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Synthesis and Characterization of Hydrogels based on Poly(vinyl alcohol) and Modified Starch as Carriers for Controlled Drug Delivery

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RESUMEN

Existe un progreso considerable con hidrogeles para aplicaciones de liberación de fármacos en años recientes, pero todavía existen retos substanciales. La síntesis y caracterización de un hidrogel fue formado utilizando PVA y almidón o almidón oxidado (con 5% cloro activo) con el método de freezing-thawing. Los hidrogeles de almidón nativo u oxidado/PVA fueron evaluados químicamente con espectroscopia infrarroja con transformada de Fourier (FTIR), y físicamente vía ensayos de absorción de agua y erosión, conjunto con análisis termo gravimétricos. La caracterización morfológica se realizó con microscopía electrónica de barrido (SEM), la cual reveló que los hidrogeles que contenían almidón nativo tenían una estructura organizada; por el otro lado, los ensayos de erosión revelaron que las formulaciones de hidrogeles que contenían almidón oxidado tenían una menor degradación. Los hidrogeles fueron evaluados como transporte para liberación de ibuprofeno y resultaron efectivos para liberación con un modo sostenible, con una cinética de liberación dependiente de cada formulación de hidrogel. La combinación de hidrogel propuesta representa un material adecuado para potenciales aplicaciones biomédicas, particularmente para liberación controlada de fármacos, y por lo tanto puede ser posteriormente probada para cyto- y biocompatibilidad.

Palabras clave: Liberación controlada de fármacos; almidón modificado; PVA; ibuprofeno.

ABSTRACT

There has been considerable progress in recent years in hydrogels for drug delivery applications but substantial challenges remain. Synthesis and characterization of a hydrogel were formed using PVA and starch or oxidized starch (with 5% active chlorine) with a freezing-thawing method. The native and oxidized starch/PVA hydrogels were evaluated chemically through Fourier transformed infrared spectroscopy (FTIR), and physically via water absorption and erosion assays, along with thermogravimetric analyses. Morphological characterization was carried out with scanning electron microscopy (SEM), which revealed that native starch hydrogels had an organized structure; on the other hand, erosion assays revealed formulations containing oxidized starch represented lower retrogradation of the hydrogels. The hydrogels were evaluated as carriers for ibuprofen release and resulted effective when delivering ibuprofen in a sustained fashion, with the release kinetics dependent on hydrogel formulation. The proposed hydrogel represent suitable materials for potential biomedical applications, particularly for controlled drug delivery, and could thereby be further tested for cyto- and biocompatibility,

Key words: controlled drug delivery; modified starch; PVA; ibuprofen.

TABLA DE CONTENIDO

Synthesis and Characterization of Hydrogels Based on Poly(vinyl alcohol) and Modified Cassava Starch as Carriers for Controlled Drug Delivery

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Abstract:

There has been considerable progress in recent years in hydrogels for drug delivery applications but substantial challenges remain. Synthesis and characterization of a hydrogel were formed using PVA and starch or oxidized starch (with 5% active chlorine) with a freezing-thawing method. The native and oxidized starch/PVA hydrogels were evaluated chemically through Fourier transformed infrared spectroscopy (FTIR), and physically via water absorption and erosion assays, along with thermogravimetric analyses. Morphological characterization was carried out with scanning electron microscopy (SEM), which revealed that native starch hydrogels had an organized structure; on the other hand, erosion assays revealed formulations containing oxidized starch represented lower retrogradation of the hydrogels. The hydrogels were evaluated as carriers for ibuprofen release and resulted effective when delivering ibuprofen in a sustained fashion, with the release kinetics dependent on hydrogel formulation. The proposed hydrogel represent suitable materials for potential biomedical applications, particularly for controlled drug delivery, and could thereby be further tested for cyto- and biocompatibility,

Keywords: controlled drug delivery; modified starch; PVA; ibuprofen.

1. Introduction

Hydrogels are polymeric materials that are non-soluble in water but have a great capacity of its absorption. While absorbing water, hydrogels grow considerably by volume, while maintain their shape and elastic characteristics (Aristizábal & Sánchez, 2007). Hydrogels can be formulated from virtually any water-soluble polymer in a variety of physical forms, including, micro particles, nanoparticles, coatings and films (Hoare & Kohane, 2007). There structures are commonly used in experimental medicine for a wide range of applications including tissue engineering, regenerative medicine, cellular immobilization and as barrier materials (Hoare & Kohane, 2007; Rosillo, 2015) The unique physical properties of these materials have sparked particular interest in their use in drug delivery applications. The chemical structure of hydrogels containing groups such as: -OH, -COOH, -$CONH_2$ and –SO_2H make them highly hydrophilic (Escobar et al., 2002). As drug delivery carriers, they offer the possibility to control physiological and biochemical processes once introduced in the organism (Sáez et al., 2003). Some models propose a system of a hydrophilic/hydrophobic solute, drug and an orientation group incorporated in the bio stable polymeric matrix (Escobar et al., 2002).

Poly(vinyl alcohol) (PVA) is a biopolymer that has been widely used in biomedical applications (Zhang, Xia and Zhao, 2012). PVA hydrogels have been extensively studied and considered as one of the most suitable hydrogels, via chemical or physical crosslinking, for biomedical applications due to its biocompatibility and non-toxicity (Zhang, Xia and Zhao, 2012). In the last decades, however, the need of physically cross-linked hydrogels has been potentially increased to avoid the use of chemical reagents (crosslinkers and initiators) that can be toxic or that can affect the nature of the entrapped substances (e.g. proteins, drugs, and cells) (Kamoun et al.,

2015). The crosslinking process of PVA by the freezing-thawing method has been used since 1975. The crosslinked polymeric matrix is obtained by exposing a PVA aqueous solution to repetitive freezing-thawing cycles which induce crystallization and result in a networked hydrogel structure (Kenawy, et al., 2014). However, to obtain structures that can have important mechanical characteristics, and can be easily manipulated, the concentrations of PVA used are usually high, and, considering that a clinical grade is needed, this would make any potential product economically unfeasible. Thus, it would be advantageous to find a material that could reinforce hydrogels made with lower PVA concentrations. Modified starches represent an example of these materials.

Starch is a natural polysaccharide composed of two structural components: amylose and amylopectin (Reis et al., 2007). This biopolymer is widely used for medical and pharmaceutical applications as its native and derivative forms are biocompatible and biodegradable (Kamoun et al., 2015). Nevertheless, the biggest technological problems and applications that hinder their use are high water vapor permeability and low mechanical resistance; thus, to improve their properties, starches can be chemically modified (Hu, Chen, & Gao, 2009). Starch oxidation allows the formation of carbonyl and carboxyl groups in the glucan chain by a hydroxyl group substitution, which increases the stability in water and film forming capacity (Fonseca et al., 2014). Starch has a poor hydrophilicity and it cannot form a stable hydrogel alone; thus, an effective method is adding natural and synthetic polymers to meet the advantages of each other (Kaetsu, 1996). Limited studies have been reported on synthetic PVA/starch hydrogels and their derivatives, and these two materials could potentially be combined in order to decrease the amount of PVA, and, at the same time, improve starch's properties. Therefore, the present study proposes the use of a freezing-thawing method to synthesize a stable hydrogel, based on PVA and oxidized cassava

starch, which can be used as a potential carrier for Ibuprofen controlled release, in order to obtain a medium with high response, minimum side effects and a prolonged efficiency in the organism.

