International Journal of Biological Macromolecules 235 (2023) 123742

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

The aging analysis of natural rubber-*Copaifera oblongifolia* extract membranes

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ARTICLE INFO

Keywords:
Latex
Copaiba
Biomaterial
Aging

ABSTRACT

Natural rubber (NR), derived from *Hevea brasiliensis*, has properties for biomedical applications. Several studies indicate that these properties can be amplified when we associate another bioproduct. However, there are no studies of aging aspects of this biomaterial regarding changes in functionality, structure and composition. The objective was to evaluate the aging process of natural rubber membranes - copaiba (NRC) subjected to controlled conditions of time, light and presence of oxygen. The NRC was prepared and stored in the presence or absence of light and vacuum, for periods of 30, 60 and 90 days. Subsequently, the membranes were characterized through the techniques of wettability, infrared spectroscopy, thermal analysis, scanning microscopy and antioxidant activity. The wettability analysis, showed that NRC membranes both in the zero time and in the aging time were hydrophilic. Through thermogravimetric analysis and differential exploratory analysis the membranes remained thermally stable. The scanning electronic microscopy, indicated no morphological alterations during the observed period. After 90 days, the packaged membranes showed satisfactory antioxidant activity. Our results suggest that the membranes were resistant to the storage period, since they maintained their chemical, thermal, morphological and antioxidant characteristics. Hence, it corroborates to use of membranes as a possible curative for biomedical applications.

1. Introduction

Polymers are some of the materials with compatible characteristics for producing biomaterials. The use of biopolymers beneficial to human health has been growing in recent decades. Studies showed their use in the food industry [1], even as a carrier matrix of probiotics for the treatment of the gastrointestinal tract [2] among others. In the biomedical area, polymers have also gained prominence, as these compounds are available in nature in large quantities, with adequate purity, reduced cost of production, and appropriate properties. Different studies have reported the incorporation of various types of drugs with different applications or plant extracts in polymeric matrices, which result in protective, antimicrobial, anti-inflammatory and antioxidant effects [3,4,5,6].

Among the materials of plant origin with promising characteristics for biomedical application, there is natural rubber latex, which is composed of particles of poly(cis-1,4-isoprene) chains extracted from the rubber tree *Hevea brasiliensis*. Natural rubber (NR) has remarkable characteristics such as biocompatibility, non-toxic nature, elastic properties, ability to promote angiogenesis [5,6,7,8]. Natural rubber biomembranes are lightweight, painless to apply and remove, impermeable to microorganisms, permeable to oxygen and water vapor, as well as easy of processing, biodegradability and biocompatibility [9,10,11].

Furthermore, studies reported the biocompatibility of natural rubber when mixed with other polymers. Shadan et al. [12] when incorporating polymers such as polyaniline and polypyrrole in the polymeric matrix of natural rubber, observed that there was an improvement in mechanical resistance, thermal stability, as well as in conductivity and resistance to water absorption.

The natural rubber has been used in association with other

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https://doi.org/10.1016/j.ijbiomac.2023.123742
Received 11 October 2022; Received in revised form 21 December 2022; Accepted 14 February 2023
Available online 17 February 2023

biomaterials to develop new materials for biomedical applications, as carried out by Silva et al. [9] when developing natural rubber membranes incorporated with propolis extract. They have observed that there was an interaction between the two compounds. Even with good interaction, changes related to hydrophobicity and morphology of membrane surfaces may be observed when incorporating propolis into the polymeric rubber matrix. These characteristics made the material attractive for the development of dressings for wound treatments.

