Radiation Physics and Chemistry 165 (2019) 108369

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Simultaneous hydrogel crosslinking and silver nanoparticle formation by using ionizing radiation to obtain antimicrobial hydrogels

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ARTICLE INFO

Keywords:
Wound dressing
Hydrogel
Multiresistant bacteria
Silver nanoparticles
Ionizing radiation

ABSTRACT

Hydrogel dressings are crosslinked hydrophilic polymers able of swelling in presence of water and can be used in many different types of wound care. In turn, in wound care, silver has been used for a long time as an effective antimicrobial agent. Recent studies have demonstrated an increase of its antimicrobial action when it is used at nanometer scale, that is, as silver nanoparticles (AgNPs), which have anti-inflammatory effect on infected wounds, rashes, and mesh skin grafts. The objective of this work was to study hydrogel dressings containing AgNPs from silver nitrate, synthesized by radiation involving the formation of silver nanoparticles with simultaneous occurrence of crosslinking and sterilization of the polymer systems. One of the hydrogels was prepared with poly(*N*-vinyl-2- pyrrolidone) (PVP) and the other with poly(vinyl alcohol) (PVA) as main studied polymers. An aqueous solution of AgNO3 was added to both polymer systems separately. The AgNPs synthesis, polymer crosslinking and dressing sterilization were achieved simultaneously by irradiating the resultant solutions with gamma-rays from a 60Co source. Gel fraction, swelling in reverse osmosis water, SEM-EDS, UV-visible spectroscopy, and antimicrobial activity were performed and characterized. The obtained results showed that the dressings have a soft consistency, high degree of crosslinking and swelling, homogeneous distribution of AgNPs with peaks of plasmonic bands about 400 nm, but only PVP hydrogel showed antimicrobial properties to *P. aeruginosa* and normal *S. aureus.* Moreover, this hydrogel also showed antimicrobial properties to *S. aureus* strain multiresistant to penicillins, cephalosporins, carbapenems, sulfonamides, tetracyclines, quinolones, and aminoglycosides, whereas the PVA hydrogel showed antimicrobial properties to *P. aeruginosa* and bacteriostatic activity to *S. aureus*. The results suggest that both synthesized dressings have potential for use in wounds and burns infected with gram-positive and gram-negative bacteria.

1. Introduction

Hydrogel is defined as a polymeric material which exhibits the ability to swell in the presence of water. It absorbs a significant water amount within its structure, without dissolving the crosslinked polymeric network. Additionally, it presents the advantage of low interfacial tension which may be exhibited between the surface of the hydrogel and an aqueous solution (Ratner and Hoffman, 1976).

The hydrophilic gels have various industrial applications: as biomedical devices, in improvement of agricola soils, in treatment of water and effluents, and in many others. Hydrogels may be prepared by using various polymers and different techniques such as polymeric chains formation by chemical reaction, physical interactions resulting formation of crystallites, entanglements and electrostatics, and by using ionizing radiation generating free radicals in the major chains that recombine by crosslinking (Ahmed, 2015). The methods of ionizing radiation are attractive for the syntheses of hydrogels because they have advantages over the use of the chemical ones since do not leave toxic residues; the process is easily controlled, promotes simultaneous crosslinking and sterilization. In addition, such methods are economically competitive when compared with other more conventional ones such as those of chemical crosslinking. In the latter, the remaining crosslinking agent, if not removed, may make difficult the use of the gel obtained for biomedical and pharmaceutical applications (Peppas et al., 2006).

Among the polymers used for synthesis of hydrogels, poly (*N*-vinyl-

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https://doi.org/10.1016/j.radphyschem.2019.108369
Received 16 April 2018; Received in revised form 18 May 2019; Accepted 15 June 2019
Available online 05 July 2019
0969-806X/

2-pyrrolidone) (PVP) stands out. It is a nonionic polymer, water soluble, presents interesting biocompatible properties, and hydrogels obtained from of its aqueous solutions with the aid of radiation techniques have been used as matrices of dressings (Rosiak et al., 1986). The hydrogels present adequate characteristics to be used as a dressing since they have all the requirements that favor an excellent epithelization of the lesions, barrier against bacteria, high elasticity, high transparency, allow a passage of oxygen, absorption of exudate. (Lugão and Malmonge, 2001).

Another polymer that can be crosslinked through radiation techniques is poly(vinyl alcohol) (PVA). It is useful in a wide variety of applications, particularly in the areas of medical and pharmaceutical science. It has a relatively simple chemical structure, is water soluble, biocompatible, and biodegradable.

The reactions in aqueous systems, such as hydrogels, using radiation techniques, occur between the solute andthe primary products of water radiolysis. The primary products are e-aq· OH•, H•, H_2, H_2O_2.Among these, OH radicals strongly react with the polymer, generating macroradicals that allow the crosslinking of PVP and PVA (Rosiak et al. 1995).

Actually, silver nanoparticles (AgNPs) widely used as a valuable nanomaterial for medical and hygiene area by incorporating them in hundreds of consumer products (Lansdown, 2010a,b). The colloidal silver products are based on the dispersion and suspension of ultrafine particles of silver in nanoscale.

The nanosilver has anti-inflammatory properties improving the wound healing (Chaloupka et al., 2010). In addition, the great interest in biomedical applications of AgNPs is due to their potent antibacterial activity against broad spectrum of gram-negative and gram-positive bacteria, encompassing antibiotic resistant strains (Rai et al., 2012; Lara et al., 2011; Franci et al., 2015).

So, considering the increase of multiresistant bacteria to many types of antibiotics, nanoparticles are a viable alternative to antibiotics (Wang et al., 2017). On the other hand, hydrogels containing silver nanoparticles (AgNPs) for use, for example, as dressings have found great scientific and commercial interest in the last three decades.