2. Materials and methods

2.1. Chemical and biological materials

Cassava was obtained from local stores (Ecuador) for starch extraction. Sodium hypochlorite solution containing 5% active chlorine was used for starch oxidation. Sodium Hydroxide (S318-1000, Fisher Scientific), Hydrochloric acid (A466-500, Fisher Scientific), Hydroxylamine hydrochloride (ACS Reagent Grade, 5470-11, ACROS Organics), Poly vinyl alcohol (Mowiol 28-99, ALDRICH), Sodium chloride (99,5% AR/ACS, LOBA CHEMIE PVT. LTD.), Sodium Phosphate, Dibasic, Anhydrous (3828-01, J.T.Baker), Potassium chloride (6858, Mallinckrodt), di-Potassium hydrogen phosphate, anhydrous GR for analysis (1.05104.1000, MERCK), Ibuprofen (≥98% GC, I4883-10G, SIGMA-ALDRICH) were used as received.

2.2. Starch extraction

Cassava starch was obtained according to the method described by Liu, weber, Currie and Yada (2003), with some modifications. Cassava tubers were washed, peeled and sliced. The samples were soaked in distilled water by a 1:2 proportion and blended for 5 minutes. The pulp was filtered and allowed to sediment for 12 h. The starch then was washed three times with distilled water and finally dried at $40°C$.

2.3. Starch Oxidation

Starch oxidation was performed according to the method described by Dias et al. (2011). A starch solution was prepared suspending 200 g of dry starch in 500 mL of distilled water and heated at $40°C$ under continuous stirring. The pH was adjusted to 7.0 with 0,5 M NaOH and 0,5 M HCl while sodium hypochlorite was added in an interval of 20 min, using a chlorine active concentration of 1 g/100 g. After the addition of sodium hypochlorite, the pH value was maintained at 7,0 for 60 min. The starch slurry was then vacuum filtered, washed thoroughly with distilled water and dried at $40°C$.

2.4. Carbonyl & Carboxyl content

Carbonyl and carboxyl content was determined as described by Guerra et al. (2011). For Carbonyl quantification, 4 g of dry basis (d.b.) starch were suspended in 100 mL of distilled water, and gelatinized at $80°C$, with continuous stirring in a heating plate, and then stored at $40°C$. The pH was adjusted to 3,2 with 0,1 M HCl, and 15 mL of hydroxylamine chlorine was added (the hydroxylamine solution was prepared by dissolving 25 g of reagent grade hydroxylamine chloride in water, adding 100 mL of 0,5 M NaOH in 500 mL distilled water). The samples were covered with parafilm, stored 4 h in an oven at $38°C$, and titrated to pH 3,2 with 0,1 M HCl. The carbonyl content is calculated by Eq. (1), expressed as the quantity of carbonyl groups per 100 glucose units (CO/100GU):

$$\frac{CO}{100GU} = \frac{(Vb-Vs)\times M\times 0{,}028\times 100}{W} \qquad (1)$$

Where, Vb is the volume of HCl used for the blank (mL), Vs is the volume of HCl required for the sample (mL), M is de molarity of HCl and W is the sample weight (d.b.).

To determine the carboxyl content, 5 g d.b. starch were suspended in 25 mL distilled water, stirred for 30 min and centrifuged for 10 min at 5000 rpm. The starch was washed with distilled water, resuspended in 300 mL of distilled water, and gelatinized in a heating plate with continuous stirring for 30 min. The heated samples were titrated to pH 8,2 with 0,01 M NaOH. The carboxyl content was expressed as the quantity of carboxyl groups per 100 glucose units (COOH/100GU), calculates by Eq. (2):

$$\frac{COOH}{100GU} = \frac{(Vs-Vb)\times M\times 0{,}045\times 100}{W} \qquad (2)$$

Where, Vs is the volume of NaOH required for the sample (mL), Vb is the volume of NaOH used to test the blank (mL), M is the molarity of NaOH and W is the sample weight (d.b.).

2.5. Hydrogel preparation

A cross-linked PVA and starch hydrogel was prepared using the freezing/thawing method described by Zhang, Xia and Zhao (2012), with some modifications given that the method was developed for PVA only. Starch, modified or native, was dissolved in distilled water in a boiling bath until complete gelatinization. PVA (Mowiol 28-99, ALDRICH) was then added under vigorous stirring at $\sim 95°C$; the mixture was maintained at constant homogenization for 120 min. After complete homogenization, formulations were poured in 2-inch diameter petri-dishes. The mold

was frozen at $-20°C$ for 3h, followed by thawing at room temperature for 12 h. The different formulations were prepared as presented in table 1. At this stage, the idea was to characterize different formulations to assess which of them would be suitable for subsequent ibuprofen encapsulation.

Table 1. Hydrogel formulations based on PVA and starch (native or modified)

Hydrogel Formulation	PVA concentration (% w/v)	Native Starch concentration (% w/v)	Oxidized Starch concentration (% w/v)	Ibuprofen concentration (mg/mL)
PVA10	10	0	0	10
PN10	10	10	0	10
PO10	10	0	10	10
PN15	10	5	0	10
PO15	10	0	5	10
PN5	5	5	0	10
PO5	5	0	5	10
PN51	5	10	0	10
PO51	5	0	10	10

2.6. FTIR Analysis

Analyses were conducted in a Fourier transformed infrared spectrometer; model Cary 630 FTIR (Agilent Technologies) for the native and oxidized starches, as well as for the different hydrogel formulations. Spectra were acquired in the region of 4000-500 cm^{-1}.

2.7. Thermal Analysis

Thermogravimetric Analyses were performed using a thermogravimetric analyzer TGA Q500, from 25 to 500 C with samples with 4x4x4 mm measurements.

2.8. Scanning Electron Microscopy (SEM)

Film morphological analyses were carried out in a JEOL JSM-IT300 Scanning Electron Microscope (Tokyo, Japan). Samples were placed on metallic stubs with carbon tape. Images were obtained at a potential of 5 kV and 30 Pa. Film samples of the different hydrogels were previously lyophilized.

2.9. Water-uptake, water absorption mechanism and erosion

Water absorption, water-uptake and erosion assays were carried out by the methods described by Castro et al. (2017), slightly modified. Water-uptake, also known as swelling degree, was determined by placing the fresh hydrogels in contact with 3 mL of a phosphate buffered saline (PBS) solution (the solution was prepared by adding 79,99 g NaCl, 2,005 g KCl, 14,394 g Na_2HPO_4 and 2,4008 g KH_2PO_4 to 1 L distilled water and the pH was adjusted to 7,2-7,4). Weight changes were registered at 5, 15, 30, 60 min, 2, 3, 4, 5, 24, 48, 72 h at room temperature and at $37°C$. Water-uptake was calculated according to Eq. (3).

$$WAM\backslash Water-uptake(\%) = \frac{(Wt-W1)}{W1} \times 100 \qquad (3)$$

Where $W1$ is initial weight of the hydrogel and Wt is the weight of the hydrogel after contact with PBS at t time.