Another biomaterial that can be associated with natural rubber that has been gaining prominence is copaiba (*Copaifera oblongifolia*). The copaiba is found mainly in the Amazon basin and Cerrado biomes. The copaiba species, *C. officinaliis*, *C. reticulata*, *C. langsdorffii*, *C. guyanensis* and *C. multijuga*, have therapeutic effects related to the presence of diterpenes and sesquiterpenes, such as copalic acid and the sesquiterpenes β-caryophyllene and α-copaene [13,14]. In addition, pharmacological properties of products obtained from the resinous oil of the copaiba trunk and their healing, anti-inflammatory, antitumor, antitetanus, urinary antiseptic, treatment of skin diseases and wound healing effects have been reported [15,16]. The antioxidant activity of the hydroalcoholic extract of the copaiba leaf was also established [17]. Several studies have suggested inhibitory oxidation processes of copaiba plant and, protection against oxidative stress processes, by avoiding the excess of free radicals in the body. This oxidative stress contributes to the processes of metagenesis, carcinogenesis, coronary heart disease and DNA damage [18].

In this context, in a previous study by our group, natural rubber membranes incorporated with copaiba fruit extract were developed, which had hydrophilic characteristics, mechanical resistance and antioxidant capacity [19]. However, studies evaluating the aging of biomembranes based on unvulcanized natural rubber are still scarce.

It is important to emphasize that biomaterials have functional groups (such as amino groups, carboxylic groups and hydroxyls) which can undergo chemical (hydrolysis, oxidation, reduction, esterification, cross-linking, *etc.*) and enzymatic modifications and, thus, interact with other molecules, thus resulting in a wide variety of products with adaptable properties [20]. When a biomaterial is exposed to the local climatic condition, interactions with other molecules and aging process may occur, which can basically be influenced by the chemical characteristics of the compound itself, resulting in intra- and intermolecular chemical reactions, thus being able to generate reactive species which in most of the cases are free radicals [21,22].

The analysis of the aging of these biomaterials is of paramount importance in directing their specific application, considering the kinetics of the process, the change in functionality, since changes occur progressively and irreversibly in the structure and composition of the biomaterial, and also analyzing the products generated during this process that can sometimes be cytotoxic and compromise the final effect of the product [23].

As there are no studies in the literature on the aging of natural rubber membranes associated with other biomaterials, the objective of this work was to evaluate the aging process of natural rubber-copaiba (NRC) membranes subjected to controlled conditions of time, luminosity and presence of oxygen, through morphological analysis, as well as thermal behavior, chemical bonds and functional groups and antioxidant activity.

2. Methods

2.1. Collecting the fruits of Copaifera oblongifolia and preparing the extract

The collection of fruits was carried out in the forests on the banks of the MT-100 road that connects the municipality of Pontal do Araguaia to the municipality of Torixoréu-MT. The collected fruits were submitted to washing and asepsis with 1 % sodium hypochlorite solution. They were then dried in an oven at 40 °C to remove moisture and placed in an airtight glass jar with a lock until the extract was prepared. The extract was prepared according to Marques-Júnior [19]. Briefly, 500 g of dry fruits were macerated and placed in 1.5 L of hydroalcoholic solution in a ratio of 7:3 (ethanol:water). The solution was kept in a place protected from light at room temperature with sporadic agitation for better incorporation of the solvent for 15 days. After this period, the solution was subjected to rotary evaporation at a temperature of 40 °C, and then the extract was dried in an oven at 40 °C until a constant weight of 74.52 g of extract was obtained. From the dry weight, a hydroalcoholic extract of *C. oblongifolia* was prepared at a concentration of 75 mg/mL.

The latex was extracted from rubber trees of the species *Hevea braziliensis*, clones of RRIM – 600, located at Fazenda Santa Isabel, in the municipality of Pontal do Araguaia – MT.

After extraction, the latex was stored in an amber glass containing 4 % by volume of ammonium hydroxide (NH_4OH) to prevent its coagulation, as well as to stabilize it at a pH of 10.2. It was then placed inside a thermal box containing ice for transport to the laboratory, where it was stored in a refrigerator during all analyzes [9].

The content of solids in the latex DRC (Drying Rubber Content) was calculated by means of the weight ratio of the content of the initial solution and the final dry content. Analysis performed in triplicate. For this, 5.0 mL of latex was added to a petri dish. Then, the plates were placed in an oven at 40 °C for 24 h. After this period, weightings were performed, which were repeated later until the material reached constant mass. The solids content was determined using Eq. (1) [24]:

$$\%DRC = \frac{100\ x\ msf}{5} \quad (1)$$

Where, msf refers to the final dry mass, in grams, of the latex after the natural rubber membranes have reached constant mass. The denominator refers to the volume of latex used for the analysis (5.0 mL).

