

## Compatibility of Cassava Starch Films as Nitric Oxide Carrier for Potential Medical Device

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**ABSTRACT:** Films prepared with bioabsorbable materials (cassava starch, glycerol, and carboxymethyl cellulose (CMC)) can be used as an external support in the myocardial revascularization surgery, releasing nitric oxide (NO) during the tissue regeneration. In the present study, these materials submitted to *in vitro* degradation tests under physiological conditions, presented the reduction of 38% of weight loss by the modification of native starch using sodium trimetaphosphate (STMP) (15 g/100g of starch). The CMC addition of 10 g/100g of cassava starch increased 119% of the tensile strength of the films in comparison with films prepared without CMC. It was verified that the ruthenium complex *trans*-[Ru(NH<sub>3</sub>)<sub>4</sub>(isn)(NO)](BF<sub>4</sub>)<sub>3</sub> incorporated in the starch based films behave similarly as in aqueous solution, releasing NO by photochemical reaction. Macrophage cells were detected in *in vivo* degradation tests in rats around the implant after 1 and 7 days, and complete biodegradation was observed after 30 days. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2015, 132, 41382.

**KEYWORDS:** biocompatibility; crosslinking; drug delivery systems

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### INTRODUCTION

Cardiovascular diseases remain the leading cause of death around the world<sup>1</sup> and myocardium revascularization surgery is the procedure most performed to promote relieve to the angina and to improve life expectance.<sup>2</sup> Saphenous vein has been widely used in coronary artery bypass surgery; however, the effectiveness of this graft is limited by the patency of the vein that is much lesser than the arterial grafts, mainly due to the functional and structural characteristics of the vein that may be affected by the high pressures distensions of the arterial environment. As a consequence of this hemodynamic alteration, the vein graft suffers radial deformation resulting in failure, occluding up to 50% of the grafts within 10 years after surgery.<sup>2,3</sup> An external, nonrestrictive polyester stent for reducing the wall thickening in porcine vein grafts has been studied.<sup>4-6</sup> Consequently, a new surgery is necessary to remove this external sheath to avoid complications of the prosthetic material implant. Because of this, it is proposed the development of an

absorbable material able to support the graft until adaptation and subsequent biodegradation.<sup>4-6</sup>

Biodegradable starch-based polymers have been reported to be a promising material to be used in medical applications.<sup>7-10</sup> Starch is an inexpensive polysaccharide, non-toxic, biodegradable and shows good biocompatibility and interaction with living cells.<sup>10</sup> Plasticizers are used mainly to overcome film brittleness of pure starch film caused by high intermolecular forces, and one of the most commonly plasticizers used is glycerol.<sup>11,12</sup> Cross-linking agents are also used to improve the properties of starch films. These agents increase chemical bonds that act as bridges between the starch molecules, modulating mechanical and barrier properties, and the degradation rate, according to the cross-linking degree of the starch.<sup>13-15</sup>

Nitric oxide (NO) is an important radical produced by human body that is actively involved in different physiological processes.<sup>16</sup> Its vasodilation property is of great relevance in the healing processes of recovery from myocardial infarction due to the

promotion of new vessel generation through stimulation of endothelial cell growth.<sup>17</sup> Among the different NO carriers, ruthenium nitrosyl complexes constitute a very promising class and have been extensively studied over the past years for the biological purposes due to their ability of releasing NO by photochemical or chemical stimulation.<sup>18</sup> The compounds with the general formula  $trans\text{-}[\text{Ru}(\text{NH}_3)_4(\text{L})(\text{NO})]^{3+}$  (wherein L = N-heterocyclic or phosphorous(III) ligands) have been studied as an exogenous NO-donors. These complexes showed ability to release NO after being reduced biologically, resulting in blood pressure suppression.<sup>19</sup> Furthermore, some of these nitrosyl complexes have been immobilized in different supporting materials,<sup>18,20</sup> in an attempt to improve the versatility of these nitric oxide donors.

In this work, the development and characterization of starch films made of native cassava starch modified with sodium trimetaphosphate (STMP), reinforced by carboxymethylcellulose (CMC) and plasticized with glycerol and water were studied. The compatibility of the material developed with the ruthenium NO-donor was assessed and an *in vitro* degradation of the films was also performed.

## EXPERIMENTAL

### Materials

Cassava starch was kindly supplied by Cargill (São Paulo, Brazil) containing 19.7% of amylose. Glycerol, carboxymethylcellulose, and sodium trimetaphosphate were purchased from Synth (São Paulo, Brazil). The ruthenium complexes with formula  $trans\text{-}[\text{Ru}(\text{NH}_3)_4(\text{isn})(\text{NO})](\text{BF}_4)_3$  were synthesized in the Inorganic and Analytical Chemistry Laboratory of the Chemistry Institute of São Carlos at University of São Paulo, following previously published procedures.<sup>21</sup>

### Preparation of Films

Initially, carboxymethylcellulose (CMC) concentrations were assessed (0, 5, 10, 15 and 20) g/100g of starch in films prepared with native cassava starch. After defined the best concentration of CMC, films were prepared with modified starch using the cross-linking agent sodium trimetaphosphate (STMP) at different concentrations (0, 1, 5, 10 and 15) g/100g of starch, in the first stage, and then (0, 15 and 30) g/100g of starch, in the second one. Finally, films with modified starch with the defined concentration of STMP were elaborated containing CMC (0 and 10) g/100g of starch and characterized.

The filmogenic solution was prepared in two steps. Firstly, CMC was slowly poured in deionized water, heated up to 75°C under stirring, to form a 1 g/100g of solution, and then, left to cool at ambient temperature (23°C). The second step consisted of the gelatinization of the starch with glycerol: 3.40 g of starch, 0.51 g of glycerol and 61.70 g of deionized water were heated up to 72°C under stirring. After that, the solution of CMC was mixed with the gelatinized starch suspension, and after homogenization, the solution was poured onto cylindrical plates (150.6 cm<sup>2</sup> of area) to obtain a constant relation of 2.5 g of starch per plate. Then, the films were dried at (40 ± 2) °C in an oven for approximately 17 h.