Water absorption mechanism was determined with the same process as water-uptake but starting with completely dry hydrogels (xerogels). Water absorption was calculated according to Eq. (3). After the samples were hydrated, they were introduced in an oven at $40°C$ for 24 h and weight variations were recorded in order to determine erosion, percentage through Eq. (4).

$$Erosion(\%) = \frac{(W1-W3)}{W1} \times 100 \qquad (4)$$

Where $W1$ is initial weight of the hydrogel and $W3$ is the weight of dry hydrogels after erosion.

2.10. Ibuprofen encapsulation and release

Ibuprofen was incorporated into the hydrogels at a concentration specified in table 1. The same process as the *hydrogel preparation* and the ibuprofen was added before the freezing stage at $40°C$ hydrogel temperature. The Ibuprofen release was performed according to a modification of the process described by Castro et al. (2017). The hydrogels with ibuprofen were cut into 5 mm diameter circles, placed at $37°C$ in 1mL of PBS. The supernatant was collected and replaced with fresh PBS at 15, 30, 60, 120, 180, 240, 300, 360 min, 24 and 72 h. Four samples of each hydrogel formulations were tested for Ibuprofen release. The supernatant was placed, with 2mL PBS, in a cuvette, and the optical density was determined at 222 nm in a CECIL CE 2041 UV-VIS spectrophotometer. A calibration curve was built with solutions of known concentrations of ibuprofen in PBS. The amount released at each time point was then calculated, and its kinetics was expressed as the cumulative release of ibuprofen in time.

2.11. Statistical analysis

All assays were performed with three samples of each formulation, and four in the case of ibuprofen release. The results are expressed as the average ± standard deviation. An analysis of variance (ANOVA) was also applied, and multiple pairwise

comparisons were carried out using the Turkey-HSD method with a 95% confidence level ($p<0.05$).

3. Results and Discussion

3.1 Oxidized Starch Characterization

The carbonyl and carboxyl contents of native and oxidized starch are shown in table 2.

Table 2. Carbonyl and carboxyl content of native and oxidized cassava starch

Starch	Carbonyl content (CO/100 GU)	Carboxyl content (COOH/100 GU)
Native	0,0330 ± 0,0321	-
Oxidized	0,0959 ± 0,0456	0,0463 ± 0,0445

GU: glucose units.

The presence of carbonyl and carboxyl groups in oxidized starches is due to oxidation of starch molecules, particularly amylose, in carbonyl groups and then the carboxyl groups (Fonseca et al., 2014). There have been reports that there is a gradual increase in the carbonyl and carboxyl contents in starch with the increasing oxidation (Spier et al., 2013). The results obtained in this assay are similar to those reported by Fonseca et al. (2011) results for the oxidation of potato starch with the 1g/100 g concentration of active chlorine. However, differences persist as oxidized starches depend on various factors such as the starch source, oxidant type and concentration, time, pH and temperature of reaction.

SEM micrographs of native and oxidized starch granules are shown in Fig. 1, displaying both granules with a semispherical form. The oxidation process affected neither the morphology nor the size of the granules. However, there are other studies that have shown that oxidation can affect the surface in the granule with the presence of pores, holes, or a very smooth surface (Kuakpetoon & Wang, 2008; Fonseca et al.,

2014). It is believed that this is because, in those studies, stronger oxidation agents, such as sodium periodate, are used, or physical processes as gelatinization are used.

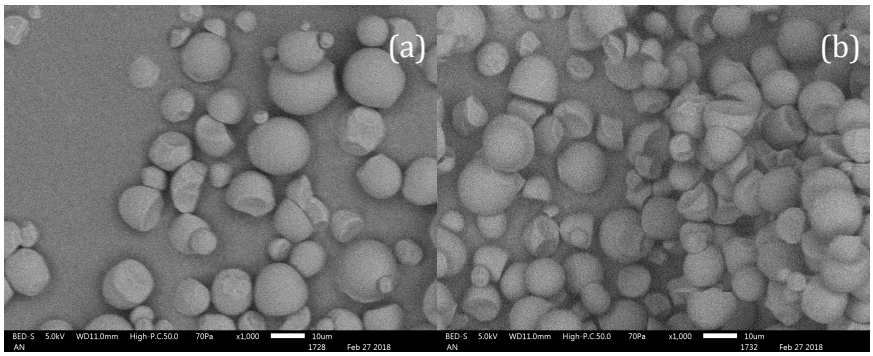

Fig. 1. Scanning electron micrographs of cassava starches: (a) native starch and (b) oxidized starch with 1g/100 g of active chlorine.

3.2. Hydrogel structural analyses

Fresh hydrogels from different formulations are presented in Fig. 2. The physical macromolecular structure varies in hydrogels containing oxidized starch and the ones containing native starch, such as texture, strength, color and opacity as shown in Fig. 2. Hydrogels containing oxidized starch presented a higher opacity and a whited color, these also had higher strength characteristics and a more stable structure when compared to the same formulations containing native starch.

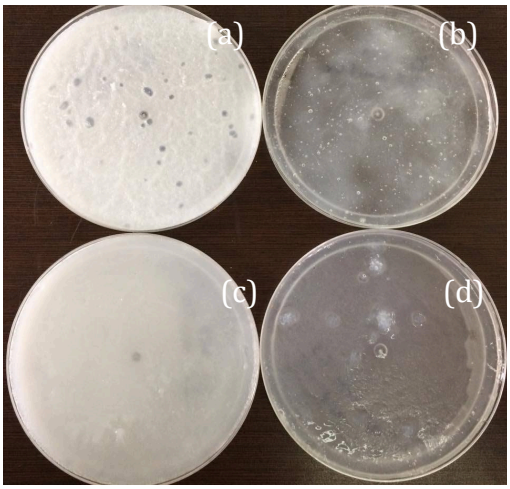

Fig. 2. Hydrogel synthesis: (a) PO10 (PVA 10% oxidized starch 10%), (b) PN10 (PVA 10% native starch 10%), (c) PO5 (PVA 5% oxidized starch 5%) and (d) PN5 (PVA 5% native starch 5%).

Morphological analysis of lyophilized hydrogels with different formulations was performed by SEM, as presented in Fig. 3.

