absorption [**37-38**].

Table 1. Thermal and Physical properties of PVOH and PVOH/Bentonite gels.

Sample	T_m °C	X_{cr} %	T_p °C	*C* %	*Porous size* µm	*GF* %	S_f %
PVOH	228.5	48.6	281.9	0	8.5 ± 4.0	83.0 ± 1.0	336.9 ± 6.4
1B	227.6	51.3	295.2	0.8	3.2 ± 1.4	86.0 ± 0.4	288.9 ± 2.5
3B	227.7	46.9	288.1	2.7	4.0 ± 2.2	89.4 ± 1.2	242.9 ± 14.5
5B	227.6	47.0	294.5	3.8	4.7 ± 2.3	88.1 ± 3.2	209.7 ± 37.5
7B	227.4	43.9	298.5	6.0	*	88.8 ± 2.0	195.8 ± 25.4

*It was not possible to measure

In addition, clay immobilizes the PVOH hydrogel, which in turns could be associated with the increase on the gel fraction (GF%). The general observation is that higher clay content induces lower capability of water absorb from the gel because more strong networks (higher gel fraction, GF %) were produced but also due to the fine dispersion of bentonite platelets that can increase the tortuosity of path for water molecules. The explanation of the observed behavior is also related with the fact that clay promotes the formation of crosslinking points, leading to a reduction in pore size that makes difficult the entrance of water molecules into the material, which it is in accordance with FESEM and XRD results. As a partial conclusion we could say that the addition of bentonite in the matrices assist their network formation and their stability **[39]**.

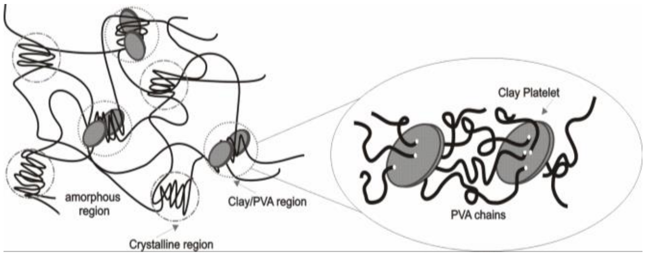

Fig. 4. Scheme of the interactions between PVOH and bentonite.

Moreover, from the table, it can be seen that insignificant changes in the degree of crystallinity (Xcr) and in the melting temperature (Tm) were found. Those indicate that the addition of bentonite was not an important influence in the form of crystallization of the PVOH. The PVOH crystals are cited in the literature as the primary mechanism responsible for the resultant mechanical properties **[40]**. Otherwise, the equilibrium water content in composites was markedly different from the PVOH gel without filler. We can suppose that the clay acts as crosslinking agent due to the H bonds interactions with the polymer **[41]** (as it was schemed in **Fig. 4**). The degradation temperature (T_p) of the nanocomposites are higher than PVOH gel, indicating a better thermal-stability than PVOH gel, it can be attributed a strong interaction between components, the polymer and the clay **[42]**.

Fig. 5 shows the residual mass (a) and its derivative (b) as a function of temperature for PVOH matrix and PVOH/bentonite nanocomposites. The derivative curves are commonly used to analyze the thermal degradation of different materials. PVOH degradation occurs in two steps, the first one is due to elimination reactions, while the latter is dominated by chain cuts and cyclization reactions **[43-44]**. In the case of PVOH/bentonite nanocomposites, the degradation process also takes place in two stages; however, the incorporation of clay into the PVOH matrix leads to a change in the mechanism of degradation as compared to the neat matrix. In the first step, removal of water and degradation of residual acetate groups generating water, non-conjugated polyenes and acetic acid take place. In addition, it is possible to observe that nanocomposites degradation occurs at higher temperatures than pure PVOH matrix, some chain cleavage reactions also occur in the first stage of degradation where acetaldehyde, acetone and furan occur. On the other hand, the reactions in the second step of degradation are chain cleavage reactions [**45**].

It can be observed that lowest concentration of clay (1 wt.%) produced a marked increase in the Tp in comparison of the neat matrix, as it was previously observed for other materials [**46**]. The increase in the degradation temperature may be related to the restrictions in the mobility of the polymer chains and reducing heat diffusion within the polymer matrix, both effects are attributed to the polymer - clay interactions **[42]**.