After dried, films were stored at controlled relative humidity of 75% and at ambient temperature (22 ± 2) °C for at least 48 h

before testing. The films were characterized in relation to mechanical, barrier, thermal and physicochemical properties, and microstructure.

### Modification of Starch

Chemically modified starch was prepared using sodium trimetaphosphate (STMP) based on Demirgöz et al.<sup>8</sup> Briefly, starch and STMP were dissolved in aqueous solution (500 mL), the pH was adjusted to 11 using NaOH solution (1M), and then, the resulting solution was stirred for 5 h at 50°C (in a thermostatic bath). After the pH adjustment to 6.8 using HCl (1M), the solution was filtered and the collected starch was resuspended twice in 500 mL of water, followed by filtration, in order to remove unreacted STMP and salt formed by the pH adjustment. Cross-linked starch was dried at 60°C for 12 h and, after reaching ambient temperature, was stored in closed glass bottle.

### Mechanical Properties

Tensile strength (*TS*), percent elongation at break (*ELO*) and modulus of elasticity (*E*) were evaluated by tensile test performed on a texture analyzer (TA.XTPlus, SMS, UK) using the A/TGT self-tightening roller grips fixture, according to ASTM D882-10.<sup>22</sup> Filmstrips (130 mm × 25 mm) were cut from each preconditioned sample and mounted between the grips of the equipment, after the measurement of the thickness [mm] at five random positions using a flat parallel surface micrometer (Mitutoyo, no. 103–137, Brazil). Initial grip separation and test speed were set to 50 mm and 0.8 mm s<sup>-1</sup>, respectively. Tensile strength at yield was calculated dividing the maximum load by the original minimum cross-sectional area of the specimen (related to the minimum thickness measured). Percent elongation at break was calculated by dividing the extension at the break point by the initial grip separation length and multiplying by 100. Modulus of elasticity was calculated as the tangent of the linear part of the stress-strain curve. At least 10 specimens of each sample were used to calculate the mean value.

### Barrier Properties

Water vapor permeability (*WVP*) was determined gravimetrically, according to ASTM E96/E96M-10,<sup>23</sup> using the Desiccant Method at 75% RH (sodium chloride saturated solution). Each film sample was sealed with paraffin over a circular opening of 44 cm<sup>2</sup> at the permeation cell (Regmed, PVA/4, Brazil) and stored in desiccator with 75% RH at ambient temperature (22 ± 2) °C. Three cells were filled with silica gel (0% RH) to create a gradient of water vapor across the film. Another two cells were kept in the same conditions but without the silica gel in order to take into account the mass of water retained in the hydrophilic film surface. The cells were weighed 10 times over 48 h. *WVP* was calculated according to the eq. (1):

$$WVP = \frac{w}{q} \cdot \frac{t}{A \cdot p_s \cdot (RH_1 - RH_2)} \quad (1)$$

wherein *WVP* is the water vapor permeability [g mm m<sup>-2</sup> d<sup>-1</sup> kPa<sup>-1</sup>]; *w/q* is the angular coefficient of the weight gain as function of time curve [g d<sup>-1</sup>]; *t* is the average film thickness [mm]; *A* is the tested area [m<sup>2</sup>]; *p<sub>s</sub>* is the saturated vapor pressure at the test temperature [kPa] and *RH<sub>1</sub>* and *RH<sub>2</sub>* are the relative humidity inside and outside the cell.

Oxygen transmission rate (*OTR*) [ $\text{cm}^3 \text{m}^{-2} \text{d}^{-1}$ ] through  $50 \text{ cm}^2$  of circular area of the films was measured for films prepared with modified starch and CMC (0 and 10) g/100g of starch at  $23^\circ\text{C}$  and 75% RH using an oxygen permeation system (OXTRAN 2/21, MOCON, USA), according to ASTM F1927-07.<sup>24</sup> The samples were sealed in the system and oxygen ( $20 \text{ mL min}^{-1}$ ) was allowed to flow at one side of the film while a stream of nitrogen carrier gas ( $10 \text{ mL min}^{-1}$ ) at the other side purged the oxygen that crossed the starch based film. A coulometric sensor determined the amount of oxygen transmitted through the film into the carrier gas. The oxygen transmission rate was determined in duplicate. The permeance ( $PO_2$ ) of the films was calculated according to eq. (2):

$$PO_2 = \frac{OTR}{p} \quad (2)$$

wherein  $PO_2$  is the permeance of the films [ $\text{cm}^3 \text{m}^{-2} \text{d}^{-1} \text{Pa}^{-1}$ ]; *OTR* is the oxygen transmission rate [ $\text{cm}^3 \text{m}^{-2} \text{d}^{-1}$ ] and  $p$  is the partial pressure of oxygen in the test gas side of the diffusion cell [Pa]. The partial pressure of  $O_2$  on the carrier side gas was considered zero.

The oxygen permeability coefficient ( $P'O_2$ ) [ $\text{cm}^3 \text{mm m}^{-2} \text{d}^{-1} \text{Pa}^{-1}$ ] was calculated as follows:

$$P'O_2 = PO_2 \cdot t \quad (3)$$

wherein  $t$  is the average thickness of the specimen [mm].

#### In Vitro Degradation

The degradation of the films was studied over a period of 60 days according to ASTM F1635-11<sup>25</sup> evaluating the weight loss of specimens in phosphate-buffered saline (PBS) solution, simulating the physiological conditions. The ionic concentrations of the PBS were  $\text{Na}^+$  (156 mM),  $\text{Cl}^-$  (101 mM),  $\text{HPO}_4^{2-}$  (24 mM), and  $\text{H}_2\text{PO}_4^-$  (6 mM) at the pH of 7.4. Each specimen was cut in ( $2.5 \text{ cm} \times 2.5 \text{ cm}$ ), conditioned at  $60^\circ\text{C}$  in an oven until constant weight, and then immersed in 80 mL of the PBS, to maintain a relation between solution and specimen volume over than 20 : 1. The sealed containers were kept at  $37^\circ\text{C}$  and four specimens of the starch based film were removed at regular intervals of (1, 3, 7, 14, 30 and 60) days, being taken out of the solution, rinsed twice with distilled water and dried at  $60^\circ\text{C}$  to constant weight. The weight loss was calculated as follows:

$$\Delta W = \frac{W_{d0} - W_d}{W_{d0}} \cdot 100 \quad (4)$$

wherein  $\Delta W$  is the weight loss [%];  $W_{d0}$  and  $W_d$  are, respectively, the dry weight of the specimen before and after the immersion time [g].