2.2. Preparation of natural rubber membranes incorporated with copaiba extract

The membranes were made by the method of "casting" by solvent evaporation, according to the methodology of Marques-Junior [19] For this, in a beaker were added 5.0 mL of latex and 3.0 mL of hydroalcoholic extract of *C. oblongifolia* (NRC). Using a magnetic stirrer, the solution was homogenized and added to a 5.0 cm diameter Petri dish and placed in an oven at 40 °C for 48 h for the polymerization of the material. Pure natural rubber membranes were prepared for control for thermal analysis and infrared spectroscopy, as well as pure copaiba extract. Subsequently, the membranes were packed in vacuum or non-vacuum plastic packaging, in the presence or absence of light for different periods to evaluate the aging process.

2.3. Aging test

The prepared membranes were stored in conventional plastic packages in the absence or presence of a vacuum. Then, these membranes were divided into environments with the presence of light (L- light) and the absence of light (D- dark). Membranes under different conditions were stored for 30, 60 and 90 days, and the membrane was also analyzed at time zero. Subsequently, the membranes were characterized in terms of wettability; surface morphology using the Scanning Electron Microscopy (SEM) technique; chemical structure of the membranes through Fourier Transform Infrared Spectroscopy (FT-IR) techniques for times 0, 30, 60 and 90 days, thermogravimetric analysis, differential scanning calorimetry and the antioxidant capacity of NRC at times 0 and 90 days.

2.4. Wettability

To analyze the wettability of the material, and to determine the hydrophilicity or hydrophilicity degree, the contact angle was measured, which is calculated from the angle formed between the liquid

in contact with the membranes. Droplets of deionized water were deposited on the surface of the membrane, in a volume of 3.0 μL, and mean values were obtained in six different locations for each of the samples. Images in JPEG format were manipulated with the ImageJ program, and the angles were determined [9]. The following criteria were adopted: wetting contact angle (θc) allows determining whether the solid surface is hydrophilic (θc < 90°), hydrophobic (90° ≤ θc ≤ 150°) or super-hydrophobic (θc > 150°) [25,26].

2.5. Scanning Electron Microscopy (SEM)

Membrane morphology was analyzed using a high-resolution scanning electron microscope from Tescan, model LMU – Vega3. Small fragments of the membranes were fixed on metal supports ("Stubs") using double-sided carbon adhesive tape. They were then coated with gold (Au). After the metallization process, the surface of the material was analyzed using a scanning electron microscope at a voltage of 5.0 Kv and 10 Pa.

2.6. Fourier transform infrared spectroscopy (FTIR)

The technique of absorption spectroscopy in the infrared region with Fourier transform (FT-IR) was used to characterize the functional groups present in the membranes. Spectrums were obtained using a PerkinElmer spectrophotometer, model Spectrum 100, with 4 cm^{-1} resolution in the region between 4000 and 600 cm^{-1}, equipped with an ATR (Total Attenuated Reflectance) accessory. For the analysis, cuts were made in the membranes to obtain small fragments, which were placed on the germanium (Ge) crystal to obtain the spectra.

2.7. Thermal analysis TGA/DSC

For the analysis of the thermal properties of the material, a METLER TOLEDO model TGA/DSC STAR System calorimeter was used. For this, 5.0 mg of each membrane sample were placed in aluminum capsules, and the analysis was performed under a dynamic atmosphere of compressed air, heating rate 10 °C/min, atmosphere flow rate 60 mL/min, in a temperature range of 25 °C to 800 °C.