Silver has a long and fascinating history for the treatment of human diseases as antibacterial agents before the introduction of antibiotics (Rai et al., 2012; Morones et al., 2005) studied the effect of silver nanoparticles on gram negative bacteria and concluded that nanoparticles in the range of 1–10 nm adhere to the surface of bacteria compromising seriously their respiration and permeability; if they penetrate into the internal structure can still interact with the sulfur and phosphorous of bacterialDNA being able to cause more damage to the microorganism. In addition, AgNPs release Ag^+ions, which further increase their bactericidal effect (Feng et al., 2000). Silver nanoparticles (AgNPs) can be prepared by various techniques, among which onecan cite chemical reduction using a variety of organic and inorganic reducing agents, electrochemical and photochemical methods, physical synthesis, microwave processing, gamma-irradiation, biological synthetic methods, among others (Iravani et al., 2014).

In the synthesis of silver nanoparticles by γ-irradiationelectrons (e^-) and H atoms, both are strong reducing agents. Thus, they may, under certainaqueousconditions, easily reduce silver ions by leading them to the zero valence state (Sheikh et al., 2009, Krkljes et al., 2007) as the following equation:

$$Ag^+ + e^-_{aq} \rightarrow Ag^0$$

$$Ag^+ + H^{\cdot} \rightarrow Ag^0 + H^+$$

In this study was obtained anantibacterial hydrogel dressing using a simple, elegant and low-cost process, once the ionizing radiation used promoted the crosslinking of the polymer base, synthesized the AgNPs “in situ” from silver nitrate and sterilized, all simultaneously. Besides that, the hydrogel of PVP was effective against resistant bacteria.

2. Methods

2.1. Hydrogel preparation

In the preparation of both hydrogels, were used aqueous solutions of PVP-glycerol-agar (Plasdone® K-90D from ISP; glycerol from Oxiteno; agar from Oxoid, respectively) and PVA-KC-agar (Mowiol 40–88 from Clariant; KC, kappa-carrageenan from Agargel; agar from Oxoid, respectively) with addition of silver nitrate (from Cennabras) to their formulations, however without any catalyst or photoinitiator. The compositions of hydrogels (without silver) were obtained from optimization after the study of experimental design, not presented in this work. The polymers and respective componentsof the mixtures were dissolved in reverse osmose water; next, solution of silver nitrate, previously prepared in the dark, was added to the mixtures separately and homogenized, emphasizing that this last operation also was performed in the dark. The silver concentrations of solution added to PVP and PVA solutions were 40 ppm and 60 ppm, respectively. The polymer crosslinking, synthesis of AgNPs and sterilization of the hydrogels obtained were carried out simultaneously induced by gamma-irradiation from a 60Co source, at a dose of 25 kGy and a dose rate of 1.58 kGy h^{-1} in an irradiator type Gammacell 220. The hydrogels obtained were named PVP-based and PVA-based, or for simplification, PVP hydrogel and PVA hydrogel, respectively along the text.

2.2. Gel fraction

The gel fraction test was performed on dry samples in quintuplicate. The extraction of the soluble fraction was affected on stainless steel bags with screen of 300 mesh dipped in distilled water, next autoclaved at 120 °C for 2 h. After this period, the samples were dried to a constant weight, and the gel fraction was calculated by Eq. (1):

$$Gel\ \ fraction(\%) = \frac{M_f}{M_0} x100 \quad (1)$$

Where *Mf* is the mass of the dried sample after extractionelimination of the soluble fraction and is the mass of dry sample before extractionsolvent removing.

2.3. Swelling in reverse osmose water

Swelling tests were performed on samples measuring 24 mm × 20 mm x 2 mm, in quintuplicate. The samples were immersed in 100 mL of reverse osmosis water at 35 °C, maintained at this temperature and weighed periodically until constantweight stabilization. Swelling results from each measure performed were calculated in accordance with Eq. (2):

$$\text{Swelling degree}(\%) = \frac{M_i - M_s}{M_s} x100 \quad (2)$$

Where M_i is the mass of the swelled hydrogel and M_s is the mass of the dry hydrogel.

2.4. Scanning electron microscopy - energy dispersive X-Ray spectroscopy (SEM-EDS)

The fracture morphology of the hydrogel was investigated on a scanning electron microscope (SEM), tabletop Hitachi TM 7000, after cryogenic fracture of the lyophilized hydrogel. With a system EDS (energy-dispersive X-ray spectrometry) coupled to a Bruker QUANTAX 70, the distribution of AgNPs was characterized on the surface of the lyophilized hydrogel.

2.5. UV-visible spectroscopy

Spectroscopic analyses were performed directly in hydrogels on an

equipment Varian Cary 50 at a wavelength of 300–800 nm.

2.6. Antimicrobial activity

2.6.1. Disk diffusion test in agar

The disc diffusion in agar test, also known as the Kirby-Bauer method, was carried out with the bacteria *Pseudomonas aeruginosa* (gram-negative) ATCC 25923 and *S. aureus* (gram-positive) ATCC 27853, as established according to NCCLS (standard). Initially the colonies were suspended in a nutrient medium (Mueller-Hinton Broth) at a concentration of 0.5 referred to McFarland scale (1×10^8 CFU/ml); next, seeding was performed in all directions in Petri dishes with Mueller-Hinton agar by using a sterile swab. Subsequently, hydrogel membranes with silver and without silver (negative control), with ca. 5 mm diameter and 2.5 mm thickness and the ciprofloxacin antibiotic disks (positive control) were placed on the surface of the inoculated medium.The plates were incubated in a bacteriological incubator at 36 °C for 18 h and then compared to eachother.

2.6.2. Broth dilution method

Individual colonies of strains of *P.aeruginosa* (ATCC 25923), *S.aureus* (ATCC 27853) were used for both hydrogels and multiresistants strains of *S.aureus* to penicillins, cephalosporins, carbapenems, sulfonamides, tetracyclines, quinolones, and aminoglycosides were used to evaluate the PVP hydrogel. The colonies were suspended in a nutrient medium and diluted in buffered isotonic glucose/1 mM phosphate until concentration of 1×10^5 CFU/mL. Then, 0.06 mL of the suspension of each bacteria set was placed on the hydrogel samples; they were subsequently immersed in a nutrient medium, and this diluted and spread on agar plates, which were maintained at 37 °C for 24 h in the presence of O_2 to evaluate antimicrobial activity of *P. aeruginosa, S. aureus* on PVP hydrogel; 24 h to evaluate antimicrobial activity of *P. aeruginosa* and 48 h to *S. aureus on* PVA hydrogel.

