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Research Article

Synthesis and Characterization of Hydrogels Cross-Linked with Gamma Radiation for use as Wound Dressings

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Abstract

In this work, the synthesis of cross-linked PVA / PVP% v / v 50% hydrogels was carried out by using ionizing energy at a dose of 30 kGy from a Co-60 source in order to be used as dressings for skin wounds. These hydrogels were characterized by various tests such as swelling, dehydration, surface pH, Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Cyto-toxicity. The results of these tests allowed us to evaluate the physical, chemical and biological properties of these gels and ensure that these hydrogels are a good tool for the treatment of skin burns.

Keywords: Hydrogels; Gamma Radiation; Biocompatibility; Wound Dressings; Crosslinking

Introduction

Burns are skin wounds that manifest themselves according to its degree from erythema to ulcers and tissue necrosis [1]. It has been shown that in general the pH of the wounds tends to be basic (pH from 6.9 to 8). This is due to the activity of proteases that are produced by the same wound and as a final product of some bacteria. A treatment that involves reducing the pH of this type of injury could generate a benefit because it would reduce the toxicity generated by bacteria and their products, increasing fibroblastic ac- tivity and controlling enzymatic activity [2].

Considering in the references [2-4], one of the methods that increase the healing of wounds is the re- duction of its pH. This affirmation was validated by health professionals specialized in burns treatment, with wide experience in the management in burns and wounds, this study therefore, is designed to devel- op hydrogels with acid pH which would not only control the moisture in the wound, necessary to prevent cell desiccation, promote autocatalytic debridement and generate thermal isolation [4] but also reducing the pH would promote angiogenesis and stimulate the synthesis of collagen, this improves the quality of healing [2].

Hydrogels are described as cross-linked polymer networks that constitute three-dimensional structures with the capacity to modify their volume in the presence of water [5]. These materials possess excellent biocompatibility properties, which are of great interest in biomedical applications such as scaffolds for cell growth, wound dressings and drug delivery [6].

There are different methods to synthesize hydrogels. Some involve the use of chemical initiators that generate covalent crosslinking between the polymer chains, these give rise to chemical gels [5,7], others use thermal cycling to initiate the formation of hydrogen bridges, Van der Waals forces or hydrophobic effect that allow the generation of physical hydrogels [5,8]. Finally, another method of synthesis of hy- drogels corresponds to the use of ionizing radiation as an initiator, which allows the formation of covalent bonds to give rise to thermostable hydrogels [6,9,10]. The synthesis of hydrogels by ionizing radiation involves a process in which the energy of this radiation is transferred to the material by ionization, gener- ating the release of free radicals that induce active sites in the polymer chains, leading to the formation of macroradicals. These macroradicals recombine themselves to macromolecules that constitute the network of cross-linked polymers [11,12]. It is important to consider that during the irradiation of the polymer solution to produce the hydrogel, two simultaneous processes need to occur: one that leads to the increase of the molecular weight through the crosslinking of the chains and the other that leads to a decrease of the molecular weight through the degradation of the polymer [6]. One of them always prevails.

In Argentina, there are no companies or institutions that develop hydrogels. Therefore, the objective is to obtain affordable products available to the most vulnerable population. As a result of this, the use of ionizing radiation for the preparation of hydrogels was considered as a simultaneous method of crosslink- ing and steril-

ization wich is fast, safe and friendly to the environment.

In this work, we develop hydrogels of poly vinyl pyrrolidone (PVP) and poly vinyl alcohol (PVA) cross-linked by gamma radiation at a dose of 30 kGy and a dose rate of 7.5 kGy h-1. The resulting hydro- gels were tested by the swelling test in order to know the percentage of swelling and the mechanism of water diffusion in the gel. There are three types of diffusion mechanisms that are representative in hydro- gels; they are based on the relative rate of water diffusion in the polymer matrix, the relaxation rate of the polymer chains, the swelling of the gel and the drug delivery in the swollen

- Fickian diffusion in which the diffusion rate of the solvent is much less than the relaxation of the gel matrix.
- Case II diffusion in which the transport mechanism is controlled only by diffusion which is faster in comparison with the processes of relaxation of the polymeric network.
- Non-Fickian diffusion also called Anomalous diffusion which arises from the simultaneous and comparable contribution of the phenomenon of diffusion of the molecules of the bioactive agent and the relaxation of the polymeric chains [14].

