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# SYNTHESIS OF POLYMERIC GELS CROSSLINKED BY IONIZING RADIATION FOR TREATMENT OF CUTANEOUS LEISHMANIASIS

**Maria José A. Oliveira, Edson G. Moreira, Pablo A. S. Vásquez, Mara T. S. Alcântara, Ademar B. Lugão**

Nuclear and Energy Research Institute (IPEN - CNEN/SP)
Av. Professor Lineu Prestes2242
05508-000 São Paulo - SP Brazil
mariajhho@yahoo.com.br

## ABSTRACT

Ionizing radiation is a very efficient, versatile and clean tool for modifying polymers for various applications, including in the biomedical area. The advantages of using radiation include the ability to process materials in any physical form, at a convenient temperature, often at room temperature. There is no need for the use of initiators or other chemicals and it is easily carried out with high reliability and does not generate any waste. In the research support area, several materials have been treated in order to find correlations between the applied dose and some property of the material susceptible to be modified with gamma radiation. This study proposes the development of polymeric gels (cream) with Glucantime (Sb V) and gel (cream) with silver nanoparticles, for alternative treatment of cutaneous *Leishmaniasis*. A cobalt-60 gamma irradiation source was used for crosslinking the polymers, forming the silver nanoparticles and simultaneous sterilization, leaving the product ready for use. Polymeric gels were characterized by physico-chemical techniques, instrumental neutron activation analysis (INAA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

## 1. INTRODUCTION

Leishmaniasis are diseases caused by several protozoan species of the order Kinetoplastida, family Trypanomastidae, genus *Leishmania*, which affects humans and different species of wild and domestic animals [1]. Leishmaniasis is classified into three clinical forms: cutaneous leishmaniasis (CL), the simplest; mucocutaneous leishmaniasis (MC), which can spread to the mucosa; and visceral leishmaniasis (VL), also known as kalazar, the most severe form of the disease which if not properly treated can be fatal and disseminate to various organs [2].

Leishmania hosts include various mammals, such as marsupials, rodents, canines, and primates and among these primates, humans [3]. In the New World (Americas) leishmaniasis are transmitted among animals and humans by the bite of the female of several species of sandflies, Phlebotominae subfamily of the genus *Lutzomya* and *Psychodopygus*. At the Old World, they are transmitted by sandflies of the genus *Phlebotomus* [4].

Integumentary Leishmaniasis (IL) is the most common form of the disease, with about 12 million infected worldwide [5]. About 95% of cases of LT occur in the Americas and around 0.7 to 1.2 million new cases occur annually around the world. More than two-thirds of the new cases of CL occur in six countries: Afghanistan, Algeria, Brazil, Colombia, Iran and Syria [3].

IL diagnosis can be done direct by tissue apposition of Giemsa-stained or Panotic fast culture in specific biphasic media (NNN-Novy, McNeal, Nicolle/BHI or Schneider supplemented with antibiotics and fetal bovine serum-SFB -Sigma) and by histopathological examinations [6].

Leishmaniasis is a neglected disease and currently available medications for the treatment of the disease are unsatisfactory due to their limited efficacy, side effects, and the resistance that the protozoa develop against these drugs [7-8]. The drug of first choice for IT treatment is N-methyl glucamine antimoniate (Glucantime). According to the World Health Organization, Glucantime dose should be calculated as mg/$Sb^{V+}$/kg/day (pentavalent antimoniate ($Sb^{V+}$). Despite considered the first choice drug pentavalent antimoniates present harmful side effects. The application takes place intravenously and patients with renal or cardiac dysfunctions cannot use the medication due to its side effects [9]. There is a need for new treatment methods for patients who cannot use the conventional treatment. In view of this information, polymeric hydrogels modified by ionizing radiation have been developed as an alternative treatment for IT [10]. These hydrogels are used as topical dressings and release the drug directly into the wound, reducing the amount of the drug in the bloodstream [11].

Modifications in polymers caused by ionizing radiation depend on the processing conditions, i.e. type of radiation, presence of oxygen or different atmospheres, additives, solvents, degree of crystallinity and homogeneity of the polymeric material that will absorb the energy [12]. The irradiation process has innumerous advantages, such as an easy process control, simultaneous preparation and sterilization of the hydrogel and it is not necessary to add any additives or initiators to the crosslinking reaction. Due to its biocompatibility, the poly(N-2-vinyl-pyrrolidone) polymer (PVP) has been used by several researchers in the preparation of hydrogels for dressings of wounds and burns [13].

