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Hybrid hydrogels produced by ionizing radiation technique

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ABSTRACT

The interest in biocompatible hydrogels with particular properties has increased considerably in recent years due to their versatile applications in biomedicine, biotechnology, pharmacy, agriculture and controlled release of drugs. The use of hydrogels matrices for particular drug-release applications has been investigated with the synthesis of modified polymeric hydrogel of PVAl and 0.5, 1.0, 1.5% nano-clay. 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 thermogravimetry analysis (TGA), infrared spectroscopy (FTIR) and swelling in solutions of different pH. The membranes have no toxicity. The nano-clay influences directly the equilibrium swelling.

1. Introduction

Interest in the preparation of biocompatible hydrogels with various properties has increased considerably in recent years due to their versatile applications in biomedicine, biotechnology, pharmacy and agriculture. Hydrogels are three-dimensional polymeric networks that can also be produced using ionizing radiation. If a polymer is subjected to ionizing radiation, reactive intermediates are formed in the macromolecules. This results from the direct effect of radiation on polymeric macromolecular chains and from indirect effects as reactions of the intermediates generated in water with macromolecules (Rosiak and Fumio, 1999). An example, is the poly(vinyl alcohol) (PVAl), a polymer of great interest due to its specific characteristics for biomedical applications. PVAl is a water-soluble polymer used in practical applications due to its excellent chemical resistance, processing properties, biodegradability and physical properties (Peppas, 1986; Pessan, 2007).

Interest in the polymeric hydrogels started in the 1950 (Guenet, 1992). Both synthetic and biologically derived polymers are used for preparation of polymeric hydrogels. Morphological changes can lead to different performance of the hydrogel and the characteristics of swelling degree and diffusion behavior may change under physiological stimuli (Hassan and Peppas, 2000) representing an important feature for controlling release of bioactive species. Biocompatible polymeric hydrogels are extensively used for biomedical/pharmaceutical applications such as controlled drug release, tissue engineering, and regenerative medicine (Jagur-Grodzinski, 2010).

Membrane development with presence of inorganic nanoparticules performs significant change in the overall morphology of the hydrogel. The adsorption of PVAl onto clay surface creates hydrogen bonding between the hydroxyl polymeric groups and oxygen atoms from silicates (Shubhangi et al., 2007).

PVAl nanocomposites obtained by ionizing radiation are not extensively reported in the literature. Therefore, the purpose of this study was to use gamma radiation to crosslink hybrid membranes in which synthesis and sterilization occurs simultaneously. The characterization of these materials includes cito-toxicity test to verify the biocompatibility.

2. Experimental

2.1. Materials and Methods

Poly(vinyl alcohol) (PVAl) (Mw=85000, degree of hydrolysis 98.4%) Celvol™ 325 provided by Dermet Agekem. PEG-300 provided by Oxiteno and clay laponite RD coding S/11176/10 provided by Buntech.

The formulations of PVAl (10% w/v) activated with PEG-300 (1%) were prepared in aqueous solution using a hot plate with magnetic stirring at temperature between 80 and 85 °C for 40 min. The clay was added to the PVAl solution, in different polymer ratios (w/w), under agitation at temperature of 85 °C for 5 min. The solution was placed in Petri dishes, deoxygenated

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under N_2 atmosphere, and irradiated at a ^{60}Co gamma source of 1.57 kGy h^{-1}dose rate, at 25 kGy dose.

2.1.1. Swelling

The dried and preweighed membranes were kept in water in a constant temperature bath of 60 °C for 24 h and reweighed after wiping the surface gently. Since membrane thickness was very small, the period of 12 h for achieve the equilibrium time was found to be sufficient. The swelling percentual (S%) was calculated according to the Eq. (A).

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

where: ms is the mass of swollen polymer and md is the mass of the dried sample prior to the swelling. The swelling was evaluated in solutions of different pH: saline solution of NaCl 0.1 mg/ml (pH=5.5), water (pH=6.5) and acetic acid (pH=3) that corresponds to different conditions of drug release applications.