The analysis of these mechanisms is realized considering the diffusional exponent n that is obtained by the following equation [15,16]:

$$Ms = ktn \quad (1)$$

Where k is a constant that depends on the diffusion coefficient and the material thickness, t is the time and Ms equals H (i) shown in the equation 2 which is calculated according to the swelling equation as a function of time, where i is the amount of data acquired.

$$H(i) = ((w(i)-w(0))/w(0)) *100= ktn \quad (2)$$

Where, w (i) is the weight increased at one time i and w0 is the weight of the xerogel (dry hydrogel). The natural logarithm is applied to equation 2 and a straight line is obtained whose slope is the diffu-sional exponent. For the case of thin films, when the value of n is 1 the diffusion mechanism is Case II, if the value of n is between 0 and 1, the transport mechanism of the water follows an Anomalous behavior, and if the n is 0.5, the diffusion follows the laws of Fick so it is the diffusion is Fickian [13,16].

In addition, to characterize the hydrogels, the pH of the surfaces was measured, FTIR spectra were ob- tained, the hydrogels images were analysed by SEM to determine porosity and pore density. Dehydration tests were conducted at room temperature and under hospitals conditions of a general care room for adult patients with skin burns. This involves imitating the temperature (32 °C +/- 1.5 °C) and humidity (50% +/-8%) of this rooms.

Material and Methods

The chemicals used were PVP k90 of Solkem of 99.98% and PVA with a hydrolysis degree of 99% of Sigma Aldrich. A PVA / PVP solution were prepared with ratios ranging from 50:50% v / v to 10% w / v. The polymers were dissolved in deionized water at 90 ° C with stirring for 2 hours. The mixture was placed in the corresponding casts inside multicamerate bags. Subsequently, the solution was subjected to gamma irradiation from a Co- 60 source at a dose of 30 kGy, with a dose rate of 7.5 kGy h^{-1}.

The resulting hydrogels were evaluated by a swelling test according to ASTM 570. In this test, the hy- drogels were taken to xerogel in an oven at 50 ° C for 24 hours. Then, the samples were placed in a con- tainer with sterile distilled water and weight measurements were taken for 72 hours. With the data ob- tained, the percentage of swelling and the diffusional exponent was calculated, this data allowed the anal- ysis of the type of water diffusion mechanism in the gel.

The dehydration test was performed at room temperature and under hospital conditions. In the first case, the hydrogel samples were placed in a petri dish maintained at a temperature of 23 °C +/- 1 °C, in the second case, the samples were placed at 36 °C +/- 1 °C and humidity of 50% +/-1% and the entire surface of the hydrogel was covered with adherent film to imitate the treatment given to this type of wounds. The weights of the samples were recorded for 72 hours and the percentage of dehydration was calculated from the data obtained. The pH was measured with a surface pH meter at different points of the elaborated hydrogels. The FTIR test was performed on a sample of xerogel and on the polymers of PVP and PVA separately so that it served as a comparative base. The fracture surface of the hydrogel was vis- ualized in the SEM at low vacuum.

The cytotoxicity of the material was evaluated with a VERO cell line through the MTT test with three extraction vehicles: physiological solution (PS), phosphate buffer (PBS) and culture medium with serum (MEM) under the guidelines of ISO 10993-5 which establishes that viability greater than 70% indicated that the sample analysed was non-cytotoxic.

Results

The results showed that the hydrogels (Figure 1) were easily manipulated and easily removable. The results of the characterization tests are shown below.

Swelling test

The (Figure 2) shows the increase in the swelling percentage as a function of the time of hydrogels. It can concluded that at 48 hrs the swelling reached saturation with a percentage of 636.58% +/- 5.09% with respect to the original weight. The calculated n expo-

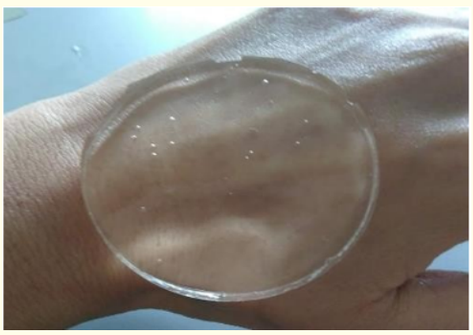

Figure 1: PVA / PVP hydrogel crosslinked by gamma radiation.

nent was 0.5, indicating that the diffusion mechanism of the water molecules in the hydrogel responds to a Fiskian behaviour, which implies that the diffusion speed of the water molecules in the hydrogel was less than the relaxation rate of the polymer matrix.