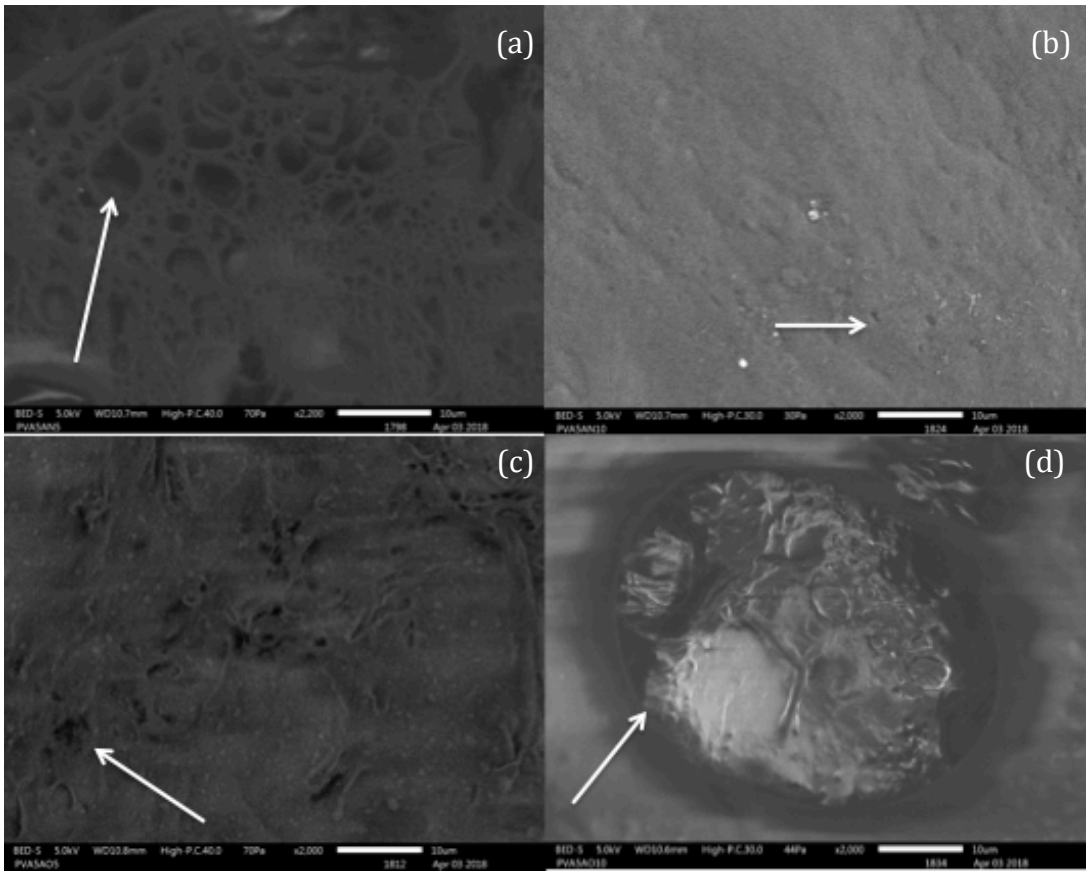

Fig. 3. Scanning electron micrographs of lyophilized hydrogels porosity (shown by the arrows): (a) PN5 (PVA 5% native starch 5%) at 2 kx, (b) PN51 (PVA 5% native starch 10%) at 2 kx, (c) PO5 (PVA 5% oxidized starch 5%) at 2 kx and (d) PO51 (PVA 5% oxidized starch 10%) at 2 kx.

For the lyophilized hydrogels, there are different structures for the different formulations. Hydrogels in Fig. 3 present different types of porosity that vary in size and structure. In Fig. 3 (a) the lyophilized hydrogel containing 5 % native starch looked more organized with an external and internal porosity that present different pore sizes, while, in Fig. 3 (b) (which contained 10% native starch) smoother area with some porosity in the hydrogel matrix can be seen, though there is a nano-porosity within its

structure and an internal porosity. Samples using oxidized starch Fig. 3 (c) and (d) presented a higher porosity in both cases but with a less organized structure, with (d) containing a larger pore size; the hydrogel matrix presented in these samples had a different plastic structure which can be ascribed on a different interaction between PVA and oxidized starch when comparing to PVA and native starch as shown in Fig. 3. The porosity arrange has similarities with a hybrid N-Siccinyl chitosan-dialdehyde starch hydrogel presented by Kamoun (2016) for that it can be seen that the structure will be subjected to changes in their porosity arrange and characteristics due to the starch content and its modification.

3.3. FTIR analysis

Fig. 4 shows the infrared absorption spectra for different hydrogel formulations. The main absorption bands were observed at 3650-3590 cm^{-1} that correspond to –OH stretching, 2880-2900 cm^{-1} that correspond to a C-H stretching, 1200-1000 cm^{-1} from a C-O stretching. The development of small peaks at 1710 cm^{-1} and at 1630 cm^{-1} is attributed to C=O and C=C stretching frequency groups that are present in starch (Reis et al., 2007; Da Róz et al., 2010). There is little variation among the spectra for the samples of each composition. In this case the percentage of PVA was maintained at 10% while starch was varied according to the samples described in table 1. Although the variation is little there are still signals from more or less stretching between the main absorption bands, as expected the spectra from the different samples were to assimilate to a Freeze-thawed PVA spectra; as explained it can be easily observed a C-H broad alkyl stretching band at 2850 cm^{-1} the strong-OH group band for free unreacted alcohol and hydrogen bonded bands at 3600-3200 cm^{-1} which are associated with freeze-thawed PVA (Kenawy et. al, 2014).

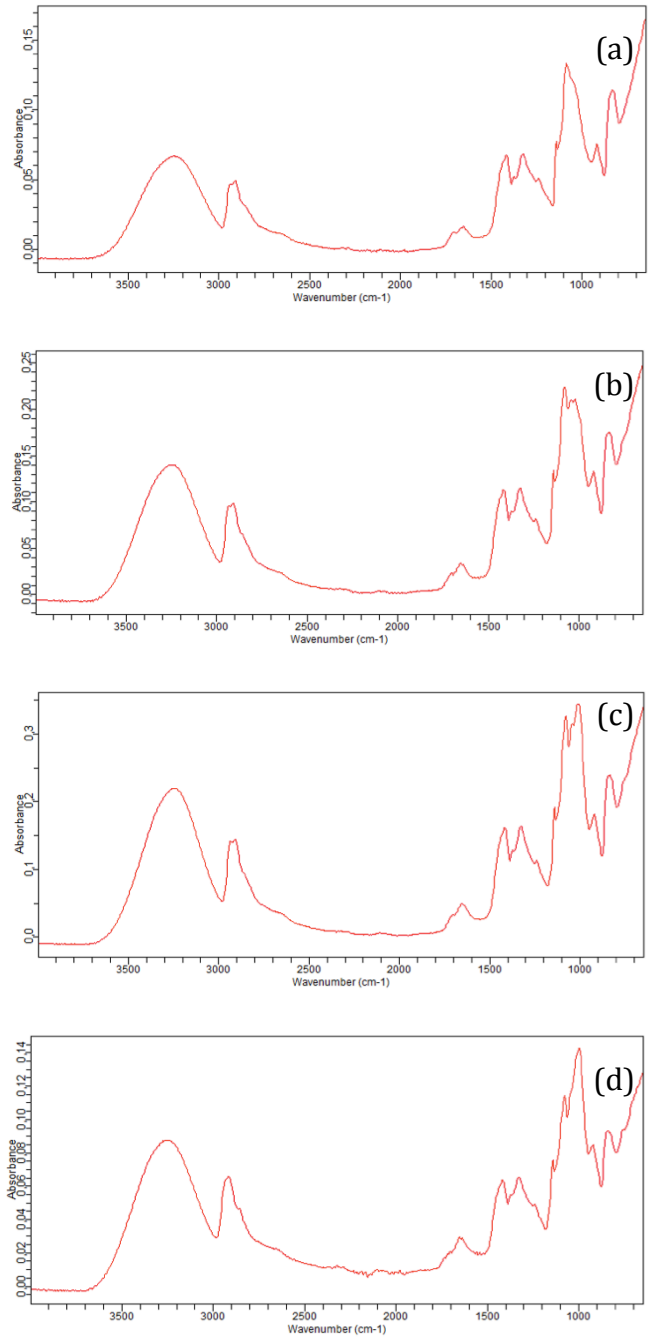

Fig. 4. Wavenumber vs absorbance; FTIR analysis. (a) PN10 (PVA10%AN10%), (b) PN15 (PVA10%AN5%,) (c) PO10 (PVA10%AO10%), (d) PO15 (PVA10%AO5%).