2.1.2. Thermogravimetry (TG)

TGA of monohybrid PVAl membranes was carried out using Mettler-Toledo TGA/SDTA 851 thermobalance, in inert atmosphere of N_2 from 25 to 600 °C at heating rate of 10 °C min^{-1}. The samples were lyophilized and about 10 mg were kept in alumina pans to perform the TGA test.

2.1.3. Cytotoxicity test

The sample extract obtained by immersion in cell culture medium (minimum Eagle's medium, Sigma Co., São Paulo, Brazil) during 24 h at 37 °C and serially diluted was exposed to NCTC L929 cell line, from mouse connective tissue purchased from American Type Culture Collection (ATCC) tissue bank, cultured on 96 wells microplate. The cytotoxic effect was evaluated by the cellular viability through the viable cells capacity of uptake neutral red and intracellular incorporation, measured in spectrophotometer with 540 nm filter in the end of the assay.

Negative and positive controls are used to determine the reference limits of the results. Negative control (no toxic compound) used was HDPE (high density polyethylene) and positive control was natural rubber latex film, which causes toxic effect to the cells.

The cell viability percentage is plotted against extract concentration and the final graphic corresponds to the viability curves of each sample and controls. Sample with viability curve above 50% cell viability line is considered no cytotoxic, as the negative control result. If viability curve is under or cross the 50% viability line is considered cytotoxic, as the positive control. In the intersection is obtained the cytotoxicity index ($IC_{50\%}$), which is the extract concentration that injures or kills 50% of cell population in the assay.

2.1.4. Fourier transform infrared analysis

FTIR analysis was done using a Thermo Nicolet FTIR-6700 Smart Diamond ATR from 4000 to 400 cm^{-1}. Pieces at about 1 mm diameter were kept in contact with the accessory window to obtain the spectrum.

3. Results and discussion

3.1. Swelling of the samples

Samples of hydrogels PVAl dried obtained without clay and hydrogels with clay content 0.5, 1.0 and 1.5% were swollen in three different solutions: water, acetic acid pH=3 and saline solution. They reached an equilibrium result up to 12 h at temperature of 20±1 °C.

According to the literature (Shubhangi et al., 2007) reticulated structures containing clay are of random nature so it is observed that swelling is a complex phenomenon involving interactions between the polymer, clay and solvent. Laponite can act as a crosslinker or in osmotic effect in face of ionic character of its composition.

Sirousazar et al. (2011) suggested that the layers of clay inside the nanocomposite hydrogel network act as physical barrier against molecules transport due to creating more tortuous paths which offers other controlling steps against mass transfer. They noted that the nanocomposite hydrogel needs longer time to reach a specified level of swelling and therefore, shows slower swelling kinetics compared to clay-free hydrogel. Swelling is associated with hydrogen bonds and laponite clay has large number of hydroxyl groups.

Fig. 1 shows that rate of water absorption is significantly higher at the immersion times less than 5 h, whereas after it, the rate of absorption only slightly increases.

Swelling is similar for PVAl hydrogels and hydrogels with 0.5% of clay. The hydrogel with 1.0 and 1.5% clay showed a significant increased swelling in saline solution, followed by water and acetic acid. The solution pH does not influence the swelling of samples at different clay concentrations but the saline concentration has an influence.

The increase in the swelling percentage observed at low concentration of clay nanoparticles can be attributed to increase of the osmotic forces in the laponite-hydrogel. The increased osmotic pressure in the polymer network promotes the increase of water flux resulting in higher swelling level, similar results shown in Fig. 1C and D are reported by Chang et al. (2010). The counter ions associated with the Laponite nanoparticles exert additional osmotic pressure in the bulk of the polymeric network. This increased osmotic pressure promotes increase of swelling by flux of water.