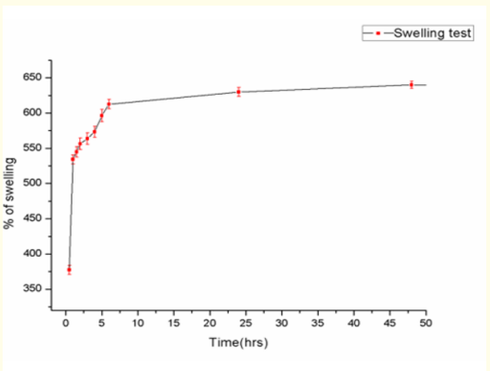

Figure 2: Swelling in hydrogels simples.

Dehydration test at room temperature

In the (Figure 3), the graph of the dehydration percentage as a function of time was observed. The dehydra- tion percentage reached 88.18% +/- 0.35% at 48 hours and stabilized at that value. In the graph it can be concluded that within the first 6 hours, the dehydration percentage reached 29.83% +/- 3.83%.

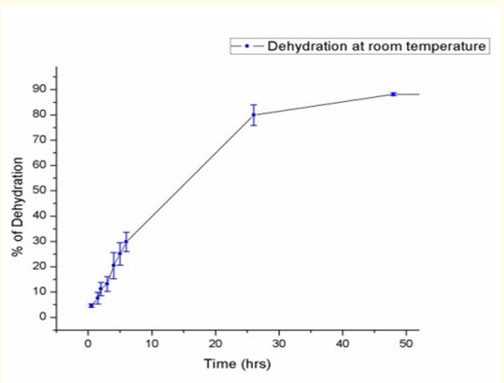

Figure 3: Dehydration of hydrogel samples in a test at room temperature.

Dehydration test at hospital conditions

In the (Figure 4) the variation of the dehydration percentage over time is exhibited. In this graph the dehy- dration rate was slower. Within the first 6 hours, the dehydration percentage was 12.7% +/- 0.70%. Just after 24 hours, dehydration percentage reached 28.4% +/- 3.13%, getting to the maximum dehydration of 56.85% +/- 5.17 at 72 hours.

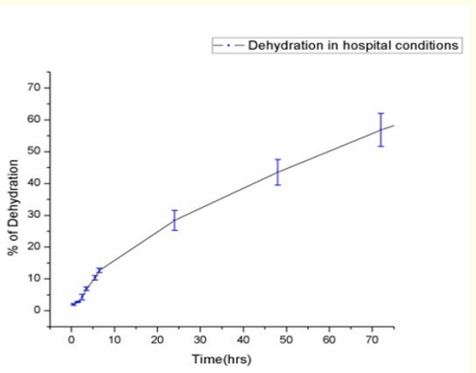

Figure 4: Dehydration of hydrogel samples under hospital conditions.

Comparing the results of the dehydration, it can be observed that the conditioning of the samples simulat- ing a hospital environment had a great influence on the results, reducing in more than 30% the dehydra- tion of the hydrogels.

PH test

An average pH value of 5.63 +/- 0.025 was obtained.

Fourier transform infrared spectroscopy (FTIR)

In the (Figure 5) the hydrogel spectrum is shown. It can be observed that the absorbance bands characteristic of each polymer were present [17,18]. The peak at 3370 cm^{-1} appeared in both polymers and corresponds to the OH- group. The band at 2925 cm^{-1} also appeared in both polymers and corresponds to the C-H. The peak at 1659 cm-1 appeared in the PVP and was associated with C = 0. The bands in 1462 cm^{-1} and 1441 cm^{-1} appeared in the PVP and were associated to the deformation of the CH. The band at 1291 cm^{-1} ap- peared in the PVP and was due to the C-N bond. The peak at 1089 cm^{-1} corresponded to the vibrational stretching of the C-O and was associated with the PVA.