2.7. In vitro citotoxicity

The *in vitro* evaluation of cytotoxicity of hydrogels was performed with extracts there of serially diluted, prepared according to the ISO10993-5 standard, and human fibroblast cell line HaCaT and permanent cell line (CLSno.300493), in accordance with neutral red capture method based on the literature (Finter, 1969; Hansen et al., 1989; Ciapetti et al., 1996; Rogero et al., 2003 and Lincopan et al., 2005).

The fibroblast cells were grown in monolayers in bottles containing 250 mL Eagle's minimum essential medium (MEM), 20 mM glutamine, 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 mg/mL); next, they were maintained at 37 °C in an atmosphere of 5% CO_2. After 24 h of culture, the preconfluent stage, the cells were harvested with trypsin, counted in a Neubauer chamber and distributed on 96-well microtiter plates, 200 μL-well at a final concentration of ca. 1×10^5 cells/mL. Subsequently, the microtiter plates were kept in an incubator for 24 h.

The extraction of hydrogels was accomplished in an oven for 24 h at 37 °C and obtained from the immersion of each of the triplicates in 1 mL MEM, where each sample was prepared with a 1 cm^2 area. As a positive control, we used 0.1% Triton X100 in D-glucose (100% of cytotoxicity), and, as a negative control, sterile D-glucose 5% (100% of viability), which were also kept in an incubator for 24 h at 37 °C.

The extracts from the samples and those from positive and negative controls were diluted in MEM to concentrations of 6.25, 12.5, 25, 50, and 100%. After 24 h of incubation of the microplates, the culture medium was aspirated from the monolayer, washed twice with PBS and replaced by the extracts obtained; the same technique was made for the positive and negative controls. The microplates containing the extracts and controls were again incubated for 1 h at 37 °C in a humid atmosphere and 5% CO_2. All samples and controls were tested in triplicate, and assays were performed in a laminar flow hood using aseptic techniques and sterilematerials.

Cell viability was determined by colorimetric method in which the capture of neural red is proportional to the viability. Thus, after 1 h of interaction, the different media were aspirated from the monolayers, the wells washed with PBS and filled with 200 μL of a solution of neutral red dye (67 mg/mL of D-glucose 5% sterile filtered through presterile Millipore 0.45μ filter).

Plates containing cells with the dye were further incubated for 2 h at 37 °C in a cell culture incubator for incorporation of the dye into cells, and the dye was discarded. The cells were again washed with PBS twice, and finally the dye taken up by the cells was extracted by addition of 0.1 mL of 50% ethanol (v/v) and acetic acid (1%), using a rotary shaker for 10 min. Then the absorbance (A) of the extracted was measured in a spectrophotometer, ELISA reader at 540 nm.

The potential toxicity (cytotoxicity index) for each of the dilutions was evaluated determined according to Eqs. (3) and (4).

$$Cell\ \ viability(\%) = \frac{A_{540}treated\ \ cells}{A_{540}NC} x100 \quad (3)$$

where A_{540} is the absorbance at $\lambda = 540$ nm, dye extracted from the cells treated with the extract and of the untreated cells (negative control - NC).

$$Cytotoxicity\ \ index(\%) = 100 - cell\ \ viability \quad (4)$$

3. Results and discussion

3.1. Hydrogel formation

The formation of hydrogels can be explained as the result of the mutual recombination and crosslinking of polymer free radicals formed, induced by the action of ionizing radiation. If the radicals are favorably positioned, they may undergo recombination through covalent bonds between the chains, Fig. 1, and if the number of such new bonds is large enough, then an insoluble gel fraction appears in the polymer system (Abad et al., 2003).

The average fraction of the synthesized gel dressing was about 85% for both hydrogels with and without silver nanoparticles. Fig. 2 shows the average results obtained from the gel fractions of the synthesized hydrogels.

The results indicated a high degree of crosslinking for both sets of synthesized hydrogels, but highlighting that gel fractions from pure PVP hydrogel have been reported with higher values using this crosslinking process: 90.68% and 90.3%, according to Khampieng et al. (2018) and Momesso et al. (2010), respectively, and about 90% to pure PVA hydrogel according to Alcântara et al. (2012). The gel fractions obtained from PVA and PVP hydrogels showed very close values with low standard deviations (± 0.5 for PVP and ± 0.3 for PVP/AgNPs, and ± 0.3 for PVA and ± 0.6 for PVA/AgNPs), indicating that, despite the difference in the concentration of the polymers, the gel fraction differences were not significant between the sets of hydrogels based on PVP and PVA with and without silver nanoparticles present in the polymeric systems.

3.2. Swelling capacity and swelling kinetics in reverse osmose water

Our swelling capacity results of PVP hydrogel were, however, quite different from those found in the literature Jovanovic et al.,2011; Yu et al., 2007), where PVP hydrogels studied with AgNPs have shown results with smaller swelling capacity than PVP without AgNPs. In our study, the results can be justified by the small concentration of silver in the hydrogels (40 ppm for the PVP-based hydrogel and 60 ppm for the PVA-based hydrogel), suggesting that the AgNPs were not sufficient to interfere on the crosslinking of the PVP-based hydrogel, differently in PVA-based hydrogel.

