Composites based on Poly (vinyl alcohol) Hydrogels for Wound Dressing.

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ABSTRACT

Wound dressing is an artificial skin that can meet the requirements such as higher vapor or gas permeation and protection of wound from infection and dehydration. Poly (vinyl alcohol) (PVA) hydrogel is one of the well-known polymer gel that due to its good biocompatibility, hydrophilicity and capability of swell in water or biological fluids has been used in several biomedical and pharmaceutical applications.

The main objective of this work was to synthesize, in order to obtain wound dressing with better properties, composite-hydrogels. These composites were prepared using combination of: PVA/bentonite PVA/cellulose, PVA/Ag nanoparticles, PVA/rosemary extract and PVA /clove extract, via the freezing/thawing method. For this purpose, chemical, thermal, swelling analyses and antimicrobial properties studies (using as indicator Escherichia coli O157:H7 and Listeria monocytogenes) were carried out. The results showed that PVA/bentonite and PVA/cellulose had the higher antimicrobial behaviour. In addition, they exhibit the permeation and swelling characteristics suitable for use as wound dressing.

Their function as barrier against microbe penetration showed that they could protect the wound from further infection; hence it could accelerate the healing process of wound.

Keywords: composite, hydrogel, poly vinyl alcohol, wound dressing.

INTRODUCTION

The hydrogels consist of a three dimensional network that swells in the presence of water or biological fluids maintaining its shape, they are soft and elastic [1]. The hydrogels can absorb an excess of wound exudates, protect a wound from secondary infection, and effectively promote the healing process by providing a moisturized wound healing environment [2]. Poly(vinyl alcohol) (PVA) hydrogels, with high water content possess good biocompatibility, non-toxicity, high elasticity and mechanical strength, have received increasing attention in recent times and have been widely used in a large variety of biomedical applications [3]. Clay based nanocomposites show notable improvements in several properties in comparison with a polymer or conventional micro- and macrocomposites. These improvements include mainly increased strength and heat resistance and decreased gas permeability and flammability [4], so these nanocomposites could be an excellent wound healing material, better than conventional hydrogels.

An improved bactericidal activity is attributed to silver nanoparticles (AgNPs) because of their electronic effects that produce a change in the local electronic structure of the surfaces of the nanosized particles, they inactivate the vital enzymes and also help in prevention of the replication of DNA [5].

Cellulose and its derivatives have been used in the medical field due to their good biocompatibility, mechanical properties similar to those of hard and soft tissue [6].

Several compounds have been proposed for antimicrobial activity in food packaging, including organic acids, enzymes such as lysozyme, and fungicides such as benomyl and natural antimicrobial compounds such as spices[7].

The aim of this work was to improve the specific properties of PVA hydrogels needed for the use as heal wounds. For this purpose, composites hydrogels based on PVA and different fillers (AgNPs, natural extracts, bentonite and cellulose) were obtained and characterized by means of swelling analyses, scanning electron microscopy and antimicrobial testes.

MATERIALS AND METHODS.

Preparation of hydrogels.

Aqueous solutions of 15 wt.% PVA were prepared by dissolving the polymer in distilled water at 85°C and slowly stirring (with magnetic stirrer). After an hour of stirring, the required amount of filler (3 wt.% bentonite (PVA/bent), 3 wt.% cellulose (PVA/cel), 0.1 ml of rosemary extract (PVA/rm) , 0.1 ml of clove extract (PVA /clove) and 1.5 wt % AgNPs (PVA/Ag) was added and the agitation continued for three hours. The polymer was entirely dissolved. After that, the solutions were placed in an ultrasonic bath for 30 minutes to remove all bubbles. The solutions were allowed to reach room temperature. Then the PVA-based solutions were cast onto anti-adherent containers and frozen for 12 h, cooling down to -18ºC and afterward placed at room temperature (25ºC, thawing process) for the same time, in order to produce crosslink. This procedure was repeated 3 times. To obtain the PVA hydrogel (without filler), the same steps were followed but without the addition of filler.

Scanning Electron Microscopy (SEM)

SEM micrographics were obtained in a JEOL JSM-6460 LV instrument in order to analyze the hydrogels morphology. The samples were dried in an oven for 24 h at 37ºC, then cryo-fractured in liquid air and gold-coated.

Antimicrobial Assays

Antibacterial activity test was carried out using the agar diffusion method according to Ponce *et al* [8]. The zone of inhibition assay on solid media was used for determination of the antibacterial effects of films against a Gram negative and positive bacteria like *Escherichia coli O157:H7* and *Listeria monocytogenes innocua* respectively. Hydrogels were cut into a disc form of 5 mm diameter and then placed on Mueller Hinton (Merck, Darmstadt, Germany) agar plates, which had been previously seeded with 0.1 ml of inoculums containing approximately 10^5 - 10^6 CFU/ml of tested bacteria. The plates were then incubated at 37^{o}C for 24 h. After that, the plates were examined for ‘zone of inhibition’ on the film discs. The diameters of inhibitory zone surrounding hydrogels discs were measured. The antibacterial effect of hydrogels was classified by the diameter of the inhibition halos as: not sensitive for diameters less than 17 mm; sensitive for diameters of 18-23 mm; very sensitive for diameters of 24-28 mm and extremely sensitive for diameters larger than 29 mm. Each assay was performed by triplicate on two separate experimental runs.