#### Water Activity

The water activity ( $a_w$ ) of the films was directly determined in an Aqualab equipment (DECAGON, USA), in triplicate, at  $25^\circ\text{C}$ .

#### Hydrophilicity Measurements

The hydrophilicity was measured by the contact angle between deionized water and the surface of the starch based films at ambient temperature ( $22^\circ\text{C}$ ) according to ATSM D7334-08,<sup>26</sup> using the measuring system OCA 15 (DataPhysics, Germany) and the software SCA 20 to analyze the images. The method

was used to observe the hydrophilicity of the films with different formulations. The results correspond to the average value of at least eight measurements.

#### Thermal Degradation

Thermogravimetric analyzer DTG-60H attached with FC-60-A flow controller (Shimadzu, Japan) was used to study the thermal behavior. The analysis was performed by heating 10 mg of the sample from ambient temperature ( $22^\circ\text{C}$ ) up to  $600^\circ\text{C}$  at a rate of  $10^\circ\text{C min}^{-1}$  in oxidative atmosphere.

#### Morphological Structure

The surface features of the starch based films were observed using an optical microscope BX60M (Olympus, Japan) by reflection imaging and analyzed by AnalySIS getIT software.

Cross-section fractures of the starch based films were observed using a scanning electron microscope (SEM) XL-30 (Phillips, USA). The samples were immersed in liquid nitrogen and then cracked using a pair of pincers. The samples were sputter coated with gold (Bal-Tec, SCD 050, Liechtenstein) and observed under the SEM at a voltage of 20 kV. The surface roughness (roughness mean square, RMS) of the starch-based films was verified by atomic force microscopy (AFM) assays. AFM were performed in tapping-mode using a model EasyScan 2 AFM device (Nanosurf Inc., USA) with Tap190AL-G cantilevers (Budget Sensors, Bulgaria). The typical force constant and resonance frequency were  $48 \text{ N m}^{-1}$  and 190 kHz, respectively. All AFM images were processed with linear fitting. The parameters evaluated to find the RMS values are described in the operating instructions of the equipment.

#### Incorporation of NO-Donor

Ruthenium nitrosyl complex with formula *trans*-[Ru(NH<sub>3</sub>)<sub>4</sub>(isn)(NO)](BF<sub>4</sub>)<sub>3</sub> (RuNOisn) was studied as NO-donor in starch based films. Complex was solubilized in water and added to the filmogenic solution prior the gelatinization and then the preparation of the films followed as above described.

The immobilization of RuNOisn into the film was verified by FT-IR spectroscopy, using a MB-102 spectrometer (ABB Bomem Inc., Canada). Cassava starch films without ruthenium complex were also verified for comparison.

#### In Vivo Implantation

Investigation of inflammation was performed by subcutaneous implantation of modified cassava starch film with SMTP at 15 g/100g of starch in Wistar rats. Animals were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) and  $1 \text{ cm}^2$  of starch based film was implanted in the dorsal area (control film, N = 4; film containing RuNOisn, N = 4). After 1 and 7 days, the implanted films were collected, washed with saline and fixed with 4 % phosphate-buffered formalin. Immunohistochemistry for CD68 was performed to identify the presence of macrophage. This study was conducted at the Laboratory of Genetics and Molecular Cardiology, Heart Institute (InCor), Medicine School, University of São Paulo. All animal procedures followed institutional guidelines for the care and use of the laboratory animals. This study protocol was approved by the local ethical committee (CAPPesq—527/04).

**Table I.** Thickness (*t*), Tensile Strength (*TS*), Percent Elongation at Break (*ELO*), Modulus of Elasticity (*E*) and Water Vapor Permeability (*WVP*) of Films Based on Native Cassava Starch as a Function of Carboxymethylcellulose (CMC) Content

CMC [g/100g of starch]	<i>t</i> [ $\mu\text{m}$ ]	<i>TS</i> [MPa]	<i>ELO</i> [%]	<i>E</i> [MPa]	<i>WVP</i> [ $\text{g mm m}^{-2} \text{d}^{-1} \text{kPa}^{-1}$ ]
0	162.6 $\pm$ 15.8 <sup>a</sup>	4.7 $\pm$ 0.4 <sup>a</sup>	141.1 $\pm$ 49.1 <sup>d</sup>	106.3 $\pm$ 20.8 <sup>a</sup>	7.8 $\pm$ 0.7 <sup>a</sup>
5	176.4 $\pm$ 5.9 <sup>b</sup>	9.4 $\pm$ 0.8 <sup>c</sup>	67.4 $\pm$ 20.3 <sup>b</sup>	164.0 $\pm$ 14.1 <sup>b</sup>	7.3 $\pm$ 0.8 <sup>a</sup>
10	185.2 $\pm$ 7.7 <sup>c</sup>	8.4 $\pm$ 0.5 <sup>b</sup>	111.4 $\pm$ 15.5 <sup>c</sup>	100.0 $\pm$ 17.6 <sup>a</sup>	8.3 $\pm$ 0.2 <sup>a</sup>
15	188.6 $\pm$ 9.9 <sup>c,d</sup>	16.7 $\pm$ 1.3 <sup>d</sup>	31.3 $\pm$ 11.9 <sup>a</sup>	235.7 $\pm$ 22.9 <sup>c</sup>	7.8 $\pm$ 0.4 <sup>a</sup>
20	194.2 $\pm$ 5.7 <sup>d</sup>	22.4 $\pm$ 0.9 <sup>e</sup>	29.1 $\pm$ 12.4 <sup>a</sup>	265.3 $\pm$ 39.5 <sup>d</sup>	12.5 $\pm$ 0.1 <sup>b</sup>
Tuckey HSD	7.6	0.7	15.4	20.6	1.4

Means in the same column with the same letter are not significantly different ( $P > 0.05$ ).

### Statistical Analysis

ANOVA analysis was performed in all results using a statistical program Statgraphics Centurion XV (Statpoint) and Tuckey test was applied to evaluate average differences at a confidence interval of 95%.