In relation to the swelling kinetics of both hydrogels, the results are

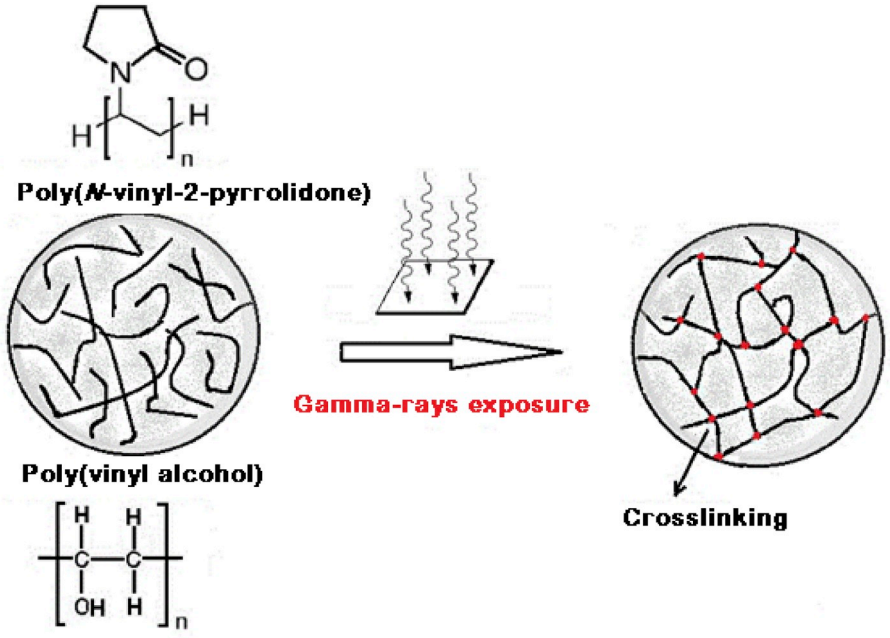

Fig. 1. -Overview of the crosslinking process that generate the network with PVP and PVA polymers.

quite different as can be seen in Fig. 3.

The equilibrium swelling occurs through osmotic stability achieved between the force that favors the absorption of the solvent and the opposing force which balances the elastic stretching of the polymer network and prevents its deformation (Ganji and Vasheghani-farahani, 2010). The profiles of the swelling kinetics curves of the hydrogels (Fig. 3) indicate that PVA hydrogel blend offers a higher degree of swelling than that of PVP hydrogel blend, although the concentration difference of both main polymers is small, i.e. 10% PVP and 11% PVA. For PVA hydrogel, after 24 h of immersion in water, the degree of swelling was ca. 230% and reached a maximum swelling capacity after 48 h with ca. 240%, reducing thereafter to ca. 200% after 144 h. In turn, for PVP hydrogel it was observed ca. 98% degree of swelling after 24 h, with a small increase after 144 h, reaching a level of swelling of 120%. In relation to the standard deviations for the results obtained from quintuplicate of the PVP/AgNPs hydrogel samples, they were insignificant, not showing any prominence after plotted in the graph.

3.3. Scanning electron microscopy - energy dispersive X-Ray spectroscopy (SEM-EDS)

Considering that the two hydrogels presented similar results regarding the gel fraction (Fig. 2), but quite different porosities based on the SEM images presented in Fig. 4 — that of the PVP hydrogel membrane is much larger than that observed for the PVA hydrogel membrane —, the results suggest a great influence of kappa-carrageenan (KC) on swelling of the PVA hydrogel. The effect of KC on the properties of hydrogel from poly (vinyl alcohol) and KC blend, prepared by application of gamma-radiation, was studied by Dafader et al. (2015). They suggest that the increase in swelling of the hydrogel is a result of KC decomposition by the action of radiation. Rupture of the KC chain in smallermolecules increases the number of hydrophilic groups of KC($-OSO_3^-$) in the PVA hydrogel networks; thus, these new hydrophilic ionized groups in PVA hydrogel networks create an electrostatic repulsion that increases the absorption of the water in the swelling medium.

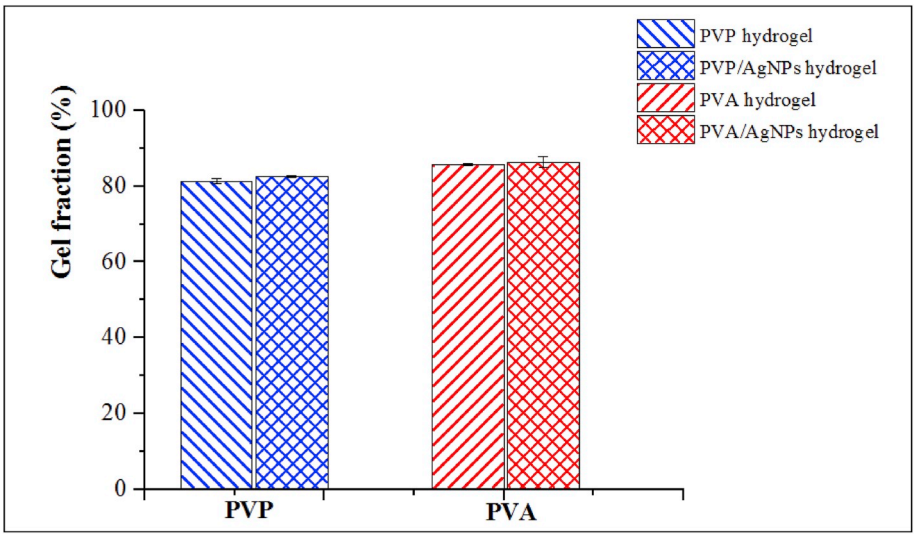

Fig. 2. Average results of gel fractions and their standard deviations obtained experimentally from hydrogels synthesized with PVP and PVA, both containing colloidal silver.