## 2. MATERIALS AND METHODS FOR POLYMERIC GELS PREPARATION

### 2.1. Materials

Poly(N-2-vinyl-pyrrolidone) (PVP) K90 by BASF, Poly(ethylene glycol) (PEG 400) by Synth, Clay by Colormix, alginate by Synth, meglumine antimoniate (GLUCANTIME®) by Sanofi-Aventis and silver ions 22 ppm prepared by water electrolysis by Khemia were used on hydrogels preparation.

### 2.2. Methods

#### 2.2.1. Gel preparation

Gels were prepared from mixture of PVP solution (3.0% m/v), clay (1.5% m/v), alginate (1.0% m/v) and PEG300 (2.0% m/v) with silver ions solution (22 ppm). The formulation was solubilized at room temperature and allowed to stand for 15 h, and then it was placed in tubes and sent to irradiation with Cobalt-60 gamma rays at total dose of 25 kGy and dose rate of 5 kGy $h^{-1}$.

#### 2.2.2. Scanning electron microscopy with Energy Dispersive Spectroscopy (SEM/EDS)

SEM/EDS were used for verification of polymeric structures. In order to obtain high resolution structures of pores in the macro and micro levels, low voltage in the range of 5 kV was applied. The device used was the Jeol JSM-6701F.

### 2.2.3. Transmission electronic microscopy (TEM)

To confirm the presence and geometry of silver nanoparticles in the gel, a sample was placed in a beaker with buffer under agitation for 24 hours to promote the release of the silver nanoparticles. No release of the nanoparticles occurred. A more dilute formulation was then provided to facilitate gel filtration, thus making the TEM analysis possible, a drop of the solution was placed on a carbon grid and analyzed. The Jeol JEM-2100 transmission electron microscope was used.

### 2.2.4. Instrumental neutron activation analysis (INAA)

The comparative INAA method was used to determine Ag and Sb (V) in the polymeric gels. The technique consists in simultaneously subjecting samples and elemental standards to the thermal neutron flux produced in a nuclear reactor. After appropriate decay period, element concentration is determined by comparison of peak areas of samples with those of standards, obtained by gamma spectrometry [13]. Samples were irradiated for 4 h at $10^{12}$ $cm^2$ $s^{-1}$ thermal neutron flux at the IEA-R1 research reactor and gamma spectra were recorded for 10 h after a 10-day decay period using a CANBERRA GC2018 HP Ge coupled to a DAS-1000 digital spectra analyzer.

The radionuclides $^{110m}$Ag (657.8, 677.6, 706.7, 763.9, 884.7, 937.5, 1384.3 and 1505.0 keV photopeaks; half life: 249.8 days); $^{122}$Sb (564.2 keV photopeak; half life: 2.7 days) and $^{124}$Sb (602.7 and 1691.0 keV photopeaks; half life: 60.2 days) were used for element identification and quantification [14].

## 3. RESULTS

### 3.1. SEM-EDS

The study was carried out on lyophilized polymeric gels containing 1.5% of laponite clay, glucantime drug and silver ions. In the synthesis of the gel containing PVP/alginate/clay, the elements silicon, magnesium and sodium (Si, Mg, Na) were observed in the fracture surface of the samples. These elements are present in the composition of the laponite clay, Zinc (Zn) was associated with clay impurity; oxygen and carbon (O, C) were associated with the polymeric part, as presented in Fig. 1.

For the gel synthesis containing PVP/alginate/clay/silver ions, Si, Mg and Na were observed in the fracture surface of the samples. Zn was associated with clay impurity and C and O were associated with the polymeric part, while silver (Ag) was from silver nanoparticles formed after irradiation, as shown in Fig. 2.

For the gel synthesis containing PVP/alginate/clay/glucantime, Si, Mg and Na were observed in the fracture surface of the samples, associated to the clay composition. Aluminum and chlorine (Al, Cl) were associated to clay impurities and C and O were associated with the

polymeric part. Antimonium (Sb) is the active ingredient of the pharmaceutical and sulfur (S) was associated with the pharmaceutical solution as presented in Fig. 3.