3.2. Thermogravimetry (TG/DTG)

Fig. 2A and B show three steps of decomposition, the first step occurs between 25 and 150 °C and is associated to the dehydration from the PVAl nanocomposite (Shubhangi et al., 2007), followed by release of water from the spheres of hydration of exchangeable sodium cations of the clay (Palkova, 2009). The second decomposition step is between 200 and 380 °C, and it is related to the PVAl dehydroxylation (Thomas et al., 2001), indicating the start of the decomposition of polymeric chains. The third step which starts at 380 °C is associated to the polymer decomposition and also to the water loss through the dehydroxylation of structural layers of clay; the loss of structural water is facilitated by the presence of polymer. The PVAl that penetrated is intercalating or exfoliating the clay according to Shubhangi et al. (2007).

PVAl has a strong tendency to form hydrogen bonding within itself as well as with other species containing highly electronegative groups. Laponite has electronegative oxygen and hydroxyl groups, which can assist the adsorption of PVAl onto laponite surface. The adsorption of PVAl onto surface of laponite is presumed to occur through hydrogen bonding. Apart from hydrogen bonding, van der Waals forces between polymer segments and clay surface would also play an important role in the overall adsorption process. This explains the change of temperature of maximum decomposition, in Fig. 2B of second step while the third step of macromolecular chains decomposition is postponed, Fig. 2A.

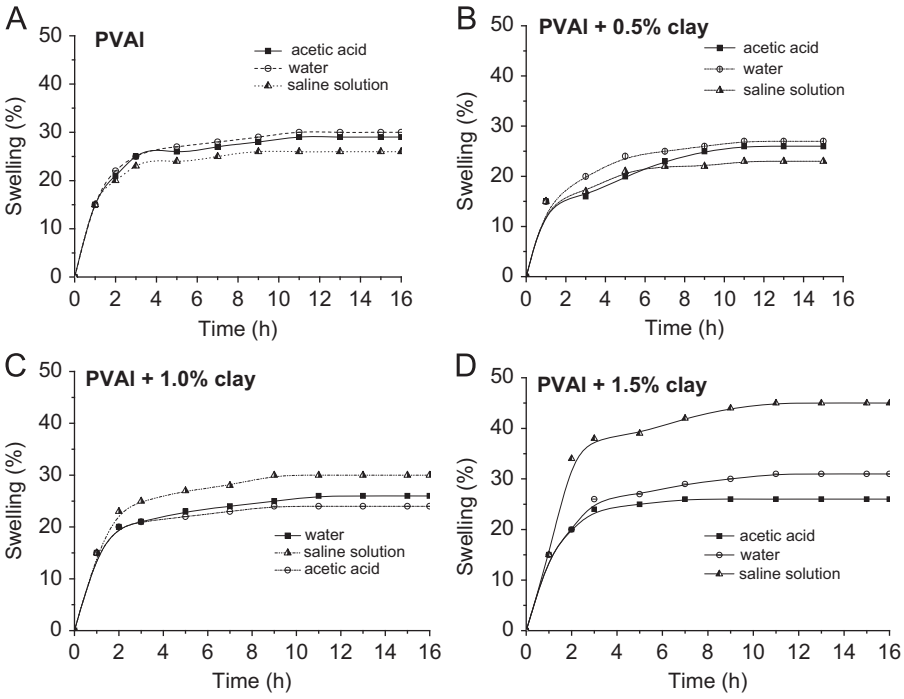

Fig. 1. Swelling behavior of hydrogels PVAl and clay laponite RD in acetic acid, water and saline solution: (A) PVAl without clay; (B) PVAl with 0.5% clay; (C) PVAl with 1.0% clay and (D) PVAl with 1.5% clay obtained by gamma irradiation at 25 kGy.

