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GLUCANTIME DRUG DELIVERY COMPARISON BETWEEN CROSSLINKED MEMBRANES IRRADIATION VERSUS ESTERIFICATION

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ABSTRACT

Pentavalent Antimony (Glucantime) is the drug of choice for the treatment of Leishmaniasis. The disease is transmitted by the female bite of *Phlebotomine sandflies*. The sandflies inject the infective stage, metacyclic promastigotes, during blood meals. The protozoan parasite causes a spectrum of clinical diseases afflicting 12 million people worldwide. The use of hydrogels matrices for particular drug-release applications has been investigated with the synthesis of modified polymeric hydrogel of poly (vinyl alcohol) (PVAl), poly (N-viny-2-pirrolidone) (PVP) and poly (ethylene glycol). They were processed using gamma radiation from Cobalt-60 source at 25 kGy dose. The characterization of the hydrogels was conducted and toxicity was evaluated. The dried hydrogel was analyzed for differential scanning calorimetry (DSC), thermogravimetry (TGA), swelling and gel content determinations. The membranes have no toxicity and gel content has revealed the crosslink degree. The chemical crosslinking depends on the acid concentration. Increase of the acid concentration increases the gel content, the thermal stability of the PVAl component and decreases the swelling capacity. The thermal stability of irradiated membranes is decreased in the presence of plasticizer. In contrast to ionizing radiation membranes described in the literature and formulated with PVAl/PEG, our new membranes composed by PVAl/PVP/PEG are more flexible and presents higher swelling capacity. The drug was immobilized in the hydrogels structures and the glucantime drug delivery was determined.

1. INTRODUCTION

Functional polymers are designed to modify the pharmaceutical function of the dosage form and to control the release of active ingredient [1]. The majority of controlled-release dosage forms can be categorized as matrix, reservoir or osmotic systems [2]. In matrix systems, the drug is embedded in the polymer matrix and release takes place by partitioning of the drug into the matrix and the release medium [3]. It may be characterized as a mass transport phenomenon. In contrast, reversion systems have a drug core surrounded by a rate-controlling membrane such as enterically coated products and implants. Factors such as pH and presence of food affect the drug release rate from reservoir devices. An increase in hydrostatic pressure drives osmotic devices, forcing the drug solution or suspension out of the device through a small delivery port [4]. Drug release is independent of pH and it is possible to modulate the release characteristics by optimizing the properties of the drug and polymer coat.

One potential application of membranes is under injured skin tissues. The cutaneous Leishmaniasis, caused by a protozoan of the genus Leishmania transmitted by mosquitoes

Phlebotomine is clinically characterized by prolonged fever, paleness, weight loss and difficult healing wounds of the skin. In the urban area, the dog is the main source of infection. The man is included in the epidemiological cycle as possible host, but in epidemic areas can be considered reservatory, and may occur in man-vector transmission-man [5].

Pentavalent antimonials, such as meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®), are the main drugs recommended in the treatment of all forms of Leishmaniasis [6]. Other alternative drugs used in the treatment are pentamidine and amphotericin B, but their use has been limited by high toxicity and cost [7]. Despite several gaps in the knowledge of action, toxicity and pharmacokinetic parameters, pentavalent antimonials have been used for over 60 years [8] and the definition of its pharmacokinetic profile may suggest a better therapeutic protocol for doses, administration interval and duration of the antimonials therapy, reducing the severe side effects [9].

Along this same line of research, based on work of literature, the purpose of this study is to prepare the hydrogels based on poly (N-vinyl-2-pyrrolidone) (PVP), poly (vinyl alcohol) (PVAl) and poly (glycol ethylene) (PEG and citric acid. The hydrogels were obtained by two different processes by gamma irradiation and esterification, from delivery of glucantime directly in vitro.

2. METHODS AND MATERIALS

PVAl (Mw = 85000 degree of hydrolysis 98.4%) from CelvolTM 325 Dermet Agekem. PEG 300 from Oxiteno. PVP *Kollidon* 90F from Basf and citric acid (99.9 %) from Merck.

PVAl (10% m/v) was prepared by the dissolution in deionized water under reflux at 85 °C for 40 minutes until total dissolution. And PVP (10% m/v) was prepared by the dissolution in deionized water under reflux at 90 °C for 5 minutes for total dissolution.

After that three different formulations were prepared by the addition of PEG in the proportions 0.5; 2.0 and 4.0%; in the PVAl / PVP solution and heated for 5 minutes at 85 °C.

From gamma irradiation and after that three different formulations were prepared by the addition of citric acid in the proportions 0.5; 2.0 and 3.0%; in the PVAl / PVP solution and heated for 5 minutes at 85 °C.