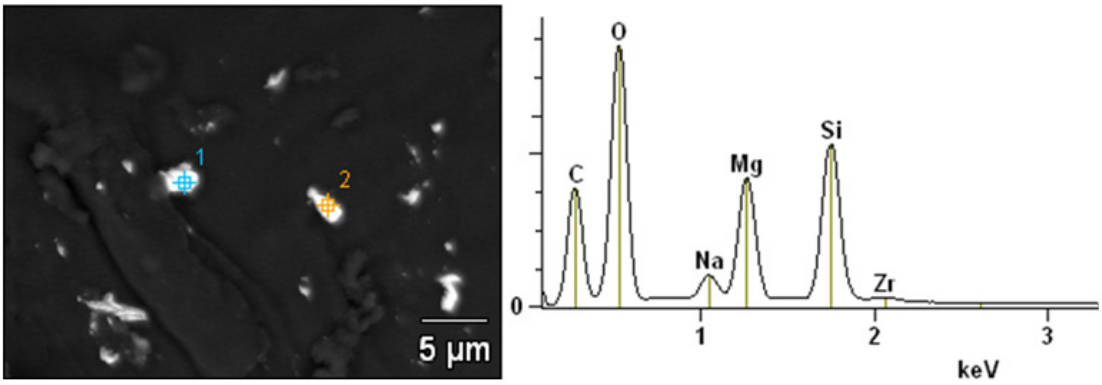


**Figure 1: EDS micrograph of lyophilized nanoclay of gel.**

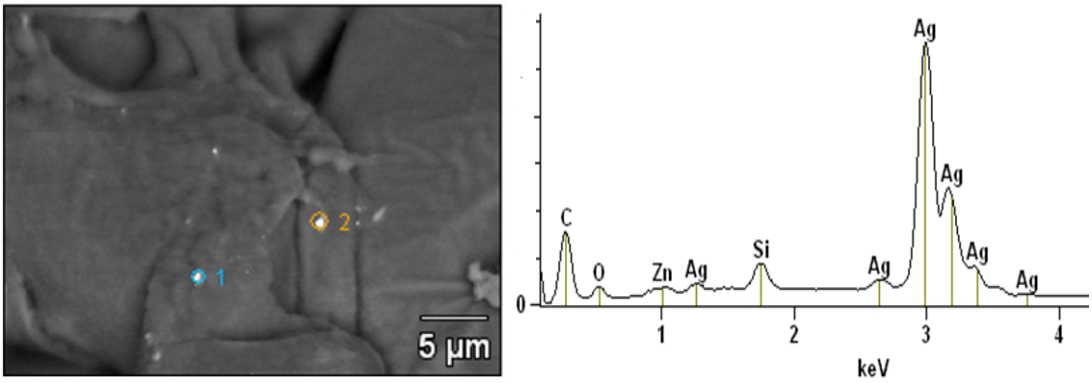


**Figure 2: EDS micrograph of lyophilized nanoclay and nanoparticle of gel.**

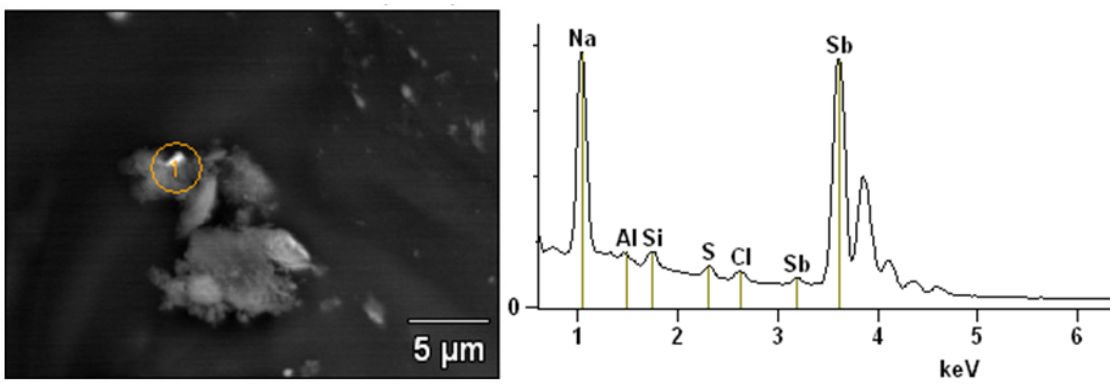


**Figure 3: EDS micrograph of lyophilized nanoclay and GLUCANTIME® (meglumine antimoniate) of gel.**

However, for the gel synthesis containing PVP/alginate/ clay/Glucantime/silver, Si observed at the fracture surface of the sample is related to clay, while S was associated to the