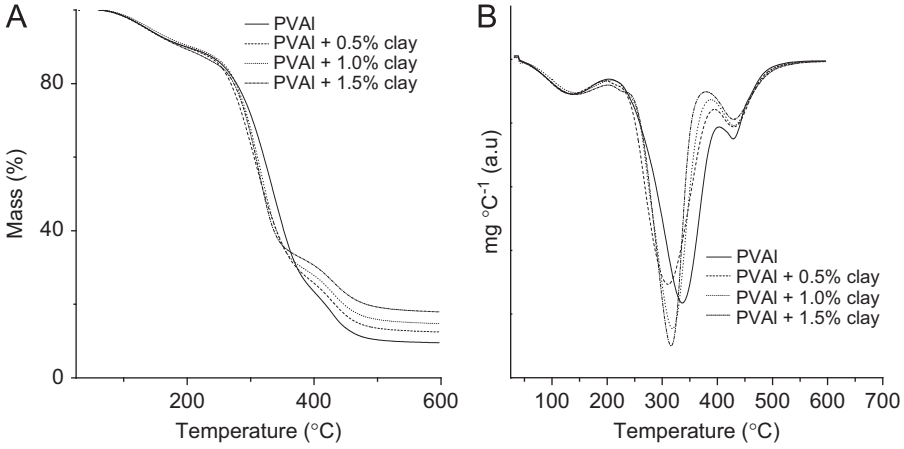

Fig. 2. Thermogravimetry results of dried hydrogels of PVAl and clay laponite RD obtained in N_2 atmosphere at 10 °C min^{-1}: (A) TGA and (B) DTG.

The data show that the ash content for PVAl membrane without clay is at about 9.54%. In presence of clay the ash content increases, for PVAl with 1.5% clay content it reaches 18.30%. This increasing residue is associated to possible intercalation of polymer and clay or a folding of the polymer chains around the particles of clay.

3.3. Infrared spectroscopy

In Fig. 3 the IR spectra of all samples are shown together with the pristine PVAl sample used as reference to the identification of the PVAl nanocomposites peaks in the interval of 1500–500 cm^{-1}. PVAl spectrum shows the out of phase C–C–O stretching band at 1072 cm^{-1} of alcohol group. Characteristic band at 958 cm^{-1} in the clay spectrum corresponds to Si–O stretching vibration (Shubhangi et al., 2007; Pessan, 2007). In the dried samples, at low clay concentration in the region of 1050–970 cm^{-1} the identification of the absorption bands is difficult due to the interaction between the polymer PVAl and the clay through Si–OH groups. However the peak at 958 cm^{-1} is attributed to Si–O stretching, and is observed as a small peak shifted at about 25 cm^{-1} (from the clay group at 958 cm^{-1}). This shift is probably due to the interaction between Si–O and hydrogen bond of PVAl.

3.4. Cytotoxicity

In vitro investigations can be used for screening biomaterials for estimations of toxic effect. Cytotoxicity in vitro assay is the first test to evaluate the biocompatibility of any material for use in biomedical devices (Rogero et al., 2003). Similar to negative control the hybrid materials of PVAl- Laponite showed no cytotoxic effect as shown in Fig.4.

4. Conclusions

Presence of clay in the hybrid hydrogel retards the decomposition of the macromolecular chains of the PVAl. The increase in

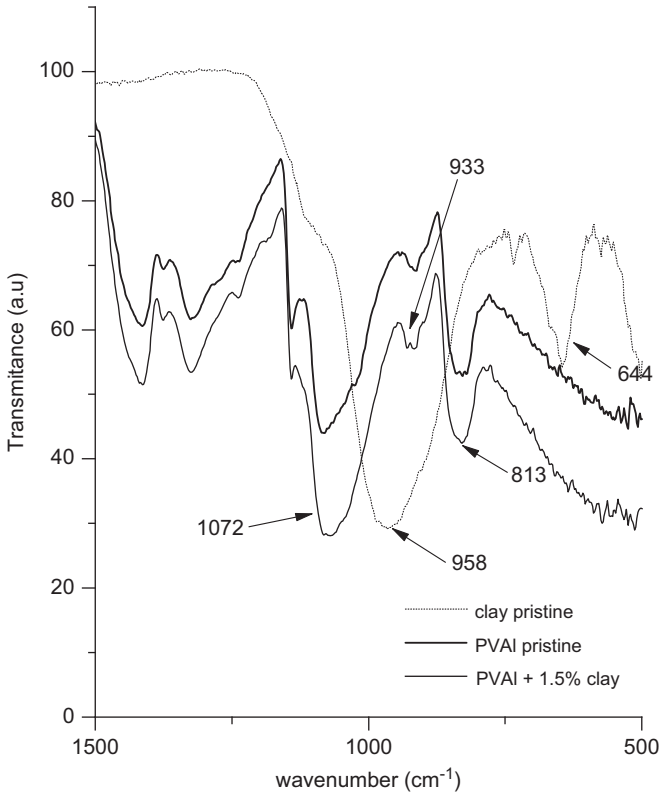

Fig. 3. Infrared spectra of PVAl, laponite RD clay and from dried film of PVAl + 1.5% clay obtained by gamma irradiation at 25 kGy.