2. 1. Swelling

After synthesis, the samples were immersed in distilled water and weighed in periods of time until 72h and the swelling was calculated according to the equation A.

$$\text{Swelling} = (ms - md)/md \ . \ 100 \ (\%H_2O \text{ per g hydrogel}) \qquad (A)$$

where: ms is the mass of swelled polymer and md is the mass of the hydrogel.

2.2. Gel content

The gel fraction was obtained by immersion of the samples in water 100°C for 10h to proceed the extraction, under stirring. The water was replaced after each 4h. After that the

samples were dried in oven (100 °C) and the gel fraction was calculated by the equation B.

$$\text{Gel fraction} = mf / ms \cdot 100 \qquad (B)$$

where: ms is the mass before extraction and mf is the mass of the dried sample after extraction.

2.3. Thermogravimetry (TGA)

TGA technique was accomplished in a Mettler-Toledo TGA/SDTA 851 thermobalance, using inert atmosphere of N_2 from 25 to 600 °C at heating rate of 10° C min^{-1}.

2.4. Differential scanning calorimetry (DSC)

The assay was carried through the DSC-822e Metter-Toledo. Samples of 10mg, under the following conditions: heating of -50 ° C at 200° C, heating rate of 10° C/min, in inert atmosphere of N_2.

2.5. Cytotoxicity

Tests for biological evaluation "in vitro" were performed according to ISO 10993-5 standards for procedures. In the cytotoxicity test, the samples were tested in culture of mammalian cells. The samples were placed on plates for cell culture and Evaluation of cytotoxicity was performed using the method of incorporation of the vital dye neutral red.

3. RESULTS AND DISCUSSION

Fig. 1A shows the swelling curves of the membranes of citric acid reticulation. The matrix PVAl + PVP + 0.5% citric acid swells relatively more than the matrices with higher crosslink agent concentration. The increasing of concentration of citric acid decreases the swelling, as effect of crosslinking. As in Fig. 1B, we observed that the concentration of PEG in the matrix PVAl + PVP directly influences the balance of swelling the higher the PEG concentration the higher is the swelling %.

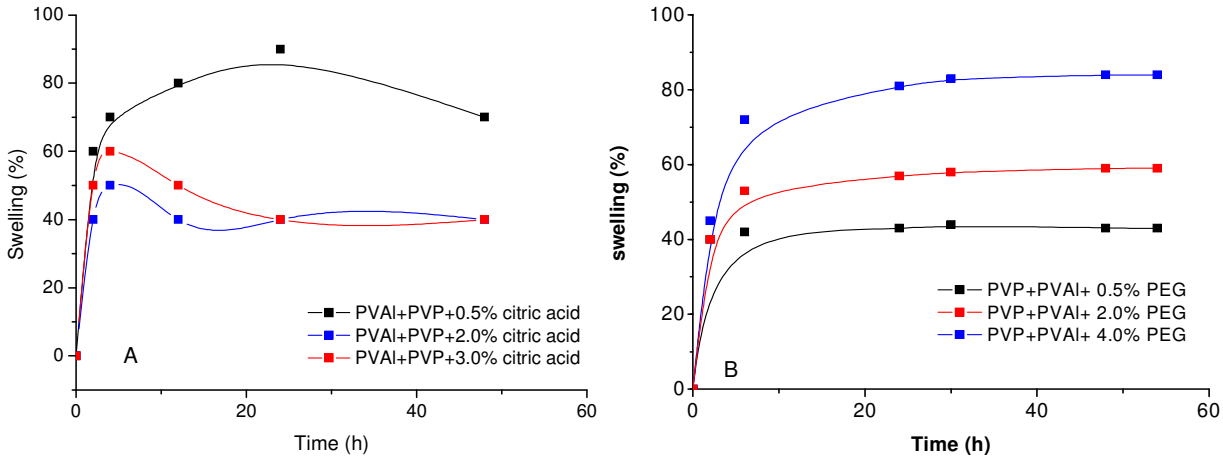

Figure 1. Swelling curves (A) hydrogels by citric acid crosslinked and (B) hydrogels by gamma irradiation.

The gel fraction content is associated to the extent of reticulation of the matrix. The results of gel fraction reported in Table 1 indicate decreasing values with increasing concentration of PEG. The PEG was used as a plasticizer to increase mobility and flexibility of the membranes. The monomeric plasticizers are generally non-volatile molecules or polymers of low molecular weight, for the most liquid, which when blended with polar polymers, is positioned between the intermolecular linkages and increase the space between adjacent links [10,11]. It is estimated that when the concentration of PEG increases, the space between adjacent links becomes more difficult to reticulation between the polymer chains and reducing the formation of the gel. For the hydrogels obtained by esterification, it was observed that increasing the concentration of citric acid increased the amount of gel fraction.