2.8. Antioxidant activity determination by the free radical sequestration method for stable, 2,2-diphenyl-1-picrylhydrazyl (DPPH)

For analysis of the antioxidant activity of 90-day NRC membranes, fragments of membranes were cut from the edge to the center, with the mass of all adjusted to 0.43 g. Then, the fragments were immersed in 10.0 mL of 40.0 mg/mL DPPH· solution. The reading of the decrease in absorbance was performed by a UV–Vis spectrophotometer at 515 nm at the following times: 1, 5, 10, 20, 30, 40, 50 and 60 min. The assay was performed in triplicate. As a standard, 3.0 mL of the hydroalcoholic extract solution of *C. oblongifolia* at a concentration of 75 mg/mL corresponding to the reaction mixture was used. To determine the antioxidant activity from the extract released from NRC membranes, Equation was used. Eq. (2) [27]:

$$\%AA = \frac{\{[ABScontrol - (ABSsample - ABSwhite)]x\ 100\}}{ABScontrol} \quad (2)$$

2.9. Statistical analysis

For statistical analysis, analysis of variance (Two-Way ANOVA) was performed, followed by the Bonferroni test. Results will be expressed as mean and standard deviation. The confidence interval was set at 95 % and differences will be considered significant for $P < 0.05$.

3. Results and discussion

In order to determine the rubber content present in the collected latex, the mean DRC values of latex had been calculated before the preparation of the membranes. Mean content of 37 % was found in accordance with the values described in the literature. In this sense, DRC in the range of 31–34 % is considered below average and values between 34 and 38 % are within the mean. DRC values between 38 and 41 % are considered above average, while values above 41 % are considered high DRC [24,28].

After preparation and polymerization of 48 h (time 0) NRC membranes obtained present translucency and flexibility. When performing a macroscopic analysis of NRC membranes at 30, 60 and 90 days, no changes were observed in translucency and flexibility during the aging period evaluated (Fig. 1).

3.1. Wettability

The wettability of the NRC membranes was analyzed at times zero, 30, 60 and 90 days, and characterized according to the contact angle, (Table 1). From the contact angles found at time zero, it was observed that the membrane has a hydrophilic characteristic, which corroborates data from a previous study by our group [19]. Krupp et al. [11], suggested that natural rubber membranes incorporated with polymerized propolis extract at temperatures of 27 °C and 60 °C, also presented hydrophilic characteristics, with contact angles of 64.7 and 67.6, respectively.

When analyzing the aging time membranes, in all conditions studied, it was observed that there was no change in relation to hydrophobicity, since the contact angles remained very similar. This hydrophilicity and high molecular weight of biopolymers can provide good biocompatibility, mechanical strength and viscosity as adhesive matrix materials [29,30].

Wettability is one of the parameters for assessing the biocompatibility of material, therefore, the results found indicate that NRC membranes are a promising material, reinforcing its potential for biomedical applications, such as for the development of dressings. Our results showed that, even after 90 days of storage, NRC membranes showed hydrophilic surfaces. This characteristic is favorable, as it causes low cell adhesion to occur in the new tissue that is being formed during the healing process, reducing pain and discomfort during changes and removal of the dressing [9].

3.2. Thermal analysis

3.2.1. Thermogravimetric analysis (TGA)

The steps of thermal degradation were analyzed in relation to the loss of mass of the NR and NRC membranes, as well as the copaiba extract, as can be seen in the thermograms below (Fig. 2).

Our results showed that between 25 °C and 250 °C there is a mass loss of 5 % in NRC, which is related to the presence of moisture. The degradation of the NRC membrane starts around 235 °C and is associated with the scission of natural rubber chains without loss of unsaturation. The maximum decomposition range occurred between 300 °C and 400 °C, with a mass loss of 72 % for NRC. This greater mass loss may be related to the depolymerization of isoprene and the scission of the main chain with the formation of small segments of chains with a radical at the end (oxidative pyrolysis of rubber), resulting in thermodegradation of hydrocarbons [31].

In the literature, studies that relate the thermodynamics of natural rubber with other compounds are scarce. However, several studies suggest that the addition of compounds to polymers can modify the thermal behavior of a polymer through specific interactions [32]. However, this type of study for natural rubber membranes is scarce in the literature.