3.4. Thermal analysis

Thermal stability is one of the important properties of a material that determines its usability at high temperature applications, but it also gives an insight into the stability of the hydrogels overall (Agustin et al., 2014). Results from the thermogravimetric analyses are presented in Fig. 5 for different formulations.

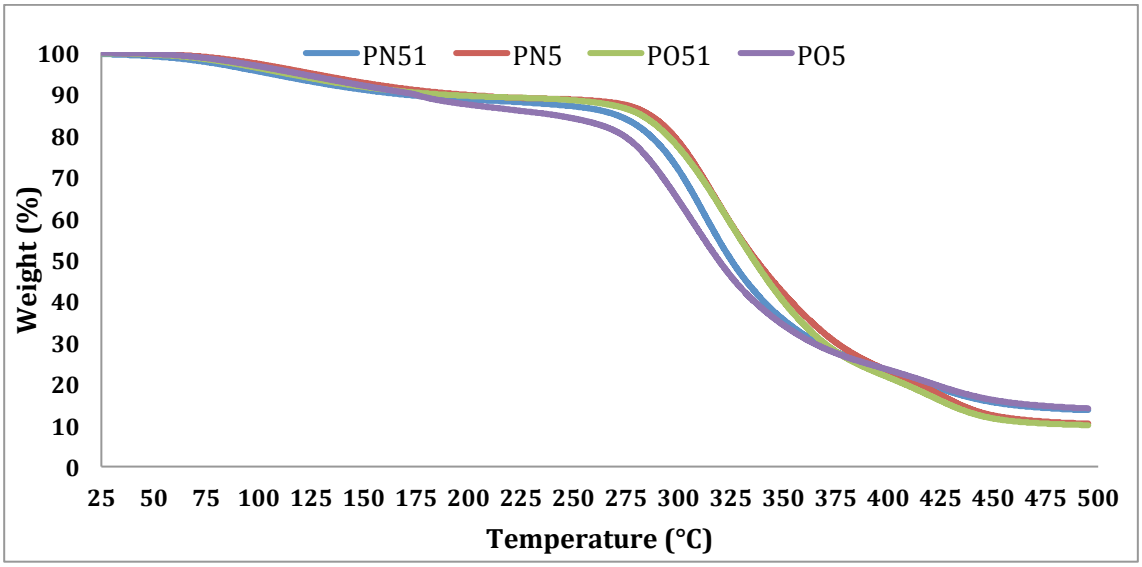

Fig. 5. TGA thermographs of PVA/starch films; Temperature vs Weight. PN51 (PVA 5% native starch 10%); PN5 (PVA 5% native starch 5%); PO51 (PVA 5% oxidized starch 10%); PO5 (PVA 5% oxidized starch 5%).

The first degradation step at $25 - 110°C$ can correspond the removal of traces of water of solvent vapor. The second degradation step at $220 - 400°C$ results in the highest residual weight loss due to decomposition and volatilization of organic components of the polymer. The loss in weight of the hydrogels varied depending of the composition of each film. After that range the third decomposition occurs where the curves become flat and mainly the organic residues are completely volatilized (Kenawy et al., 2014). Fig. 5 represents the different degradation profiles as percent of weight loss; at the second degradation step the sample containing 5% PVA and 5% oxidized starch had the

highest weight-loss rate while he curves that contain 5%PVA with 5% native starch had a notorious similarity with the ones from 5%PVA with 10% oxidized starch. The degradation profiles are similar to the degradation curve for pure PVA reported by Minhas et al. (2013), where the second degradation step was at $225 - 325°C$ where it showed a 20% weight at $325°C$.

When compared to cassava starch film degradation profile presented by Perotti et al (2014) the curves showed a great difference as the pure starch presented one more degradation step and a second degradation rate at $275 - 320°C$ where it showed a 40% weight at $320°C$. It seems that the formulation with 5% oxidized starch had a high degradation, but it leaves a higher residue as shown in the third step from the curve; when adding a higher percentage of oxidized starch it shows a lower degradation over temperature and also leaves less residue. The opposite occurred in the formulations containing native starch, as the formulation containing 5% native starch presented a lower degradation over time and a lower residue than the composition with 10% native cassava starch. Fig. 5 showed that the formed hydrogels could be processed at reasonably higher temperature than the PVA component presenting PO51 and PN5 formulations with a higher temperature stability.

3.5. Water-uptake, water absorption and erosion

The water-uptake (%) capacity is indicated in Fig. 6 and 7 for assays at $37°C$ in PBS solution. Fig. 6 indicates a significantly different water-uptake capacity between formulations containing native starch; the highest water-uptake profile between these formulations is the one with the PVA 10% and native starch 10% followed by the PVA 5% and native starch 10%. The hydrogel formulation PVA 10% and native starch 5% showed that a lower composition of native starch results in a lower water-uptake capacity; furthermore this formulation had a very similar water-uptake capacity as the PVA 10% pure hydrogel (without starch), and the other formulations have a higher capacity when compared to the PVA 10%.