## RESULTS AND DISCUSSION

### Addition of CMC

Thickness of the films based on native cassava starch increased with the increasing content of CMC, this fact would be expected as the proportion of all other components was maintained constant. As it can be observed in Table I, an increase of CMC content caused an increase of *TS*, *E* and *WVP*, and a decrease of *ELO*. The increasing tensile strength and modulus of elasticity of cassava starch films is mainly caused by the intermolecular interactions between hydroxyl and carboxyl groups from the CMC.<sup>27</sup> Hydrogen bonds originally formed between starch molecules can be substituted by hydrogen bonds among hydroxyl groups from starch and hydroxyl and carboxyl groups from the carboxymethylcellulose. The tensile strength and elongation at break were inversely related and as the concentration of CMC increased, the first significantly increased while elongation decreased. Tongdeesontorn et al.<sup>27</sup> presented similar results in the study of biodegradable films as an alternative of conventional packaging.

Water vapor permeability was poorly influenced by the addition of CMC in cassava starch films up to the concentration of CMC of 15 g/100g of starch. These values were similar to those

obtained by Tongdeesontorn et al.<sup>27</sup> and Souza et al.<sup>28</sup> in the study of biodegradable cassava starch films. However, *WVP* was significantly higher for films prepared with the highest concentration of CMC, as can be seen in Table I. Ghanbarzadeh et al.<sup>29</sup> had similar results studying *WVP* in films based on maize with different contents of CMC. At low concentration, CMC is well dispersed in the matrix acting like a barrier to water vapor transmission. Although, higher concentrations of CMC would lead to the formation of clusters that might decrease the effectiveness of the CMC.<sup>29</sup>

The difficulty in preparing the films also increased with the increasing content of CMC due to higher filmogenic solution viscosity restraining the release of the entrapped bubbles. Moreover, the degradation study in PBS immersion showed that the concentration of CMC of 10 g/100g of starch produced films that better behaved in aqueous environment in comparison to those prepared with 15 g/100g of starch. Therefore, the concentration of CMC 10 g/100g of starch was chosen to continue this study, improving the mechanical properties of the cassava starch film mainly at low relative humidity environment (Table I).

### Starch Modification with STMP

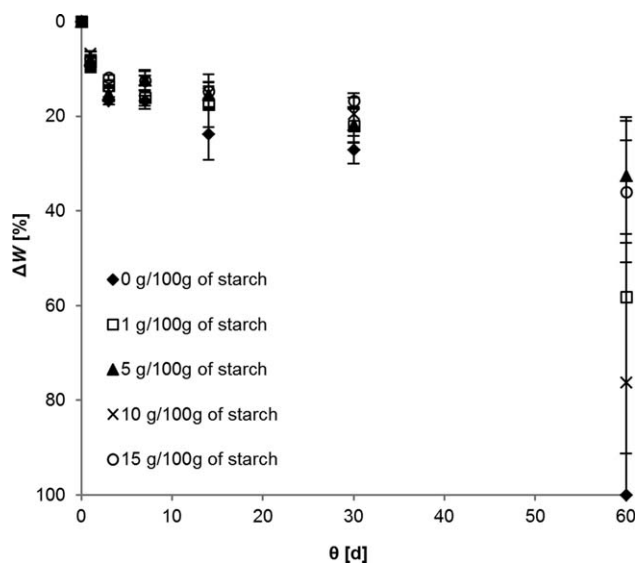
For modification of native starch with sodium trimetaphosphate (STMP), films were prepared with CMC (10 g/100g of starch). Thickness, *TS*, and *WVP* were poorly influenced by the concentration of the cross-linking agent, as can be seen in Table II. However, an increase of STMP content caused significant decrease of *ELO* and increase of *E*, fact that can be attributed to

**Table II.** Thickness (*t*), Tensile Strength (*TS*), Percent Elongation at Break (*ELO*), Modulus of Elasticity (*E*) and Water Vapor Permeability (*WVP*) of Films with Carboxymethylcellulose (CMC) at 10 g/100g of Starch as a Function of Sodium Trimetaphosphate (STMP) Content for Starch Modification

STMP (g/100g of starch)	<i>t</i> [ $\mu\text{m}$ ]	<i>TS</i> [MPa]	<i>ELO</i> [%]	<i>E</i> [MPa]	<i>WVP</i> [ $\text{g mm m}^{-2} \text{d}^{-1} \text{kPa}^{-1}$ ]
0	171.6 $\pm$ 7.2 <sup>a</sup>	4.5 $\pm$ 0.3 <sup>a</sup>	134.7 $\pm$ 4.3 <sup>b</sup>	24.6 $\pm$ 2.1 <sup>a</sup>	8.0 $\pm$ 0.9 <sup>a</sup>
1	183.2 $\pm$ 7.5 <sup>b,c</sup>	5.4 $\pm$ 0.4 <sup>b</sup>	109.0 $\pm$ 13.3 <sup>a</sup>	58.4 $\pm$ 12.2 <sup>c</sup>	7.1 $\pm$ 0.7 <sup>a</sup>
5	186.0 $\pm$ 11.4 <sup>c</sup>	4.5 $\pm$ 0.5 <sup>a</sup>	122.2 $\pm$ 15.6 <sup>a,b</sup>	39.5 $\pm$ 8.4 <sup>b</sup>	8.1 $\pm$ 0.5 <sup>a,b</sup>
10	177.2 $\pm$ 10.5 <sup>a,b</sup>	4.5 $\pm$ 0.9 <sup>a</sup>	117.2 $\pm$ 16.7 <sup>a,b</sup>	43.4 $\pm$ 12.5 <sup>b</sup>	9.8 $\pm$ 0.7 <sup>b</sup>
15	180.0 $\pm$ 7.1 <sup>b,c</sup>	4.9 $\pm$ 0.7 <sup>a,b</sup>	107.7 $\pm$ 15.7 <sup>a</sup>	47.1 $\pm$ 5.0 <sup>b,c</sup>	9.8 $\pm$ 0.5 <sup>b</sup>
Tuckey HSD	6.9	0.8	18.2	12.1	1.7

Means in the same column with the same letter are not significantly different ( $P > 0.05$ ).