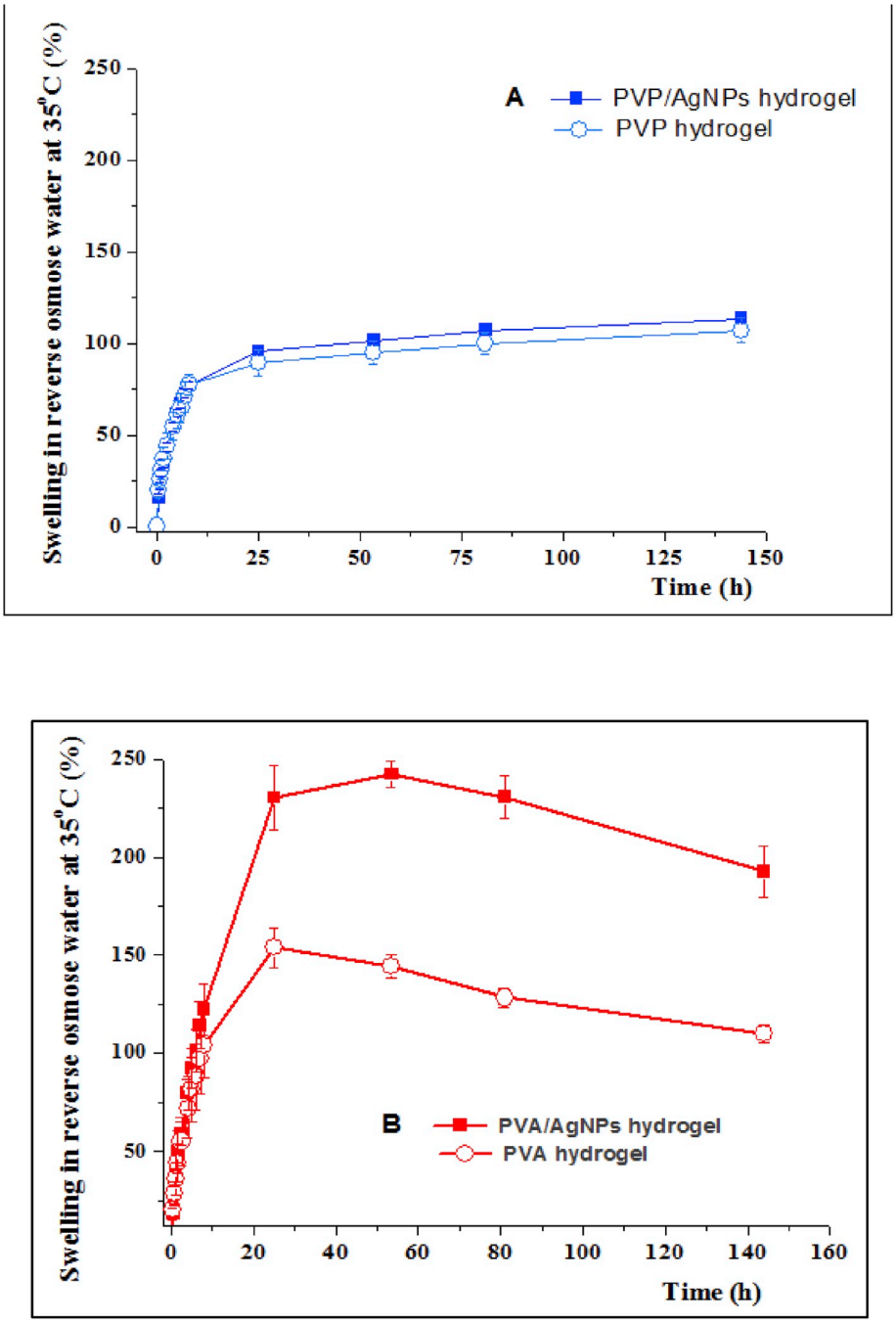

Fig. 3. Kinetics of swelling of PVP and PVA hydrogels synthesized with monocrystalline silver. (A) PVP-based hydrogel with and without AgNPs and (B) PVA-based hydrogel with and without AgNPs.

Fig. 4 presents images of scanning electron microscopy (SEM) of lyophilized hydrogels (cross-sections), where 3A and the 3B show the inner morphological structures of the PVP and PVA dressings, respectively. The PVP hydrogel (4A) shows a more porous microstructure with more cavities, which, in principle, also would favor its greater swelling with more water absorption, what didn't occur simply analyzing the SEM images. Figs. 4C and 3D show the surface images obtained by SEM coupled with EDS, where the distribution of silver on the surface of the hydrogels can be seen.

Analyzing the fracture surface of the hydrogels, it is observed in Fig. 4A, PVP hydrogel, a structure with large pores irregularly shaped, thick walls and heterogeneously distributed, while in Fig. 4B, the structure of PVA hydrogel is more closed, with few small pores and they are forming a rough surface structure, however, Fig. 4C and D suggest that the silver is uniformly distributed on the surface of the dressing, without the formation of agglomerates of metal.

To prove the reduction of silver ion (Ag^{+}) to colloidal silver (Ag°), UV-visible plasmonic bands of the two obtained hydrogels were observed and measured as shown in Fig. 5. The absorption spectra at ~400 nm indicated for the occurrence of spherical AgNPs and the absorption spectra obtained show peaks centered at 402.9 nm for the PVA hydrogel and 404.8 nm for the PVP hydrogel, suggesting that there was a reduction of Ag + to Ag°, and confirming the in situ formation of AgNPs.The wavelengths at which the two peaks were observed in the UV-visible spectra are characteristic for AgNPs with spherical shape according to the study by Mock et al. (2002).

Although there is only a small offset between the two peaks of the plasmonic bands, one observes very similar profiles of the two curves