Scanning electron microscopy (SEM)

In the (Figure 6) an SEM image of the hydrogel fracture surface is shown. By analyzing it with the ImageJ® software it was observed that the porosity range covered as from 0.625 µm to 2.075 µm. The pore size that appeared most frequently was 1.17 µm and the average pore size calculated was 1.16 µm +/- 0.38 µm.

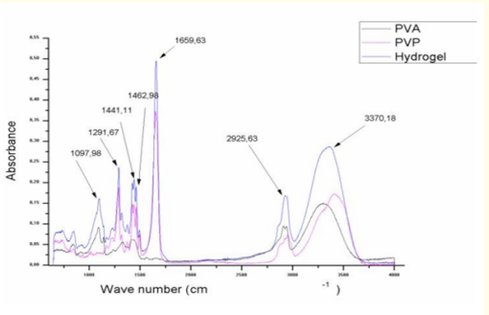

Figure 5: Spectra of hydrogel (blue color), PVP (magenta color) and PVA (black color).

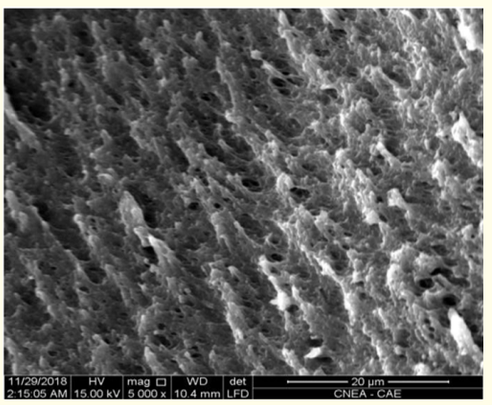

Figure 6: Image of the fracture surface of a hydrogel with magnification of 5000X

Cytotoxicity test

The cytotoxicity test showed the following cell viability percentages: PBS 103.72% +/- 0.03%; MEM 101.55% +/- 0.02% and PS 102.84% +/- 0.05%. which indicated that the hydrogels were non-cytotoxic.

Discussion

The hydrogels that resulted from the radiation crosslinking process compared to those generated by other methods such as the chemical or physical method, had greater advantages from the point of view of the ease and speed with the generation of the product that occurred simultaneously with the sterilization. The procedures for obtaining physical hydrogels by thermal cycling are extensive since at least more than two cycles are needed to generate a hydrogel and this implies a time greater than 48 hours [19]. Another ad- vantage of the hydrogels generated by radiation in the absence of toxic elements, was one of the main disadvantages of chemical processes [12]. Also other advantage was that these hydrogels had thermosta- bility compared to physical hydrogels which dissolve at certain temperatures, therefore the gel would not support a process of heat sterilization.

The hydrogels characterization was crucial to demonstrate that the gels were apt to be used as a medical product. The swelling test was of great importance to know how much liquid the gel can absorbed and how the transport mechanisms with which liquid were moving in the hydrogel mesh. The dehydration tests under two conditions could demonstrate that under laboratory conditions (room temperature) similar to those under hospital conditions, the hydrogels had the capacity to have a minimum dehydration during the first 8 hours of treatment, which was the duration of a conventional bandage.

The analysis of the FTIR spectra allowed us to appreciate that there is no other chemical element that can result from the synthesis process and that can generate toxicity. Both polymers presented supplier certifi- cation as biocompatible materials; however in the final product the cytotoxicity test verified that the hy- drogel was non-cytotoxic therefore was suitable for use as a medical product.

Finally, surface pH measurements indicated that hydrogels generated with PVA/PVP polymers present acidic surfaces that could reduce the basic environment present in wounds as a result of bacterial and enzymatic activity.

Conclusions

The hydrogels generated in this project present a simple, economical synthesis alternative procedure, which was independent of the use of chemical substances for the generation of hydrogels, friendly to the environment, biocompatible and with acid surface pH characteristics that could induce an accelerated recovery of patients with dermal wounds.

Interest conflict declaration

Our research group considers that this work does not present conflicts of interest.

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