**Figure 1.** Weight loss ( $\Delta W$ ) in phosphate-buffered saline (PBS) solution as a function of the immersion time ( $t$ ) of modified starch films with different concentrations of sodium trimetaphosphate (STMP) [g/100g of starch].

the reinforcement of the film by chemical bonds between the starch molecules,<sup>13,15</sup> constraining the chain flow (lower values of *ELO*) and improving stiffness (higher values of *E*).

Concerning mechanical properties, the concentrations of (1 and 15) g/100g of starch presented higher *TS*, lower *E* and higher *ELO*. As it can be observed in Table II, ANOVA indicated that these concentrations are not significantly different.

Figure 1 shows the *in vitro* degradation results of starch modification indicating that the highest concentration of STMP resulted in slower degradation of the film. Demirgöz et al.<sup>8</sup> had similar results when comparing the degradation behavior of blends of different materials and corn starch cross-linked with sodium trimetaphosphate in simulated physiological condition. Cross-linked samples had slower weight loss and the final values

were significantly different in relation to the non-reacted samples with a mean of 8% of difference.

Degradation behavior is one of the most important properties of materials that are aimed to be used in biomedical applications.<sup>8</sup> Because of this, the concentration of STMP (15 g/100g of starch) was considered for further studies. In addition, a higher concentration of the cross-linking (30 g/100g of starch) was also tested. Instead of what would be expected, the use of STMP of 30 g/100g of starch in the modification of cassava starch resulted in films with higher degradation rate in PBS immersion, showing complete degradation after 30 days of the beginning of the test (data not shown). Thickness, *WVP* and *E* showed no significant difference. *TS* was reduced while *ELO* increased with increasing concentration of STMP of 30 g/100g of starch. As the developed material is intended to remain its properties for at least 1 month, time of neointimal formation after vascular graft surgery,<sup>4,5</sup> the better concentration of STMP was found to be 15 g/100g of starch.

### Characterization of the Films

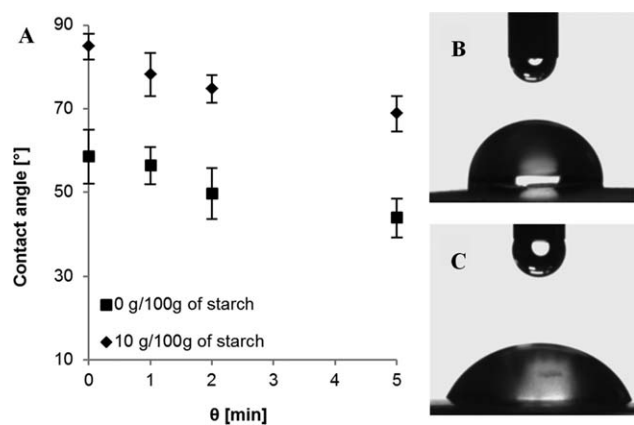
Table III shows thickness, mechanical and barrier properties, water activity, contact angle, and water content of the films prepared with modified starch with STMP (15 g/100g of starch) and CMC (0 and 10) g/100g of starch. According to the previous study of CMC addition into films prepared with native starch, thickness and mechanical properties were significantly influenced by the reinforcement of the CMC ( $P < 0.05$ ).

As reported by others researchers,<sup>30,31</sup> gas and water vapor permeabilities of the films depend on diverse factors, as the ratio of crystalline and amorphous zones, polymeric chain mobility and the specific functional groups interactions between the matrix and the gases. In this work, *WVP* and oxygen permeability coefficient were not influenced by the amount of CMC added to the films ( $P > 0.05$ ). Water activity ( $a_w$ ), which is a parameter that measures the available water for chemical reactions and growth of microorganisms, measured at 75 % RH and 25 °C, was also not influenced by the presence of CMC.

**Table III.** Thickness ( $t$ ), Tensile Strength (*TS*), Elongation at Break (*ELO*), Modulus of Elasticity (*E*), Water Vapor Permeability (*WVP*), Oxygen Permeability Coefficient ( $P'_{O_2}$ ), Water Activity ( $a_w$ ), Contact Angle, Water Content of Films Prepared with Modified Starch with Sodium Trimetaphosphate (STMP) (15 g/100g of Starch) Containing Carboxymethylcellulose (CMC) (0 and 10) g/100g of Starch

	CMC (g/100g of starch)		Tuckey HSD
	0	10	
$t$ [ $\mu\text{m}$ ]	$149.6 \pm 7.6^a$	$164.6 \pm 6.3^b$	3.9
<i>TS</i> [MPa]	$11.8 \pm 1.4^a$	$25.9 \pm 0.6^b$	0.9
<i>ELO</i> [%]	$72.8 \pm 33.6^b$	$20.9 \pm 2.9^a$	21.2
<i>E</i> [MPa]	$208.1 \pm 14.4^a$	$282.3 \pm 6.9^b$	10.1
<i>WVP</i> [g·mm/m <sup>2</sup> d kPa]	$9.7 \pm 0.1^a$	$9.6 \pm 0.6^a$	0.9
$P'_{O_2} \times 10^{-9}$ [cm <sup>3</sup> /m d Pa]	$14.6 \pm 1.9^a$	$11.7 \pm 0.2^a$	5.9
$a_w$	$0.708 \pm 0.002^a$	$0.708 \pm 0.001^a$	0.004
contact angle [°]	$58.2 \pm 6.4^a$	$84.9 \pm 3.1^b$	5.2
water content [%]	$13.4 \pm 0.1^a$	$13.8 \pm 1.3^a$	3.9

Means in the same row with the same letter are not significantly different ( $P > 0.05$ ).