Glucantime vehicle solution and Ag was associated to the Ag ions added to the formulation. Samples were analyzed at three scales: 5, 25 e 50 µm but it was not possible to observe Sb between 3.2 and 3.7 keV at Fig. 4. It was assumed that the Ag intensity present in this interval superimposed Sb signal as it was present in Fig. 3 at the energy range.

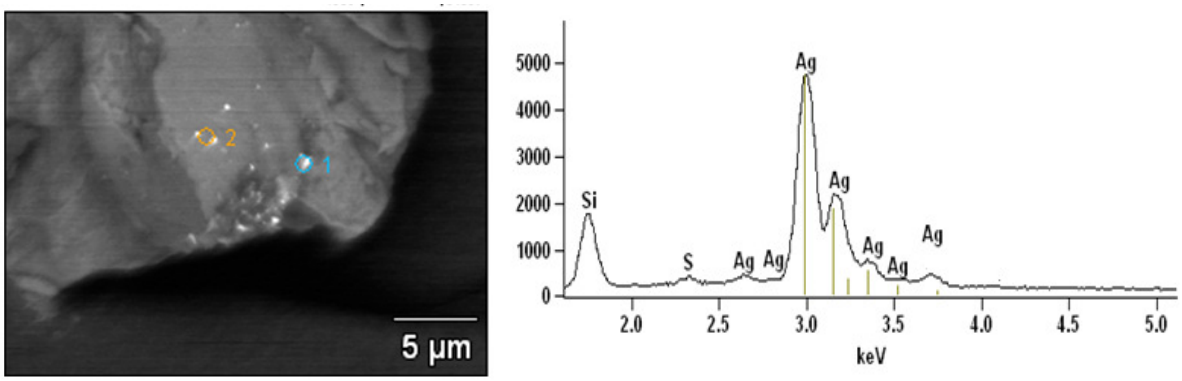


**Figure 4: EDS micrograph of lyophilized nanoclay, silver nanoparticle and Sb (V) pharmaceutical of gel.**

### 3.2. TEM

TEM confirms the formation of silver nanoparticles as shown in Fig. 5, as already seen in Fig. 2 by EDS analysis. It can be observed that the silver nanoparticles exhibit a monodisperse structure with spherical shapes. It was not possible to improve the image visualization because the focus was not precise due to the presence of the polymer gel in the solution.

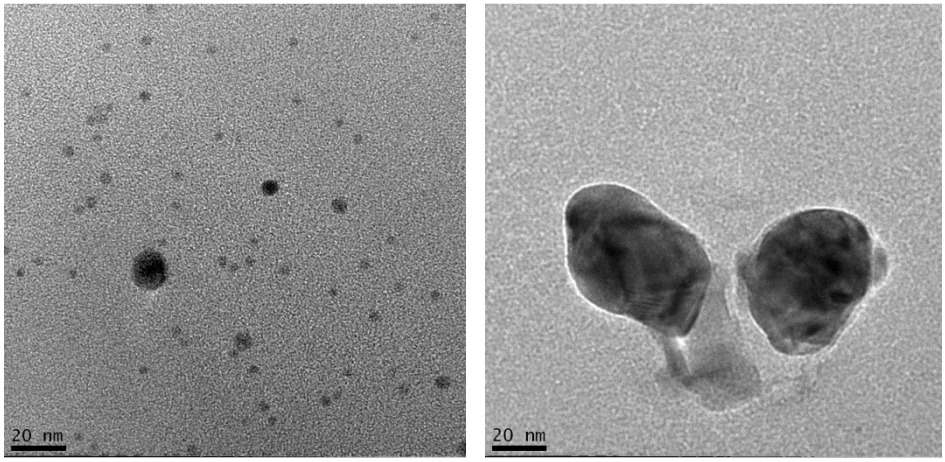


**Figure 5: TEM images of Ag nanoparticles distributed at the polymeric gel solubilized in aqueous solution.**

### 3.4. INAA

Ag and Sb were determined in freeze dried gels and in phosphate buffer pH =7 after exposition times varying between 1 to 30 h. Good correlations were observed among INAA