In relation to natural rubber, Dall'Antonia et al. [33] observed that

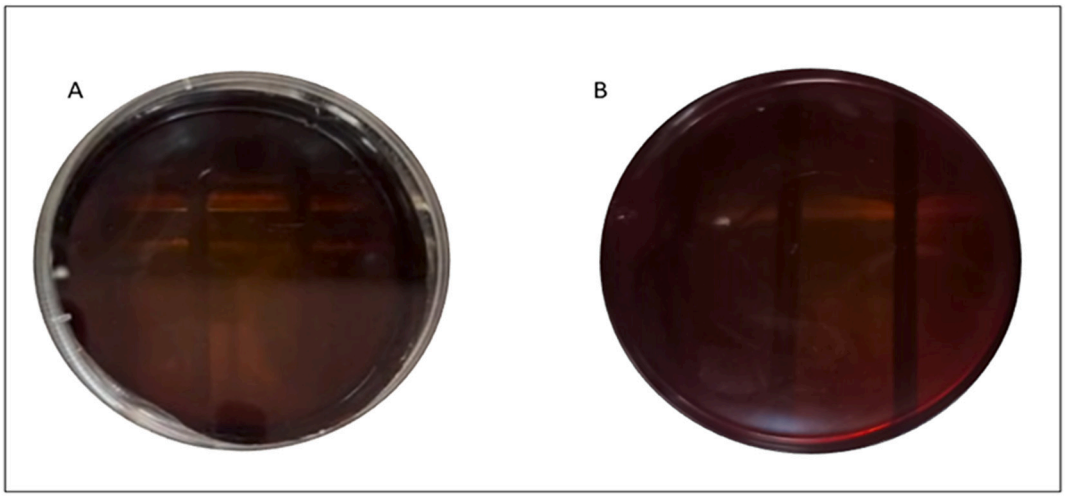

Fig. 1. Macroscopic analysis of natural rubber-copaiba (NRC) membranes A - Time zero and B - Packed for 90 days.

Table 1
Mean values and standard deviation of contact angles of natural rubber membranes - copaiba (NRC) at times zero, 30, 60 and 90 vacuum packed and in the absence of vacuum and presence or absence of light. Group 30 days - NRC30 (NRC30VD – natural rubber - copaiba membranes 30 days vacuum packed and stored in the absence of light; NRC30D – natural rubber - copaiba membranes 30 days packaged in the absence of vacuum and stored in the absence of light; NRC30VL – natural rubber - copaiba membranes 30 days vacuum packed and stored in the presence of light; NRC30L - natural rubber - copaiba membranes 30 days packed in the absence of vacuum and stored in the presence of light), Group 60 days - NRC60 (NRC60VD – natural rubber - copaiba membranes 60 days vacuum packed and stored in the absence of light; NRC60D – natural rubber - copaiba membranes 60 days packaged in the absence of vacuum and stored in the absence of light; NRC60VL – natural rubber - copaiba membranes 60 days vacuum packed and stored in the presence of light; NRC60L - natural rubber - copaiba membranes 60 days packed in the absence of vacuum and stored in the presence of light) and group 90 days - NRC90 (NRC90VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC90D – natural rubber - copaiba membranes 90 days packaged in the absence of vacuum and stored in the absence of light; NRC90VL – natural rubber - copaiba membranes 90 days vacuum packed and stored in the presence of light; NRC90L - natural rubber - copaiba membranes 90 days packed in the absence of vacuum and stored in the presence of light) compared to time zero membrane.

Membranes	Contact angle ($\theta c \pm \sigma$)
NRC0	68.92 ± 6.7
NRC30L	63.25 ± 1.6
NRC30VL	74.46 ± 0.8
NRC30D	55.57 ± 1.7
NRC30VD	66.68 ± 2.5
NRC60L	68.55 ± 1.1
NRC60VL	64.09 ± 3.0
NRC60D	67.70 ± 1.0
NRC60VD	58.77 ± 1.1
NRC90L	59.12 ± 1.7
NRC90VL	76.01 ± 0.9
NRC90D	62.44 ± 1.8
NRC90VD	61.38 ± 2.1

the loss of mass of natural rubber is accentuated at a temperature close to 300 °C, where the structural degradation of the rubber begins, which loses about 50 % of mass up to a temperature of 360 °C, corroborating data found in the present study, since natural rubber was used as a matrix for incorporating the copaiba extract.