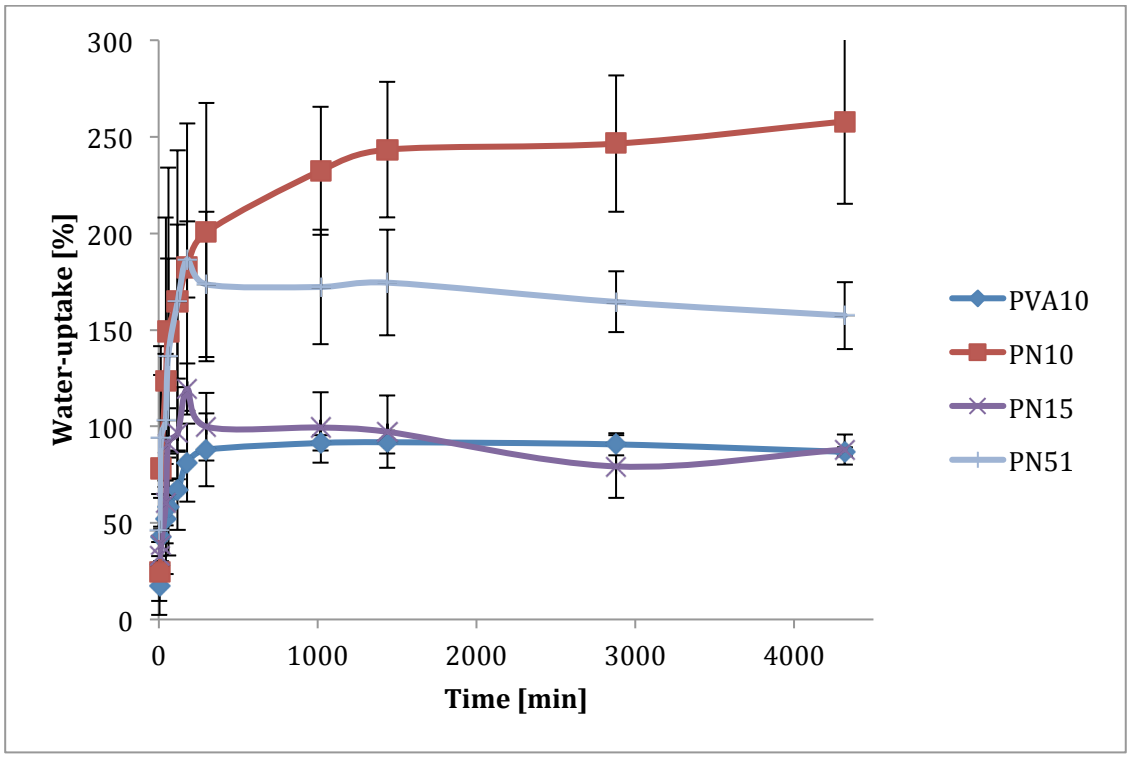

Fig. 6. Water uptake percentage for the hydrogel formulations: PVA10: PVA10%, PN10: PVA 10% Native starch 10%, PN15: PVA10% Native starch 5%, PN51: PVA 5% Native starch 10%.

Fig. 7 shows the water uptake capacity for formulations containing oxidized starch. For both formulations containing PVA 10% and PVA 5% the water-uptake

capacity increase as the amount of oxidized starch increase in the composition of the hydrogel. The hydrogel with PVA 5% and oxidized starch 10% was the profile, which resembles the most to the pure PVA 10% hydrogel.

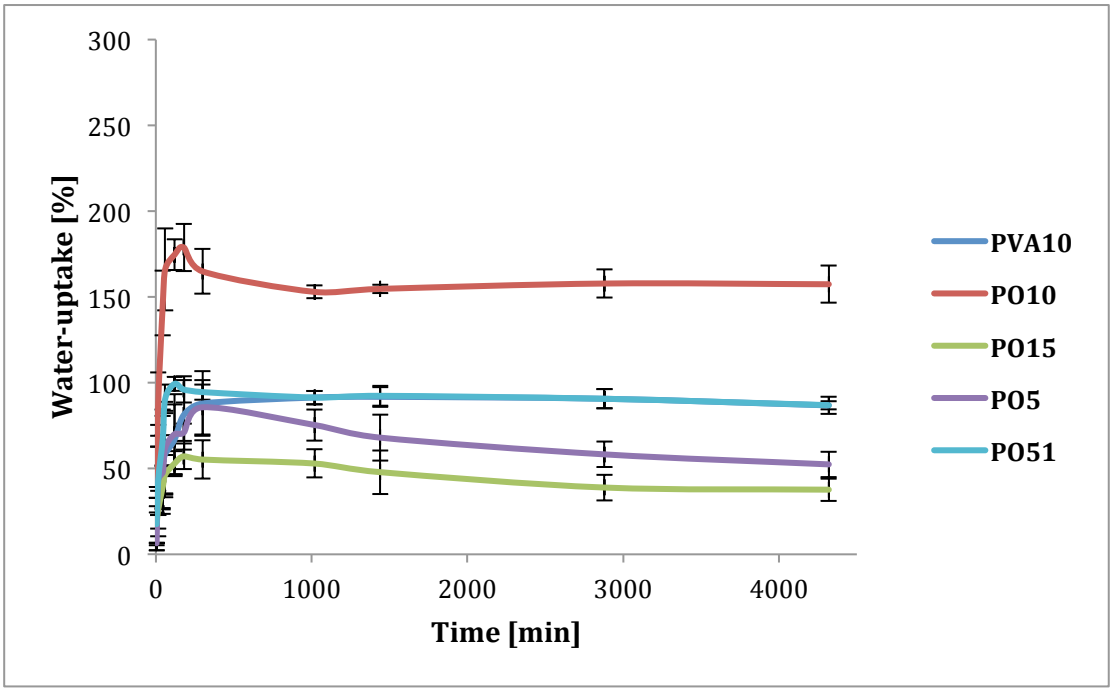

Fig. 7. Water uptake percentage for the hydrogel formulations: PVA10: PVA10%, PO10: PVA 10% Oxidized starch 10%, PO15: PVA10% Oxidized starch 5%, PO5: PVA 5% Oxidized starch 5%, PO51: PVA 5% Oxidized starch 10%.

Water-uptake capacity in both formulations containing native and oxidized starch presented the highest water-uptake the first 5 hours, a pattern similar to that reported by Reis et al. (2007) on films of corn starch modified with glycidyl methacrylate. After this period, most of the formulations reached equilibrium until 72 h. When compared both Fig. 7 and 8 it is shown that the formulations containing native starch had higher water uptake than the ones containing oxidized starch; nevertheless, hydrogels with oxidized starch seemed to be more stable as the standard deviations are clearly lower, this result was suspected as the structural analysis for the formulations correlated the content of oxidized starch with a higher stability, as observed in SEM.

Water absorption capacity is presented in Fig. 8. Unlike the water-uptake the hydrogel that presented the least capacity of water absorption was the one with the formulation of PVA 10% and native starch 10%, while formulations containing PVA 5% presented a relation with the quantity of starch and its water absorption capacity: when the content of the starch was higher the capacity was lower. Nonetheless the hydrogel containing native starch 5% had an unstable water absorption capacity as its curve shows a decrease over time. The water absorption for the hydrogels formulations containing oxidized starch presented in Fig. 8 (b) presented a similar profile form. The water absorption capacity in this case revealed that when a higher percentage of starch the water absorption capacity decrease as shown in Fig. 8 (b) for hydrogels containing PVA 5%.

As in the case of water-uptake capacity, the water absorption assay showed that the hydrogels formulations containing native starch had a higher water absorption capacity; however, while in the assay, the native starch hydrogels were not stable and seem to start dissolving in the PBS. The assay also showed that the formulations with oxidized starch presented a clearly higher stability over time, and presented lower standard deviations as shown in Fig. 8. The reduction in water solubility of the films of oxidized cassava starch can be attributed to the strong intermolecular bond promoted by the oxidation of the starch, which results in a reduce of the capacity to absorb water of the film (Zavareze et al., 2012). This assay showed that the higher water absorption occurs in the first 3 hours and then it stabilizes at 5 hours in most of the cases. The hydrogels presented a great capacity of water-uptake and water absorption as expected, thereby making all the formulations good candidates as a carrier for a drug delivery system.