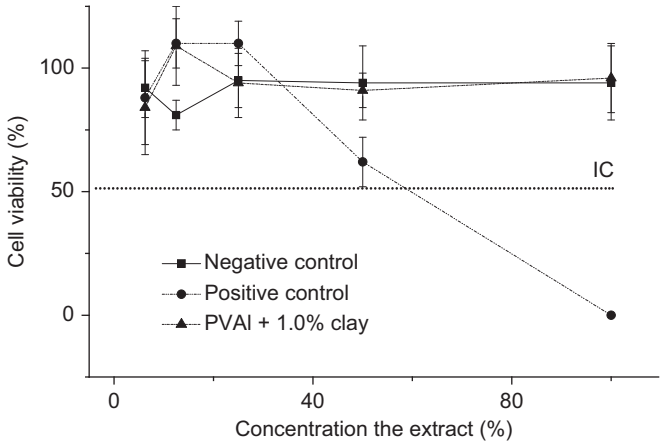

Fig. 4. Curves of cytotoxicity for hydrogel of PVAl and clay.

ash content is associated to intercalation of polymer and clay or a folding of the polymer chains around the particles of clay.

This polymer-clay interaction is observed as Si–O stretching at small peak shifted by about 25 cm^{-1} and is considered to be associated with the interaction between SiO- and hydrogen bond of PVAl. The presence of clay changes the swelling behavior of the PVAl hydrogel resulting in a considerable increase in swelling in saline solution. A percentage of 1.0–1.5% clay increased osmotic pressure; the counter ions associated with the clay exerts additional osmotic pressure within the polymer network, and promotes the increase of water flux resulting in higher swelling level. The samples prepared showed no cytotoxic effect.

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References

Guenet, J.M., 1992. Thermorevesible gelation of polymers and biopolymers. Acad. Press, London 287-289.
Hassan, C.M., Peppas, N.A., 2000. Cellular PVA hydrogels produce by freeze/thawing. J. Appl. Polym. Sci. 76, 2075–2079.
Jagur-Grodzinski, J., 2010. Polymeric gels and hydrogels for biomedical and pharmaceutical applications. Rev. Polym. Adv. Technol. 21, 27–47.
Palkova, H., 2009. Laponite-derived porous clay heterostructures: I. synthesis and physicochemical characterization. Micropor. Mesopor. Mater. 127, 228–236.
Peppas, N.A., 1986. Hydrogels in medicine and pharmacy. In: Press, C.R.C. (Ed.), , pp. 1.
Pessan, L.A., 2007. Poly(vinyl alcohol)/clay-based nanocomposite hydrogels: swelling behavior and characterization. Macromol. Mater. Eng. 292, 620–626.
Rosiak, J.M., Fumio, Y., 1999. Swelling agents and devices in oral drug delivery. Nucl. Instr. Meth. B 151, 56–64.
Rogero, S.O., et al., 2003. Bicompatibility study of polymeric biomaterials. Artif. Organs 27, 397–499.
Shubhangi, H.N., et al., 2007. Swelling and mechanical behavior of modified poly(vinyl alcohol)/ laponite nanocomposite membranes. J. Appl. Polym. Sci. 103, 2896–2903.
Sirousazar, M., et al., 2011. Swelling Behavior and structural characteristics of polyvinyl alcohol/montmorillonite nanocomposite hydrogels. J. Appl. Polym. Sci. 123, 50–58.
Thomas, P.S., et al., 2001. FTIR study of the thermal degradation of poly(vinyl alcohol). J. Therm. Anal. Calorim. 64, 501–508.