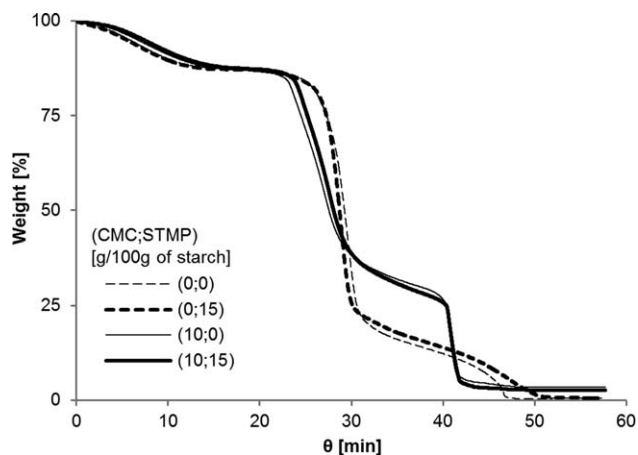
**Figure 2.** Contact angle of the modified starch films with STMP (15 g/100g of starch) measured as a function of time for films containing different concentrations of CMC [g/100g of starch] (A); in detail, images of the drops of water immediately after the fall over the surface (B) and (C).

Because the obtained values are lower than 0.8, this reveals unfavorable conditions for the growth of microorganisms.<sup>32</sup>

The hydrophilicity of a material can be quantitatively measured by analysis of the contact angle between its surface and a drop of water.<sup>26,33</sup> As can be observed in Figure 2, films prepared without CMC exhibited more affinity with water in comparison with films prepared with CMC (10 g/100g of starch) due to the lower value measured of the contact angle ( $P < 0.05$ ). Because of the values of contact angle were higher than  $45^\circ$ , the starch films showed similar characteristics to hydrophobic materials [Figure 2(B,C)]. However, when the drop of water touched the surface of the material, the area in contact immediately swelled, taking the shape of it. It was observed that the swell was very fast and, after that, the contact angles decreased with time as a consequence of the absorption of water by the films, characteristic of hydrophilic materials [Figure 2(A)]. Similar behavior was verified by Shi et al.<sup>34</sup> studying the contact angles of corn maize films prepared with polyvinyl alcohol and glycerol to be used as membrane in tissue regeneration.

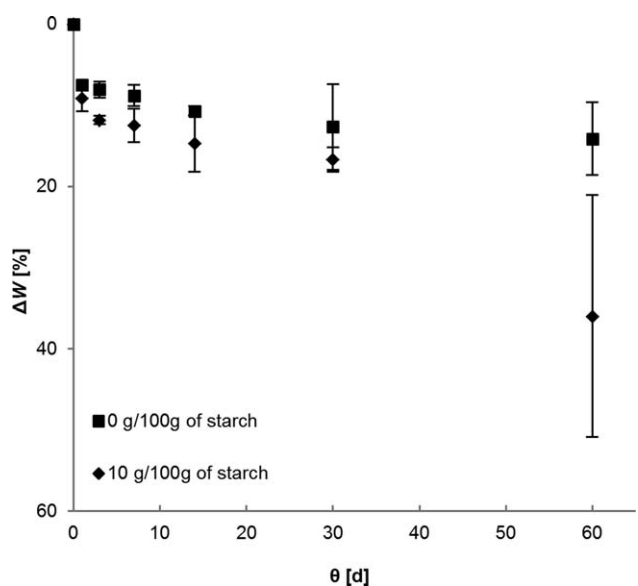
Figure 3 shows the thermogravimetric analysis (TGA) diagrams of films prepared with modified starch with STMP (0 and 15) g/100g of starch and CMC (0 and 10) g/100g of starch in order to compare the effect of each component on the thermal stability of the films. It can be observed that there are three main degradation steps, which agree with reports of other authors<sup>35–37</sup> about glycerol-plasticized starch, wherein the first of them corresponds to the water loss; the second one, to the degradation of the starch and glycerol; and the third to the oxidation of the partially decomposed starch. For films containing CMC, the second step appears as a shoulder representing two events superimposed with peaks at  $262^\circ\text{C}$  and  $293^\circ\text{C}$ , detected by the first derivative (data not shown), suggesting that in this system, the thermal stability of glycerol-plasticized starch, with degradation peak around  $320^\circ\text{C}$ , is reduced by the addition of CMC, as the degradation slightly shifts to lower temperature. Results showed that the addition of CMC (10 g/100g of starch) did not influence the water content of the films (see Table III).

Figure 4 shows the weight loss profile in PBS (pH 7.4 and  $37^\circ\text{C}$ ) of films elaborated with STMP of 15 g/100g of starch and