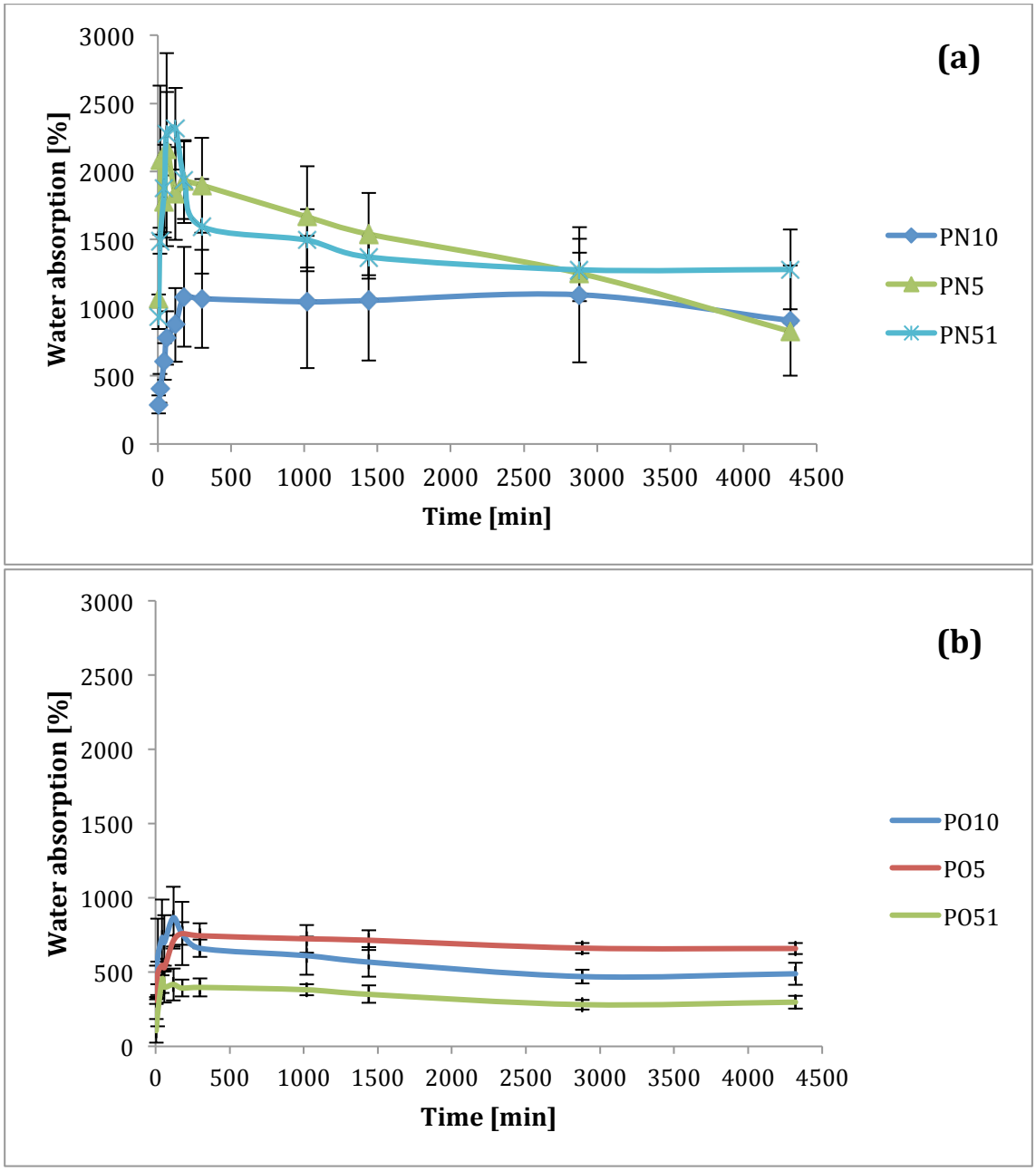

Fig. 8. Water absorption percentage for the hydrogel formulations. (a) Formulations containing native starch PN10: PVA 10% Native starch 10%, PN5: PVA5% Native starch 5%, PN51: PVA 5% Native starch 10%. (b) Formulations containing oxidized starch: PVA10: PVA10%, PO10: PVA 10% Oxidized starch 10%, PO15: PVA10% Oxidized starch 5%, PO5: PVA 5% Oxidized starch 5%, PO51: PVA 5% Oxidized starch 10%.

Erosion revealed to be less pronounced for all formulations that contained oxidized starch, as shown in table 3. There is a correlation between the starch content and the % of erosion; in compositions containing PVA 5% in both cases for native and oxidized starch the erosion decreases as the percentage of starch increases in the hydrogel composition. Oxidized starch has an increased quantity of carbonyl and carboxyl groups, as shown in table 2, which result in lower retrogradation of the hydrogel (Fonseca et al., 2014); hence they resulted in stronger and more stable hydrogel structures; this can also be seen as the standard deviation of the materials with oxidized starch are relatively low when compared to the ones containing native starch. The oxidation with active chlorine affects differently the characteristics of starches, it present a lower water solubility, which enables the use of the hydrogels in product with higher water activity as compared to the native starch hydrogels, thus the erosion will be lower in the hydrogels with oxidized starch.

Table 3. Erosion of hydrogels after water absorption analyses

Hydrogel	Composition	Erosion (%)
PN10	PVA 10% native starch 10%	65,78 ± 21,23
PO10	PVA 10% oxidized starch 10%	30,07 ± 3,87
PN5	PVA 5% native starch 5%	58 ± 19,59
PO5	PVA 5% oxidized starch 5%	22,82 ± 8,48
PN51	PVA 5% native starch 10%	30,48 ± 17,12
PO51	PVA 5% oxidized starch 10%	22,64 ± 5,40

3.6. Ibuprofen release assay

Release assay analyses provided a preliminary approach for ibuprofen release as illustrated in Fig. 9 and 10, for which a calibration curve for ibuprofen in PBS was made. Release assay revealed a similar ibuprofen profile in every hydrogel formulation. The high water content of most hydrogels typically results in relative rapid release of drugs from the gel matrix (Hoare & Kohane, 2007). Fig. 9 and 10 shows an increasing drug release in 72 hours, the drug in every composition keeps a constant dissolution rate over time leading to an order 0 kinetic release (Sáez, 2003). Thus making all the hydrogel formulations viable as carriers for a controlled drug release.