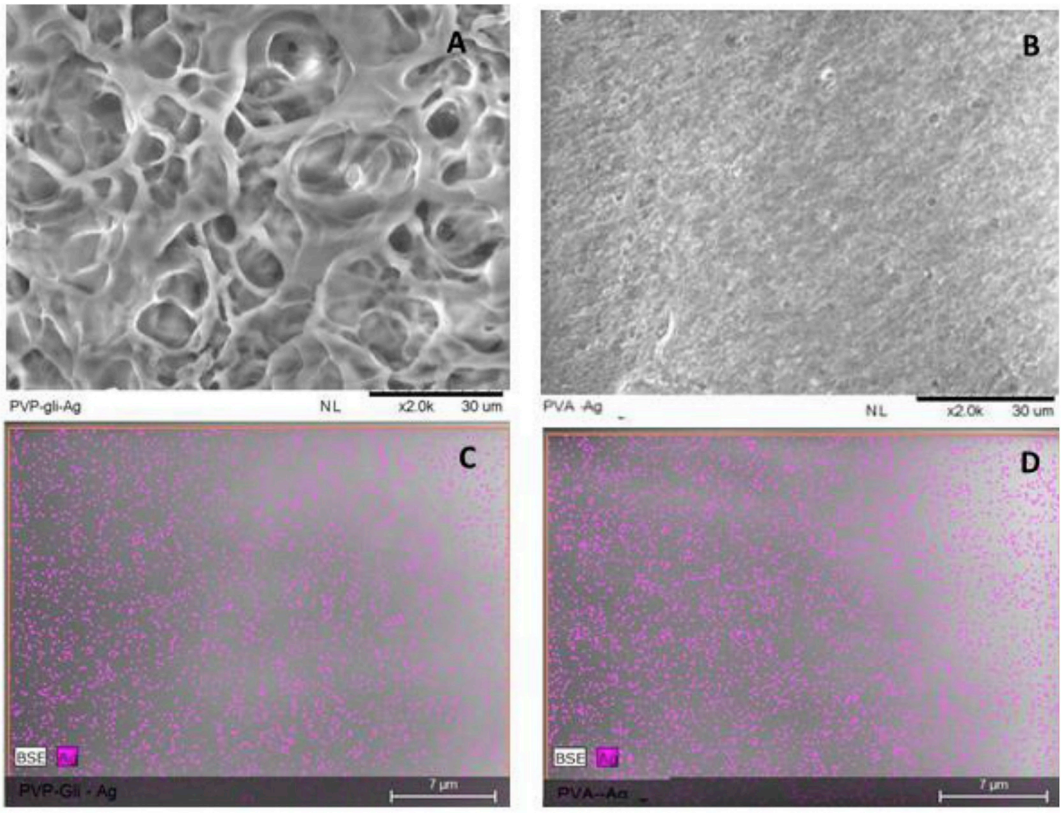

Fig. 4. SEM morphology of the lyophilized hydrogel fracture: (A) PVP/AgNPs and (B) PVA/AgNPs; Images of SEM coupled with EDS, showing the distribution of silver on the surface: (C) PVP/AgNPs and (D) PVA/AgNPs.

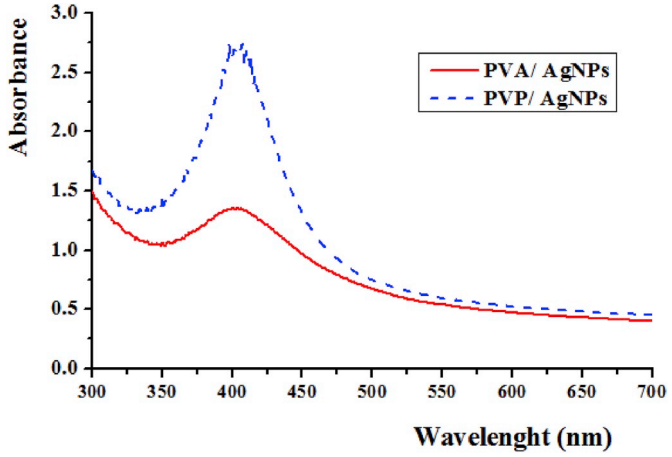

Fig. 5. Plasmon resonance spectra obtained from the synthesized hydrogels: PVA/AgNP and PVP/AgNPs.

indicating a very similar distribution of the sizes of the nanoparticles formed in the two hydrogels.

Fig. 5 exhibits the plasmonic band obtained in the UV-visible analysis and indicates that there was a reduction of Ag + to Ag° as a result of irradiation process at 25 kGy, confirming the in situ formation of AgNPs.

It is known that the surface plasmon resonance peak present in silver nanoparticles is about 400 nm for spherical shape; when the nanoparticles agglomerate, and consequently increase in size, or they precipitate, a wavelength increase for the surface plasmon resonance peak is observed (Grassian, V.H., 2010; Stebounova, L. et al., 2011; MacCuspie, R.I., 2011). The spectra show peaks centered at 402.9 for PVA hydrogeland 404.8 nmfor nanoparticles from PVP hydrogel, characteristic of AgNPs with spherical shape, however with different levels of absorbance.

The extinction peak wavelength for silver nanoparticles suggest, according to the studies of Agnihotri et al. (2014), an average size of between 15 and 20 nm to both hydrogels. In addition, the full width at the half maximum of the corresponding peaks determines dispersity of the nanoparticles, where a large part is attributed to peak broadening, hence to polydispersity (Agnihotri et al., 2014; Yu et al., 2007). The results shown in Fig. 5 hint that the synthesized nanoparticles in situ in the PVA hydrogel present greater dispersity than those synthesized in situ in the PVP hydrogel.

It is also possible to observe much higher absorbance for silver nanoparticles synthetized in PVP hydrogel, although the silver concentration in the PVA hydrogel is 50% higher. The smaller absorbance on surface plasmon resonance peak for PVA hydrogels suggests a use of smaller concentration of AgNPs in this hydrogel; however, the silver concentration in this hydrogel (60 ppm) was higher than that of the hydrogel (40 ppm). On the other hand, the area under the absorbance curve frequently increases with the agglomeration increase, which was not observed for the PVA/AgNPs hydrogel in the experiment (Zook et al., 2011). Considering these facts, the results suggest that not all silver ions added suffered reduction, forming the silver nanoparticles; and if that happened, they would not be stable, and the Ag° quickly would vote for your initial state (Ag $^{+}$).

3.4. Antimicrobial activity

In the antimicrobial susceptibility test by disk diffusion,no halo formationat any of the tested hydrogels against *S. aureus* and *P. aeruginosa*, unlike that observed onthe positive control performed with ciprofloxacin for comparison.The tests were repeated, and the results confirmed, but different from the results obtained by Boonkaew et al. (2014), who studied dressings based on hydrogel for burn wound,