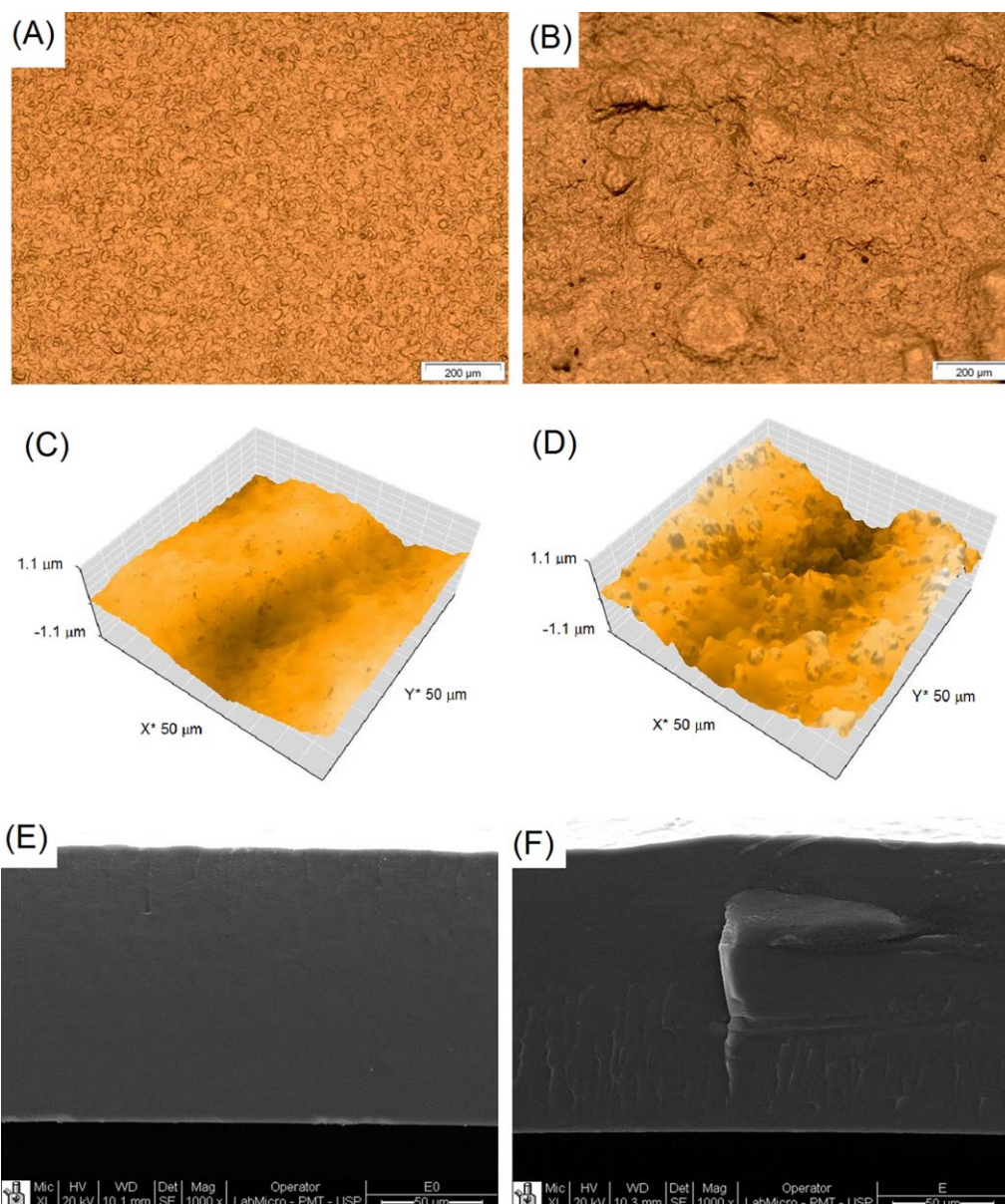


**Figure 3.** Thermogravimetric diagrams of the films containing different concentrations of STMP (0 and 15) g/100g of starch and CMC (0 and 10) g/100g of starch.

CMC (0 and 10) g/100g of starch. It was observed that both films had similar behavior presenting high weight loss from the beginning of the test up to the third day with daily rates of (3.1 and 4.4) % for films containing CMC (0 and 10) g/100g of starch, respectively, followed by a slower weight loss with daily rates of (0.1 and 0.4) % until the end of the test. The addition of CMC increased the weight loss mainly after 30 days of immersion, while films without CMC resisted more the test conditions. These results can be attributed to the fact that CMC increases the amount of available hydroxyl groups to be associated with water molecules, promoting hydrolysis of the films. Furthermore, the presence of CMC in starch films enhances the swell ability of the material, increasing the contact area with the PBS solution, raising the rate of weight loss of the biomaterial.



**Figure 4.** Weight loss ( $\Delta W$ ) in phosphate-buffered saline (PBS) solution as a function of the immersion time ( $\theta$ ) of modified starch films with STMP (15 g/100g of starch) containing different concentrations of CMC [g/100g of starch].



**Figure 5.** Surface images of cassava starch films with different contents of CMC: OM images— (A) 0 g/100g of starch and (B) 10 g/100g of starch; and AFM images— (C) 0 g/100g of starch and (D) 10 g/100g of starch; and SEM images of cross-section fractures of cassava starch films— (E) 0 g/100g of starch and (F) 10 g/100g of starch. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

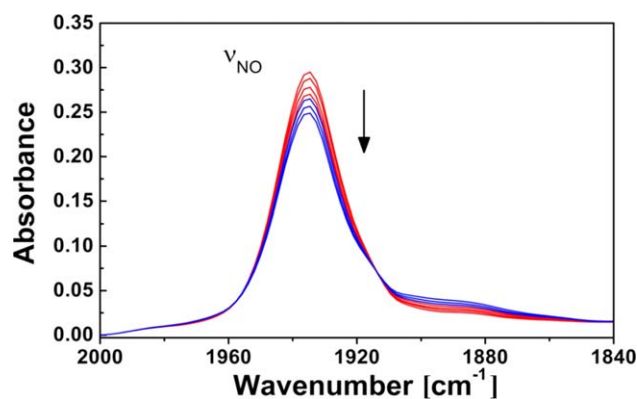
Figure 5 shows the optical microscope (OM) and atomic force microscopy (AFM) images, as well as the SEM micrographs of the films with CMC (0 and 10) g/100g of starch. Native cassava starch films exhibit similar OM images (not shown) to the modified starch films with STMP (15 g/100g of starch), varying only the shape of the small granules that became ring-shaped. On the other hand, the addition of CMC clearly changed the surface characteristics, being possible to observe an increase of roughness, presented as particulate prominences, fact also noticed during manipulation of the films [Figure 5(A,B)]. The increased roughness of the films containing CMC was confirmed by AFM assays, as shown in Figure 5(C,D): the roughness mean square (RMS) of the films produced with CMC was 324.1 nm, whereas the RMS for the films without CMC was 227.5 nm.

SEM micrographs of the cross-section fractures showed characteristic compact structures of plasticized starch films without pores or cracks, also reported by other authors.<sup>31,33,34</sup> By comparing the micrographs, it is possible to observe that the fracture surface of films containing CMC presents elevations on the surface, as detected by OM, and also inside the matrix of the films, showing significant difference between the two formulations of modified starch films [Figure 5(E,F)].

#### Incorporation of NO-Donor

The diverse physiological and pathological roles of NO have lead many researchers to develop new NO-donors devices.<sup>18,38</sup> In this context, cassava starch films present interesting characteristics as easy degradation, biocompatibility, low cost, and





**Figure 6.** FT-IR spectral decay of NO peak ( $\sim 1935\text{ cm}^{-1}$ ) after photolysis of the film containing RuNOisn (31.9 mg/g of starch), each line representing the spectrum after 50 pulses ( $\lambda = 355\text{ nm}$ ). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

owing to these facts are potential platforms to be used in the development of NO-releasing medical device.<sup>39,40</sup>

The ruthenium(II) nitrosyl complex with formula *trans*-[Ru(NH<sub>3</sub>)<sub>4</sub>(isn)(NO)](BF<sub>4</sub>)<sub>3</sub> (RuNOisn) was incorporated into the cassava starch films, which was prepared with modified starch with STMP (15 g/100g of starch) containing or not CMC (10 g/100g of starch), as above described. The resulting materials had similar aspect to those prepared without the complex.