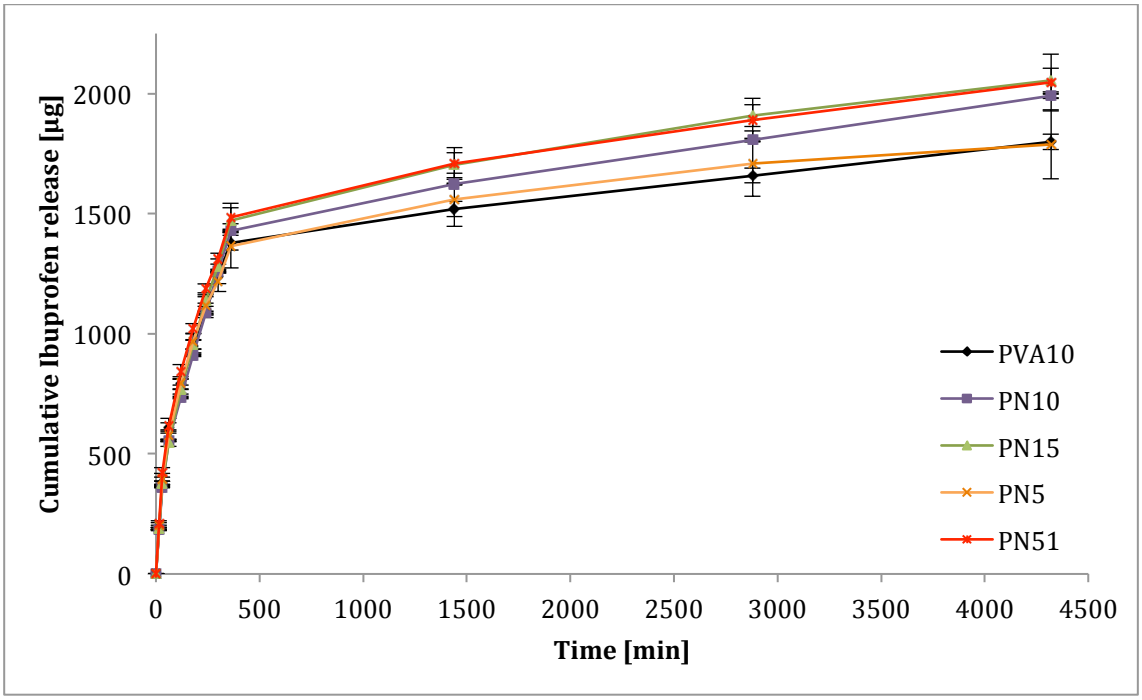

Fig. 9. Ibuprofen cumulative release profile for the hydrogel formulations: PVA10 (PVA10%), PN10 (PVA 10% Native starch 10%), PN15 (PVA10% Native starch 5%), PN5 (PVA 5% Native starch 5%), PN51 (PVA 5% Native starch 10%).

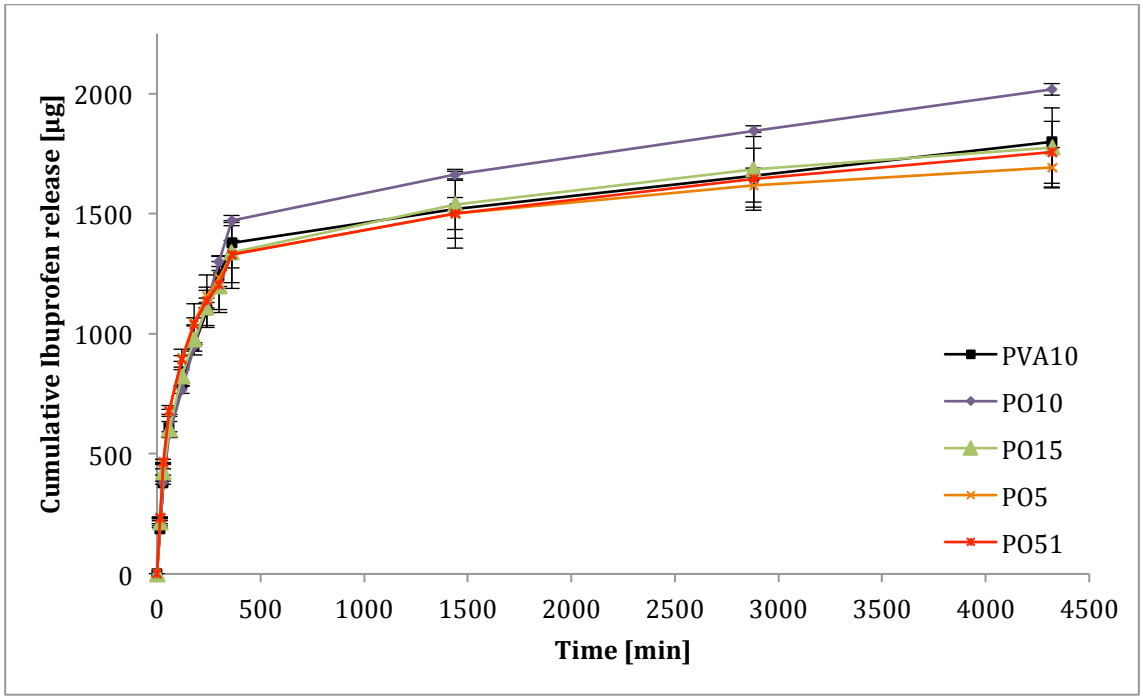

Fig. 10. Ibuprofen cumulative release profile for the hydrogel formulations: PVA10 (PVA10%), PO10 (PVA 10% Oxidized starch 10%), PO15 (PVA10% Oxidized starch 5%), PO5 (PVA 5% Oxidized starch 5%), PO51 (PVA 5% Oxidized starch 10%).

Ibuprofen release assays revealed that hydrogels containing native starch and oxidized starch in most of the cases presented that in when the formulations increased the percentage of starch, the ibuprofen cumulative release profile also increase; this result may be due to its dependence to the water-uptake capacity of each of the formulations so its relation will be similar. Similar to water-uptake, the release of ibuprofen also presented the higher release the first 6 hours and then an almost linear increase as expected (in this case is a cumulative release profile, in the case of the water-uptake this would represent the stability or equilibrium profile). The release profile is similar to those reported by Apopei et al. (2016) where the cumulative release presented an increasing rate over time in an oxidized starch/poly(N,N-dimethylaminoethyl methacrylate) cryogels in a controlled release evaluation. The ibuprofen release profiles resemble the ones from PVA-hydroxyethyl starch presented

in Kenawy et al. (2014). The relation with the pure PVA release and the rest of the curves had also a similar pattern than the swelling capacity profiles presented in Fig. 6 and 7 as the curves with native starch represented a higher cumulative release profiles, while the oxidized starch hydrogels had lower cumulative release profiles. The higher water-uptake and water released could be correlated to the internal and external porosity on each hydrogel formulations. As seen in Fig. 3 native starch formulations contained a higher porosity and a more organized structure in the polymeric matrix, which could lead to a higher capacity of water-uptake, hence a higher drug release rate. Nevertheless, as shown in the water uptake, water absorption and erosion assays the formulations with oxidized starch provide more stable hydrogels with lower dissolution in PBS.

4. Conclusions

The oxidation with active chlorine affects the characteristics of cassava starch, which seem to have high implications in the evaluated physical and chemical hydrogel characteristics. All hydrogels formulations presented an optimal kinetic release profile for ibuprofen, extending the duration of release. Slight variations in the release profile could be of benefit for applications varying doses of a drug over time and could widen the range of applications of these hydrogels. Notably, PVA hydrogels containing oxidized starch presented a lower water solubility while in PBS solution at $37°C$ when compared to native starch/PVA hydrogels, demonstrating, a potential for improved stability in a physiological environment. Hydrogels with vary degradation may help to address different kinetic issues. Additionally, these hydrogels could expand their usefulness in medical applications, such as drug release and tissue-engineering-based applications, depending on the success of future biocompatibility tests.

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