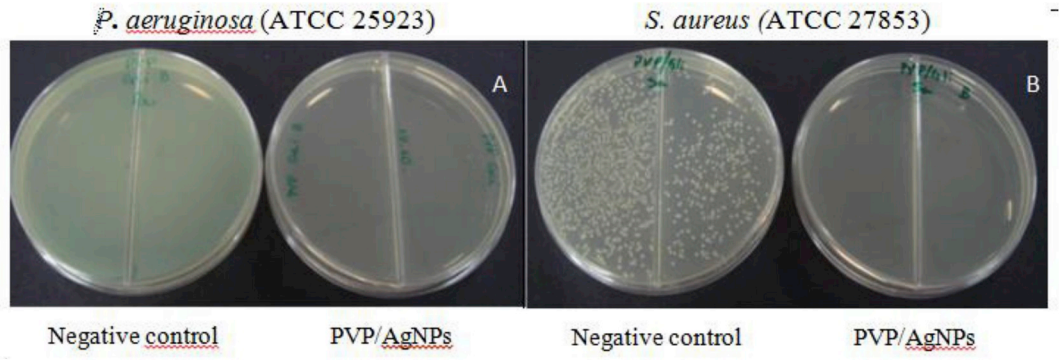

Fig. 6. Results of sample tested and negative control obtained in the evaluation test for microbial activity of hydrogel PVP/glycerol/agar against (A) *P. aeruginosa e (B) S. aureus.*

composed of 2-acrylamido-2-methylpropane sulfonic acid sodium salt with silver nanoparticles. In terms of antimicrobial efficacy, they were compared with two samples commercially available for silver dressings: Acticoat TM and PolyMem Silver®.

3.4.1. Broth dilution

The results obtained by broth dilution method in tests of microbial activity for the synthesized PVP/AgNPs hydrogel indicated that the samples tested showed antimicrobial activity against *P. aeruginosa* and *S. aureus*, as show Fig. 6A and B.

The same results were obtained with the multiresistant strain *S. aureus*, although such bacteria present resistance to penicillins, cephalosporins, carbapenems, sulfonamides, tetracyclines, fluoroquinolones and aminoglycosides. However, they did not show to be resistant to silver nanoparticles synthesized in situ by ionizing radiation.

The results from PVA hydrogel shown in Fig. 7A indicated that this hydrogel with AgNPs showed antibacterial activity against *P.aeruginosa*. However, for activity against *S. aureus*, the results were very different from those obtained from the PVP hydrogels tested, showing an atypical response.

As can be seen in Fig. 7B, no colony of *S. aureus* growth at 24 h of incubation on the PVA hydrogel with AgNPs tested, neither on PVA hydrogel without AgNPs used as negative control. However, on the plates kept in an incubator for 48 h one observed growth of colonies on the two plates, suggesting that the PVA/KC/agar hydrogel exhibited bacteriostatic activity against *S. aureus*. The tests were repeated, and the results were confirmed.

The results of antimicrobial activity of PVA-based hydrogel, in relation to PVP-based hydrogel, pointed out, as we reported previously, thatall silver added was not reduced to form the silver nanoparticles. If that had happened, they would not be stable, and Ag° quickly would return to ionic silver, that is, to its initial state (Ag^{+}) since the ionic silver presents less antibacterial potential than that of Ag°.

So, failure in halo formation along the tests suggests that the PVP-

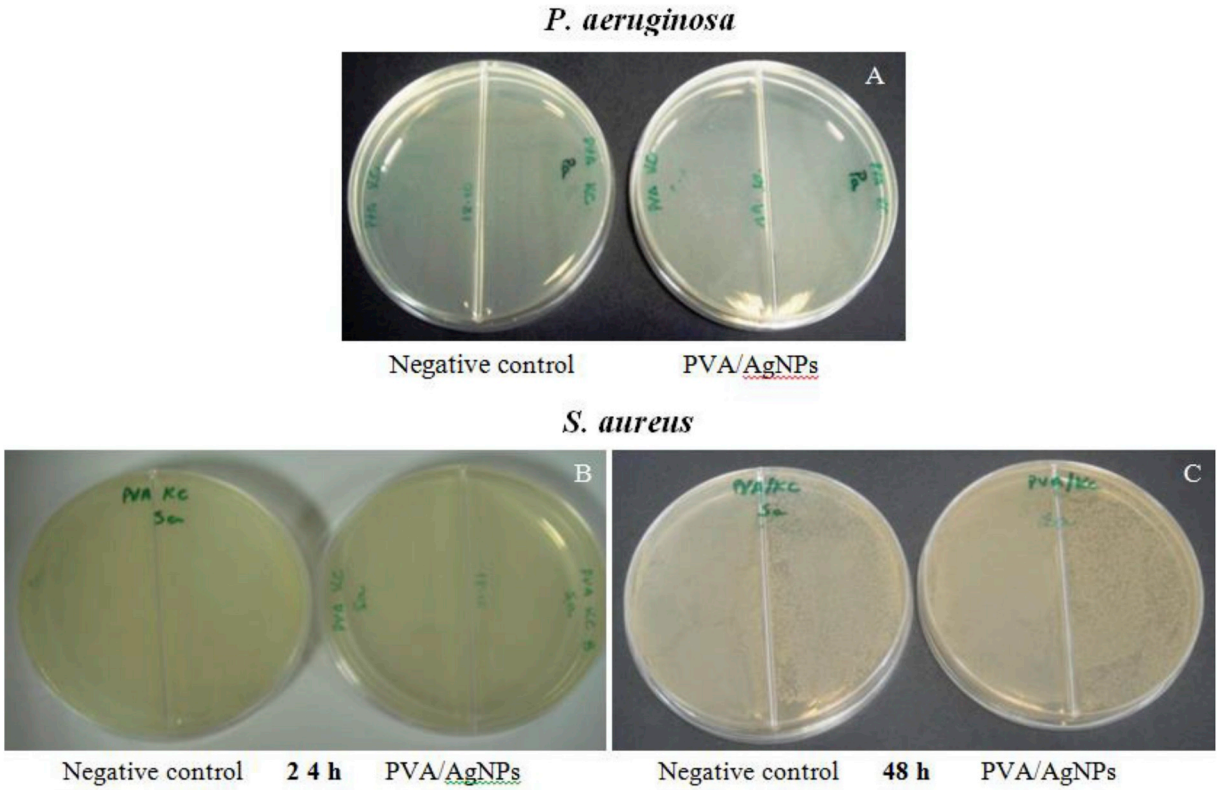

Fig. 7. Results of sample tested and negative control obtained in the evaluation of the microbial activities for PVA-based hydrogel against *S. aureus* for incubation time of 24 h (A and B) and 48 h (C).