results obtained with the various photopeaks ($R^2$ = 0.9999 for 657.8 and 884.7 keV of $^{110m}$Ag; $R^2$= 0.997 for 564.2 keV of $^{122}$Sb and 1691.0 keV of $^{124}$Sb) assuring that no interference was observed and enhancing the confidence in the obtained data. Then the most intense energies for Ag and Sb were used in the remaining of the study.

Table 1, presents INAA results for the freeze dried gels. It was possible to detect small amounts of Ag and Sb in the gels that were not spiked with these elements. It might be associated to some cross-contamination during gel preparation and is explained by the good sensibility of INAA for these elements.

**Table 1: Element in freeze-dried polymeric gels (µg $g^{-1}$) determined by INAA**

| Element | polymeric gels | | | |
|---|---|---|---|---|
| | No spike | + Ag | + Sb | +Ag/Sb |
| Ag | 0.181 ± 0.029 | 406.5 ± 1.4 | ND | 560 ± 25 |
| Sb | 0.407 ± 0.004 | 0.016 ± 0.001 | 601 ± 27 | 474 ± 3 |

ND – not detected;

The pattern of element liberation to the phosphate buffer pH = 7 from polymeric gels is presented in Fig. 6. As expected, element liberation increased with time for both elements and a maximum was observed around 24 h of exposition. One element seems not to interfere in the liberation of the other but it should be further investigated with repetitions of the liberation study. It was also observed that Sb liberation was higher than Ag liberation and it is associated to the higher concentration of this element in the polymeric gels but also possibly to their different chemical forms and diffusion coefficients.

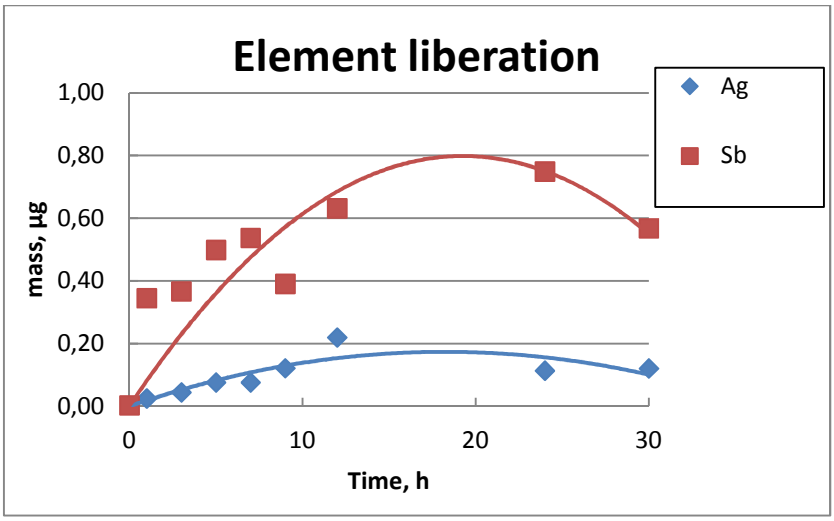


**Figure 6: Element liberation to phosphate buffer pH = 7, the polymeric gels.**

## 4. CONCLUSIONS

The polymer gels were synthesized by ionizing radiation and the results presented: transmission electron microscopy (TEM) showed the formation of silver nanoparticles from gamma irradiation. EDS micrographs presented the distribution of the elements Si, Ag and

Sb. Sb was not observed at the gel containing PVP/alginate/clay/Glucantime/silver. It was assumed that the Ag intensity in this interval 3.2 and 3.7 keV superimposed Sb signal as it is presented in Fig.4. However Instrumental neutron activation analysis (INAA) confirmed that one element seems not to interfere in the liberation of the other but it should be further investigated with repetitions of the liberation study. It was also observed that Sb liberation was higher than Ag liberation and it is associated with the higher concentration of this element in the polymeric gels but also possibly to their different chemical forms and diffusion coefficients. A comparison to other analytical techniques can be interesting to complement and confirm this study and thus become applicable

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