Table 1. Gel fraction (%) results of hydrogels obtained by gamma radiation (25 kGy).

Sample (irradiation)	Gel fraction %	Sample (esterification)	Gel fraction (%)
PVAl + PVP + PEG 0.5 %	78.1	PVP + PVAl + 0.5 citric acid	40.0
PVAl + PVP + PEG 2.0 %	60.9	PVP + PVAl + 2.0 citric acid	59.6
PVAl + PVP + PEG 4.0 %	49.8	PVP + PVAl + 3.0 citric acid	67.1

It is important to emphasize that increasing the citric acid concentration the membrane crosslinked by esterification gives a result of higher thermal stability with, as observed in Fig. 2. Te first decomposition event is related to residual water. The second event of the PVAl decomposition beginning in 273 °C and is shifted to a temperature above 350 ºC after reticulation. The event of PVP decomposition does not change significantly. It was observed that the thermal stability of irradiated membranes is decreased in the presence of plasticizer, which is attributed to the disorganization of the polymer chain and accessibility by oxygen requiring lower temperatures to degrade.

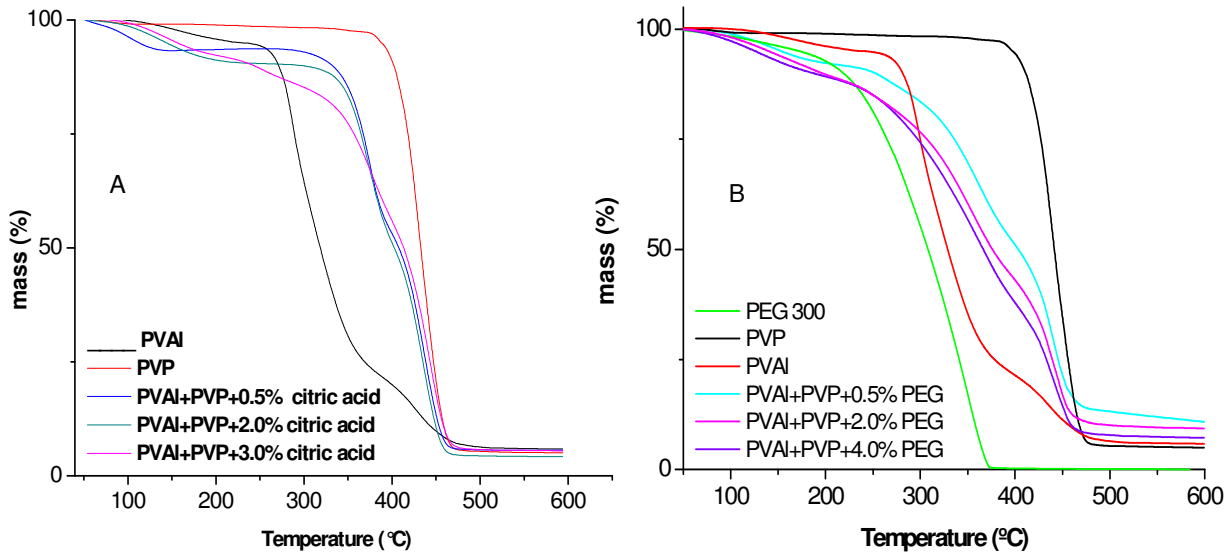

Figure 2. TGA results of (A) hydrogels by esterification and (B) gamma irradiated.

The cytotoxicity tests showed similar behavior to the negative control, and not toxicity effect as seen in Fig. 3.