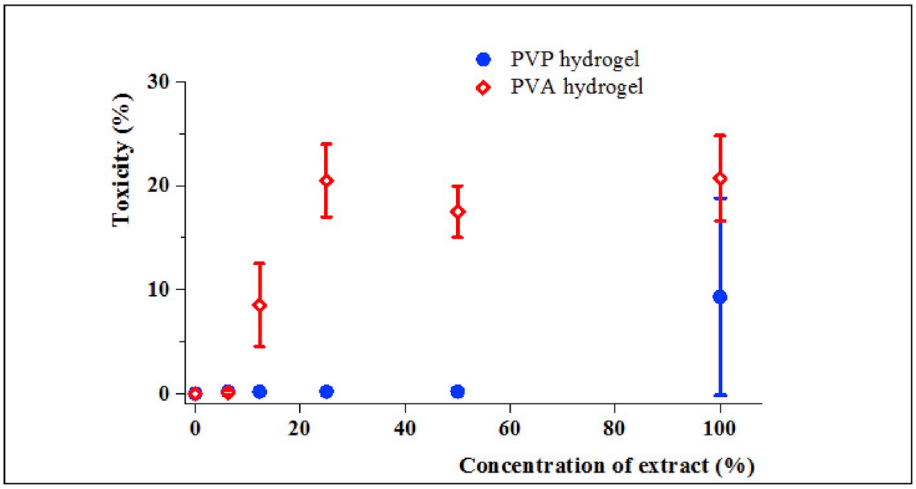

Fig. 8. Cytotoxicity results obtained by the method of capture of neutral red to extract solutions of PVP-based and PVA-based hydrogels with AgNPs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

based and PVA-based hydrogels did not release AgNPs in sufficient quantities, so that they could spread and kill the pathogens present in the agar around the samples. Reiterating, such an event demands the necessary amount of the antimicrobial agent to be released (Yang et al., 2018). Nevertheless, both hydrogels showed antimicrobial efficacy by contact.

3.4.2. *In vitro cytotoxicity*

The cytotoxicity is an important consideration for the hydrogel in view of the proposal is to be used as a dressing for infected wounds and burns infected with gram-positive and gram-negative bacteria.The toxicity of the hydrogels with silver nanoparticles would be caused by the release of hydrogels components, including the AgNPs whose toxicity depends on dose, time, size, shape, surface chemistry, and cell type (Zhang et al., 2014); therefore, we chose human fibroblast cells since those are the most common connective tissue cells and are also involved in the production of growth factors, which control cell growth and differentiation.

The AgNPs antimicrobial capacity is attributed to the strong oxidative activity of nanoparticle surfaces and the release of silver ions into biological environments (He W. et al., 2012). These factors are believed to be responsible for triggering a number of negative effects on cell structures and functions, inducing cytotoxicity (Chernousova and Epple, 2013).

Fig. 8 presents results of toxicity tests to PVP-based and PVA-based hydrogels with AgNPs. In tests, the extract with the released hydrogels components were diluted to 6.25, 12.5, 25, and 50%, and it was tested pure, too.

The results indicated that the PVP-based hydrogel with AgNPs exhibited low toxicity to cell fibroblasts. The PVP-based hydrogel with 40 ppm of AgNPs showed to be even less toxic than PVA-based hydrogel to all dilutions of the extract, suggesting, therefore, that the PVA-based and PVP-based hydrogels with AgNPs are biocompatible.

The results also pointed out that the PVP-based and PVA-based hydrogels with AgNPs exhibited low toxicity to human fibroblast cells. The PVP-based hydrogel with an amount of 40 ppm of AgNPs showed low toxicity, approx. 10%, that is, 90% of studied cells, kept in contact the pure extract, didn't die.

For the PVA-based hydrogel with AgNPs, the results presented indicated that there was cellular rupture in cells that came in contact with the extract from 12.5% concentration. Cellular death was observed of up to just over 20% of the tested fibroblasts, results that were also considered low so that the hydrogel could be considered cytotoxic. Such results suggest, therefore, that the PVA-based and PVP-based hydrogels with AgNPs showed to be biocompatible, and they were considered nontoxic.

4. Conclusions

The process conditions suggest that Ag + ions can be efficiently reduced by gamma-irradiation. Preparation of hydrogels from hydro soluble polymers, such as PVP and PVA, containing Ag + ions in their solutions, can be performed efficiently by using gamma-irradiation as a profitable tool, showing advantages with simultaneous synthesis of AgNPs and sterilization of the obtained hydrogel. PVP-based hydrogel showed antimicrobial properties against *P. aeruginosa*, normal *S.aureus*, and *S. aureus* strain multiresistant to penicillins, cephalosporins, carbapenems, sulfonamides, tetracyclines, quinolones and aminoglycosides. In turn, PVA-based hydrogel showed antimicrobial properties against *P. aeruginosa* and bacteriostatic activity to *S. aureus*. The results hint that both dressing hydrogels have potential for use in wounds and burns infected with gram-positive and gram-negative bacteria; moreover, PVP hydrogel showed to be very effective against multidrug-resistant *S. aureus*.

In summary, the total results obtained from antimicrobial tests point out that both hydrogel dressings tested herein exhibited antimicrobial action by contact. This indicates a more security for the use of such hydrogels, however, according to Santos et al. (2014) and Wei et al. (2015), the use of AgNPs in therapy of human body, their manipulation, toxicity, security, accumulation and disposal still need many in-depth multidisciplinary studies in order their efficacy to be better evaluated and understood.

Acknowledgements

The authors thank the CTR-IPEN, especially the engineers Elizabeth Somessari and Carlos Gaia da Silveira, for irradiation of the samples; the group of functional nanomaterials of CQMA-IPEN, especially PhD Jorge Vaz, for the spectrophotometric analyses (FAPESP project 02/01131-7)the CCTM-IPEN for the MEV analyses; CAPES for financial support, and Washington Luiz Oliani for helpful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radphyschem.2019.108369.

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