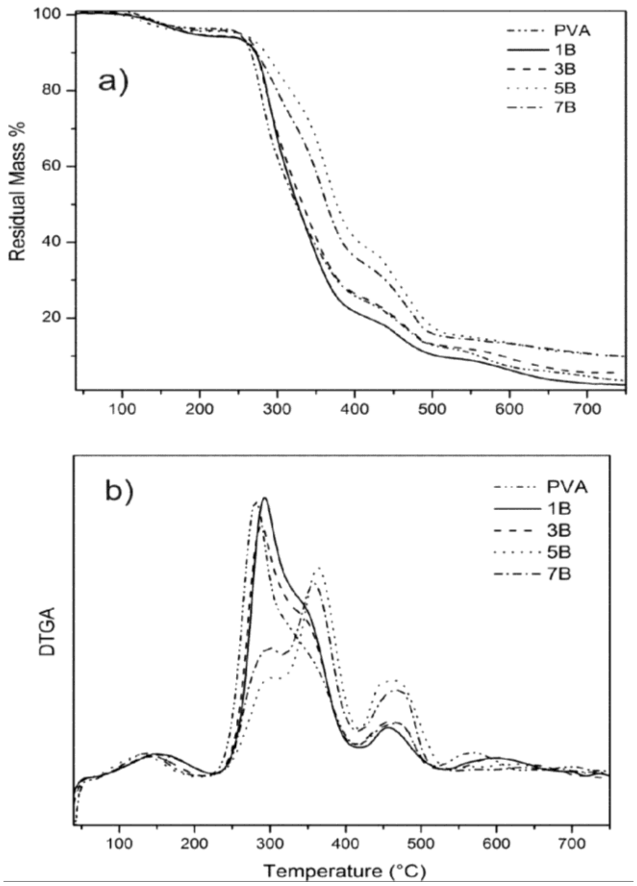

Fig. 5. (a) Residual mass and (b) Derivative of residual mass as a function of temperature for PVOH matrix and PVOH/bentonite nanocomposites with different clay contents.

Generally, nanoclays can act as a heat barrier thus nanocomposites can be described by barrier model [**46-47**]. Micrographs **(Fig. 6)** were used to analyze pore morphology. The introduction of clay inside the PVOH hydrogel matrix produced a clear decrease on the pore size. So, the decrease in the water uptake capability was attributed to the fact that clay promotes the formation of crosslinking points, leading to a reduction in pore size that makes difficult the entrance of water molecules into the material.

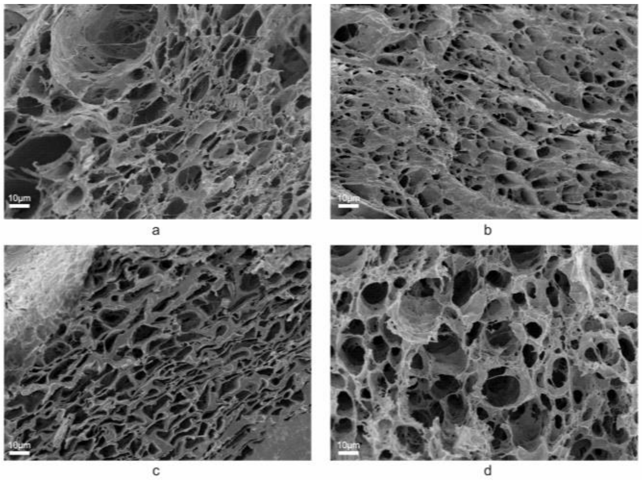

Fig. 6. FESEM micrographs of hydrogels: (a) PVOH and PVOH/bentonite composites: (b) 1B, (c) 3B y (d) 5B.

All results indicate that well-dispersed clays within the matrix of PVOH are able to act as crosslinking agents **[48-49]**.

Table 2. Tensile and barrier properties of PVOH matrix and nanocomposites with different bentonite contents.

Sample	E MPa	σ_{max} MPa	ε %	WVTR $g/m^2.h$	ℜ $g.mm/m^2.h$
PVOH	0.8 ± 0.2	2.6 ± 1.1	294 ± 10	19.7 ± 1.0	2.6 ± 0.3
1B	0.6 ± 0.1	2.1 ± 0.3	205 ± 40	22.0 ± 2.3	2.8 ± 0.6
3B	0.9 ± 0.2	3.5 ± 0.5	309 ± 10	21.5 ± 2.3	2.5 ± 0.5
5B	0.8 ± 0.2	2.8 ± 0.8	295 ± 82	22.6 ± 0.7	2.3 ± 0.1
7B	0.5 ± 0.1	1.7 ± 0.3	205 ± 63	21.2 ± 2.8	2.8 ± 0.5

Water vapor transmission rates (WVTR)

An ideal dressing has to control the evaporative water loss from a wound at an optimal rate. This rate should be in the mild-range of loss rates from injured skin (11 to 200 $g/m^2.h$, depending of the degree of burn) and normal skin (8.5 $g/m^2.h$) **[50-51]**. The hydrogel wound dressing must avoid or at least reduce the body liquid lost by controlling absorption and transmission as well as by maintaining the wound area wet, in order to accelerate the formation of granule and epitelesation process **[28]**. A higher value of WVTR causes a faster drying of the wound and a slower WVTR produce an accumulation of exudates. **Table 2** shows the WVTR and R values obtained for PVOH matrix and PVOH/bentonite composites with different clay contents. In all cases WVTR values are in the optimal range for the application proposed (around 20 $g/m^2.h$). When the hydrogel has higher water absorption capacity (higher S_f), indicating a higher porosity and / or larger pores, prevent the water vapor to pass through them, holding the humidity in the wound; similar results are found in the literature for hydrogels of poly (vinylpyrrolidone) / PVOH [**29**].