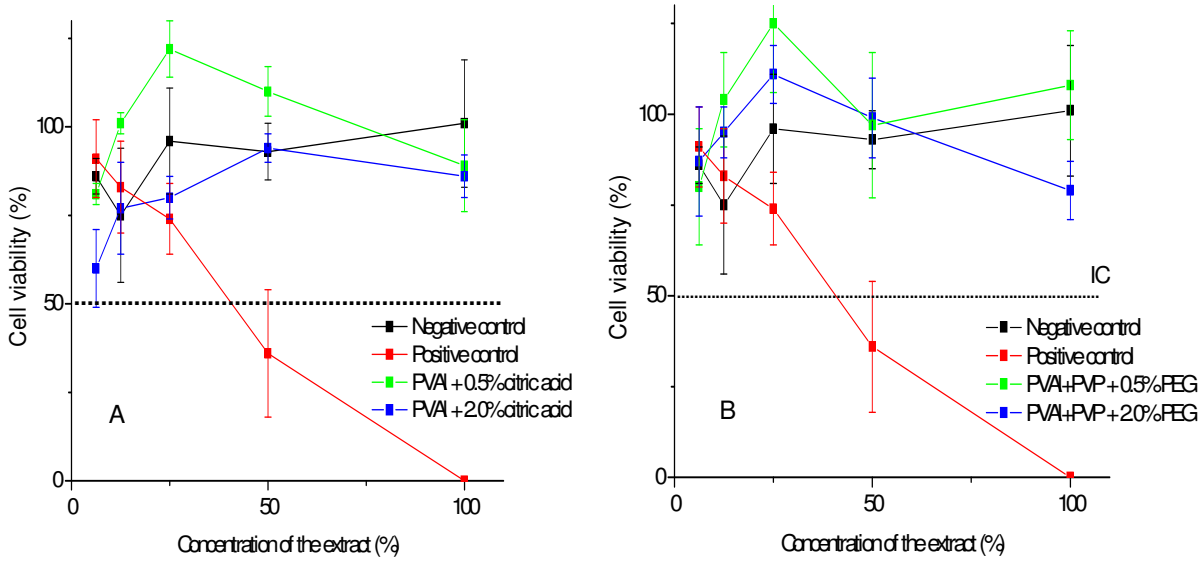

Figure 3. Curve cytotoxicity (A) hydrogels crosslinked chemistry and (B) hydrogels crosslink gamma

The crosslinked hydrogels were selected as matrices for glucantime release in vitro. According Y. Ikeda et al [12] the area available for diffusion of the solute is the free space that exists between the macromolecular chains of hydrogels. The more reticulated is the hydrogel, smaller will be the spaces between the macromolecular chains. The analysis of the kinetics of this process showed a value close to the release of the drug concentration between the matrices tested. The collection time of glucantime releasing results was long enough to detect differences between the matrices produced by the various crosslinking processes, according to the Fig. 4.

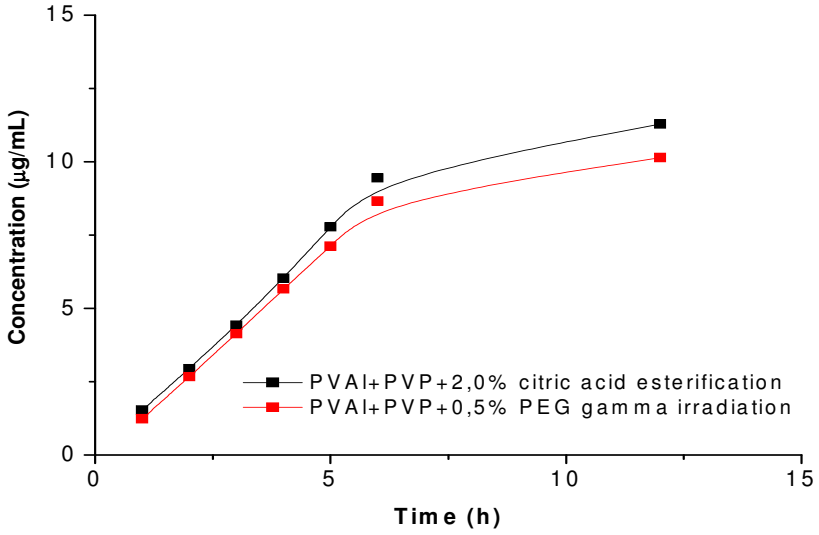

Figure 4. Kinetics of glucantime release in matrices of hydrogels obtained by esterification and gamma irradiation.

4. CONCLUSIONS

The cytotoxicity of irradiated or chemically crosslinking membranes tests showed similar behavior to the negative control, no cytotoxicity of the membranes.

The chemical crosslinking depends on the acid concentration. Increase of the acid concentration increase the gel content, the thermal stability of the PVAl component and decreases the swelling capacity. The thermal stability of irradiated membranes is decreased in the presence of plasticizer, which is attributed to the disorganization which is attributed to the disorganization of the polymer chain and oxygen accessibility requiring lower temperatures to degrade.

Kinetics studies of glucantime were developed in the membranes obtained by gamma irradiation and esterification. The drug immobilized has released with success and the release from gamma irradiated membranes was slower than the other.

The characteristics of integrity, not cytotoxicity, uniformity and swelling, observed in the membranes obtained are evidence that synthesis can be used for different applications such as release of drug. According to the degree of reticulation can be used for rapid release or slow release

ACKNOWLEDGMENTS

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