Gel fraction

To perform gel fraction measurements, a slice of each sample was placed in an oven before and after rinsing at 37ºC until no change on its mass was observed. After that, each sample was immersed in distilled water at room temperature for 4 days to rinse away unreacted species. Subsequently, the immersed sample was removed from distilled water and dried at 37ºC until constant weight was reached. Therefore the gel fraction can be calculated as follows:

$$GF\% = \frac{W_f - W_F}{W_i - W_F} \times 100 \qquad (1)$$

where W_i and W_f are the weights of the dried hydrogels before and after immersion respectively and W_F is the weight of the filler incorporated into de hydrogel.

Swelling studies

Swelling determinations were carried out in saline solution and buffer solutions (pH 4 and 10) at 37ºC. All samples were dried before immersion until constant weight at 37ºC. The maximum swelling degree ($M_\infty\%$) percentage was determined by the following equation:

$$M_\infty\% = \frac{M_f - M_i}{M_i} \times 100 \qquad (2)$$

where M_i and M_f are the weights of the sample before and after immersion respectively.

Permeation Analyses

For these measurements, dried hydrogels samples with a diameter of 28 ± 2 mm and a thickness of 0.16 ± 0.04 mm were cut and then put as a cap with adhesive on the mouth of a flask with a diameter of about 26 mm containing 20 ml of distilled water. The flask was then placed in a constant temperature-humidity homemade chamber for 72 h (37ºC at 75% RH). The mass loss of the system was considered as an index of water vapour transmission rate (WVTR).

The WVTR of each sample was calculated by using the following equation [9]:

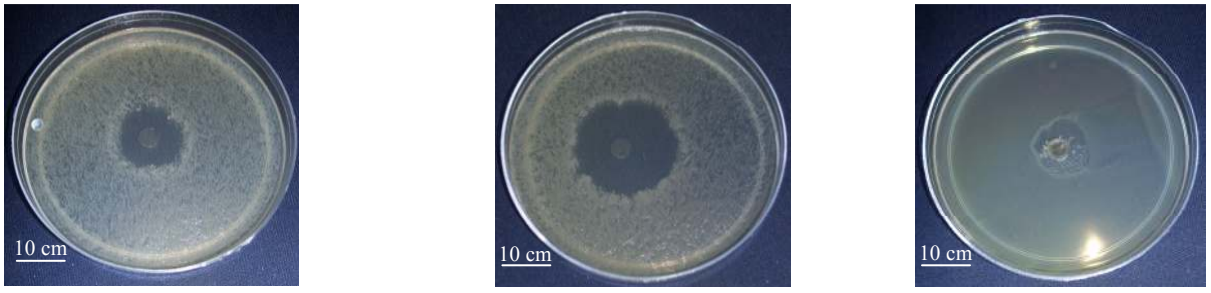
$$WVTR\left(\frac{g}{m^2 \times h}\right) = \frac{M_0 - M_1}{72 \times A} \times 10^6 \qquad (3)$$

where A is the area of flask mouth (mm^2), M_0 and M_1 are the mass of the system (flask and hydrogel cap) before and after placing in the chamber, respectively.

RESULTS AND DISCUSSIONS

The PVA/rm and PVA /clove hydrogels had an inhomogeneous aspect and the extract was exudated from the matrix. Moreover, antimicrobial activity was not showed.

Figures 1 (a) and (b) show the inhibition halos obtained by the agar diffusion method for different non sterilized hydrogles in *L. monocytogenes* media. It can be noted that PVA /bent and PVA/cell displayed an inhibitory halo, showing very sensitivity for this bacteria. In the case of (c) the PVA/Ag show a sensitive behavior for *E.coli*.



a) b) c)

Figure 1. (a)PVA /bent in *L. monocygenes* media.(b)PVA/cell in *L. monocygenes* media (c) PVA/Ag in *E. coli* media

It was not possible to measure the WVTR and mechanical properties to PVA/Ag because the samples were non-uniform

The SEM images (not shown) indicate that bentonite and cellulose was dispersed in the matrix.

Table 1 summarized the main characteristics of the best composites (obtained until this moment). It is known that an ideal dressing would control the evaporative water loss from a wound at an optimal rate. The rate for normal skin is 8.5 g/m^2 per hour, while that for injured skin can range from 11.6 g/m^2 per hour [10]. Therefore the wound dressing should have a WVTR value between 8.5 and 11.6 $g/m^2.h$.

In addition the *GF* and the M_∞ were markedly high; hence these composite hydrogels are ideal for wound dressing.

Table 1. Main characteristic of selected composite hydrogels.

Sample	*GF* %	*WVTR* g/(h.m^2)	M_∞ (%)
PVA/bent	93.9 ± 0.7	7.71 ± 0.52	226.50 ± 4.65
PVA/cel	94.4 ± 0.8	8.66 ± 1.30	230.30 ± 7.43

CONCLUSIONS

The developed composite hydrogels was found to possess high water absorption capacity, gel content, and a water vapor transmission rate similar to the natural skin, and excellent antimicrobial activity indicating its ability to act as an effective wound dressing material.
Mechanical characterization of composite hydrogels is being carried out.

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