Mechanical results

Fig. 7 shows the stress–strain curves for PVOH matrix and the PVOH/bentonite nanocomposites. The hydrogels behave under stress as a non-linear elastic material [**52-53**]. Tensile properties of PVOH matrix and nanocomposites with different bentonite contents are summarized in the **Table 2**. Where E is the Young's modulus, σ_{max} is the stress-at break and ε is the maximum elongation-at-break.

The mechanical properties of PVOH hydrogel were improved in comparison with the neat matrix. It seems that the optimal concentration in terms of mechanical properties is the PVOH hydrogel reinforced with 3 wt. % of bentonite. Higher clay content could produce aggregates that are in detriment on the mechanical properties and lower clay content could not be enough to improve the mechanical properties. These improvements can be attributed to several factors. An important one is the interaction of the reinforcement with the matrix; in the studied system the improvement in mechanical properties can be attributed to a homogeneous dispersion of the clay within the matrix. If the clay sheets are well dispersed and distributed in the matrix the load transfer from the matrix to the reinforcement (highly rigid) is favored **[54]**. As we have previously explained, both components (filler and matrix) are hydrophilic, so the dispersion and distribution of the bentonite in the PVOH are benefited, and there is an

excellent adhesion due to the several hydrogen bonds formed between them. These results are in agreement with the morphological, chemical and physical characterization.
The values of the tensile strength of the skin are usually in the range 2.5–16 MPa [**55**] and the elongation is approximately 70% in the most flexible zones [**56-57**]. It can be partially concluded that these hydrogels can be used at wound dressing in all skin zones from the most rigid to the most flexible zones.

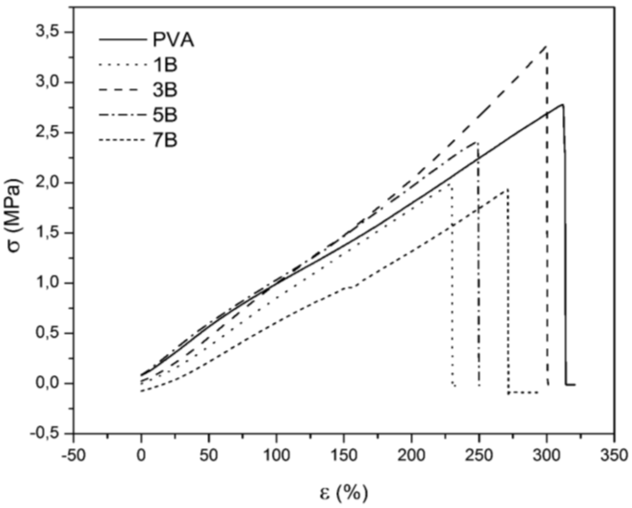

Fig. 7. Stress–strain curves for PVOH matrix and the PVOH/bentonite nanocomposites with different clay contents.

Bacterial penetration

The observations indicated that no bacteria were passed through all the PVOH/Bentonite hydrogel for the entire assay (15 days). It can be partially concluded that the hydrogels dressing could protect wound from bacterial penetration. These hydrogels should help the wound from possible infections **[39, 58]**.

Conclusion

Poly (vinyl alcohol)/ bentonite nanocomposite hydrogels with several bentonite contents (0, 1, 3, 5 and 7 wt. %) to be used for wound dressing were successfully obtained by freezing-thawing technique. It was found that the bentonite possesses compatibility with the hydrogel matrix because both are hydrophilic. The best dispersion was found for 1 and 3% of bentonite content (from XRD spectrums). In addition, their function as barriers against bacterial penetration showed that they could protect the wound from further infection. That filler/matrix affinity was responsible for the improved mechanical properties of nanocomposites. The most appropriate mechanical properties were obtained from 3 and 5% of bentonite content however, 5% is not well dispersed and homogeneous material. All obtained results seem to show that the hydrogel produced with PVOH and 3 wt.% of bentonite is a potential material to be used as wound dressing. Further works regarding in vitro and in vivo assays of a poly (vinyl alcohol)/clay nanocomposite hydrogel will be carried out. Hydrogels are present in commercial products though their potential has not been fully explored yet. We believe that these hydrogels for wound dressings will not only improve the quality of life of patients, but also it will help to develop this boundary topic that remains unexplored. Moreover, the regulatory and cost-effective aspects should be properly addressed in the design of novel skin substitutes.

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