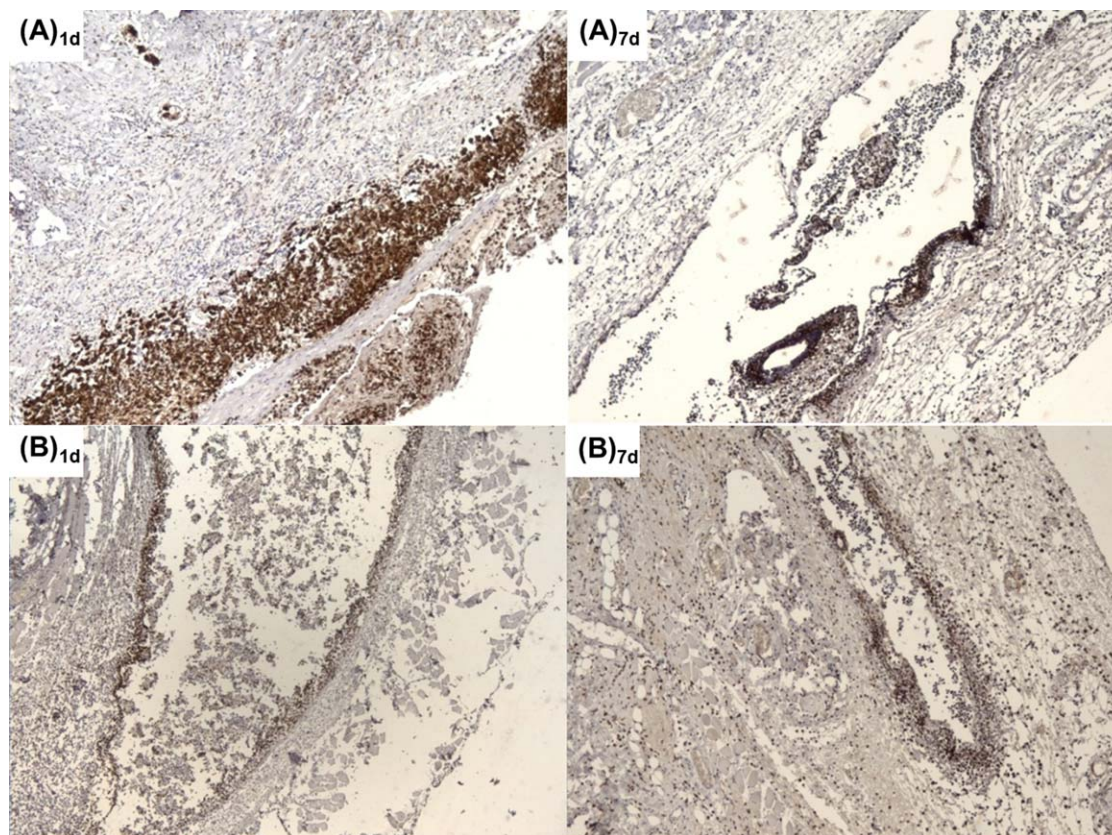
Modified cassava starch films without CMC containing RuNOisn (31.9 mg/g of starch) were irradiated with Nd:YAG Laser (Continuum, model Surelite-II) ( $\lambda = 355\text{ nm}$ ) in order to trigger the NO release from the films. The reaction progress was monitored by FT-IR spectroscopy, in which the intensity of the stretching frequency of NO ( $\nu_{\text{NO}}$ ) was measured after determined pulse laser intervals, as shown in Figure 6. This result is in agreement with the predicted reaction in aqueous media described in literature.<sup>21</sup>

The incorporation of the complex did not change the morphological characteristics of the films, mainly due to the small amount of complex used.

#### *In Vivo* Implantation

*In vivo* assays in rats were performed as a pilot study to direct the future steps of this research. Initial tests showed that the films prepared with CMC (10 g/100g of starch) and modified cassava starch (15 g/100g of starch) were completely bioabsorbed after 30 days of implantation.

In Figure 7(A) the microscope images of the tissue surrounding the area of implanted films of modified cassava starch with STMP (15 g/100g of starch) and without the NO-complex removed after (1 and 7) days of implantation, are shown. It can be observed a great aggregation of macrophages surrounding the implanted area and also inside the film, suggesting the degradation of the material. Similar results were observed for the tissue



**Figure 7.** Microscope images (5X) of the implanted films surrounded by inflammatory cells. Modified cassava starch film without RuNOisn: (A<sub>1d</sub>) after 1 day and (A<sub>7d</sub>) after 7 days of implantation; and containing 31.2 mg/g of starch of RuNOisn: (B<sub>1d</sub>) after 1 day and (B<sub>7d</sub>) after 7 days of implantation. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



surrounding the area of implanted films with RuNO<sub>is</sub>n (at a concentration of 31.2 mg/g of starch, 0.52 mg cm<sup>-2</sup>), which were also removed after (1 and 7) days of implantation [Figure 7 (B)]. It was not possible to notice difference between the amount of macrophages in the tissue implanted with the film containing RuNO<sub>is</sub>n complex in relation to the control. This fact may be attributed to the low concentration of the complex used. For future studies, higher concentrations of RuNO<sub>is</sub>n will be tested in attempt to verify a dose-response relationship.

## CONCLUSIONS

Natural derived materials have been developed for a variety of biomedical applications, especially due to the hemocompatible characteristics. Until now, few researches have aimed the development of a new material to be used as a bioabsorbable external sheath in the myocardial revascularization. The first major achievement of this study was the developed film composed with natural compounds considered as biocompatible materials able to resist for over 30 days under physiological conditions (pH 7.4 at 37°C). The results also demonstrated that this material can be modulated by using different concentrations of CMC for reinforcement and further by cross-linking starch with STMP for better behavior in high moisture environment. The second major accomplishment is the ability of the developed material to incorporate the ruthenium nitrosyl complex that reacted according to the predicted reaction described in literature. Furthermore, the *in vivo* pilot study showed that the cassava starch film is completely absorbed after 30 days and the presence of the ruthenium nitrosyl complex did not affect the immune response to the implanted film in rats in relation to the control films.

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