In the interval between 400 °C and 500 °C, another stage of mass loss occurred, which may be related to the thermal decomposition of the carbon residues. After this stage, no additional mass loss occurs, suggesting that only inorganic species remain, such as oxides, carbonates, phosphates, which may be related to impurities present in the latex.

Copaiba hydroalcoholic extract has its first stage of mass loss around 101 °C, which is associated with loss of moisture and volatile compounds from the extract. A second stage between 135 °C and 365 °C is associated with greater mass loss of the copaiba extract and the third stage is between 418 °C and 515 °C. These last two stages may be related to copaiba pyrolysis which occurs at temperatures close to 400 °C. Debone et al. [17] also observed copaiba pyrolysis around this temperature in chitosan films incorporated with copaiba oleoresin, suggesting that the compounds present in the copaiba fruit extract have thermal characteristics similar to those of copaiba oil.

Similarly, Pascoal et al. [34], impregnating bioactive compounds of *Copaifera* sp. in three-dimensional gelatin dressings, it was observed that in the extract of the copaiba leaf at 96 °C there was an evaporation process, and the degradation at 307 °C and 419 °C, when purchased with the oleoresin of the same study, copaiba oil presented two characteristic degradation events. The first event at 167 °C and the second around 302 °C. When relating to the fruit extract adopted in our study, it was observed that it presented a profile more similar to the extract of copaiba leaves.

In the analysis of NRC membranes of the times 30, 60 and 90 days, it was observed that the members of the different time points presented a profile similar to the zero-time membrane (Fig. 3).

All membranes obtained the same maximal decomposition results in the temperature range between 300 °C and 400 °C. By analyzing at lower temperatures, it was observed that the initial mass loss up to 100 °C was very small for all membranes. These results indicate high thermal stability of the membranes, both zero-time membranes and 90-day membranes.

3.2.2. *Differential exploratory analysis (DSC)*

In Fig. 4, the DSC data for the NRC membrane is shown. An endothermic peak at 368 °C is observed, which is characteristic of fusion; already at 391 °C, an exothermic peak of oxidation processes can be noticed. An endothermic event was observed at 102 °C of fusion and two exothermic peaks at 333 °C and 456.66 °C represent the oxidation and degradation processes of the copaiba extract constituents.

The exothermic peaks in the membrane may be related to the degradation process of the polymeric matrix of the isoprene chain.

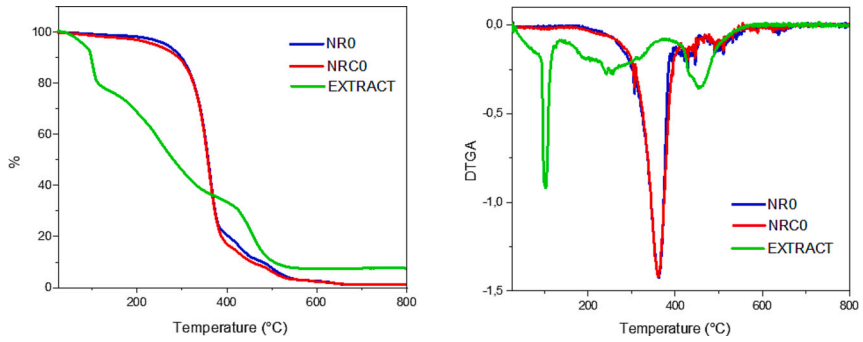

Fig. 2. A - Thermogravimetric analysis (TGA) of pure natural rubber (NR) membranes and natural rubber membrane - copaiba (NRC0) at time zero and copaiba extract. B- DTGA of pure natural rubber membranes (NR) and natural rubber membrane - copaiba (NRC0) at time zero and copaiba extract.

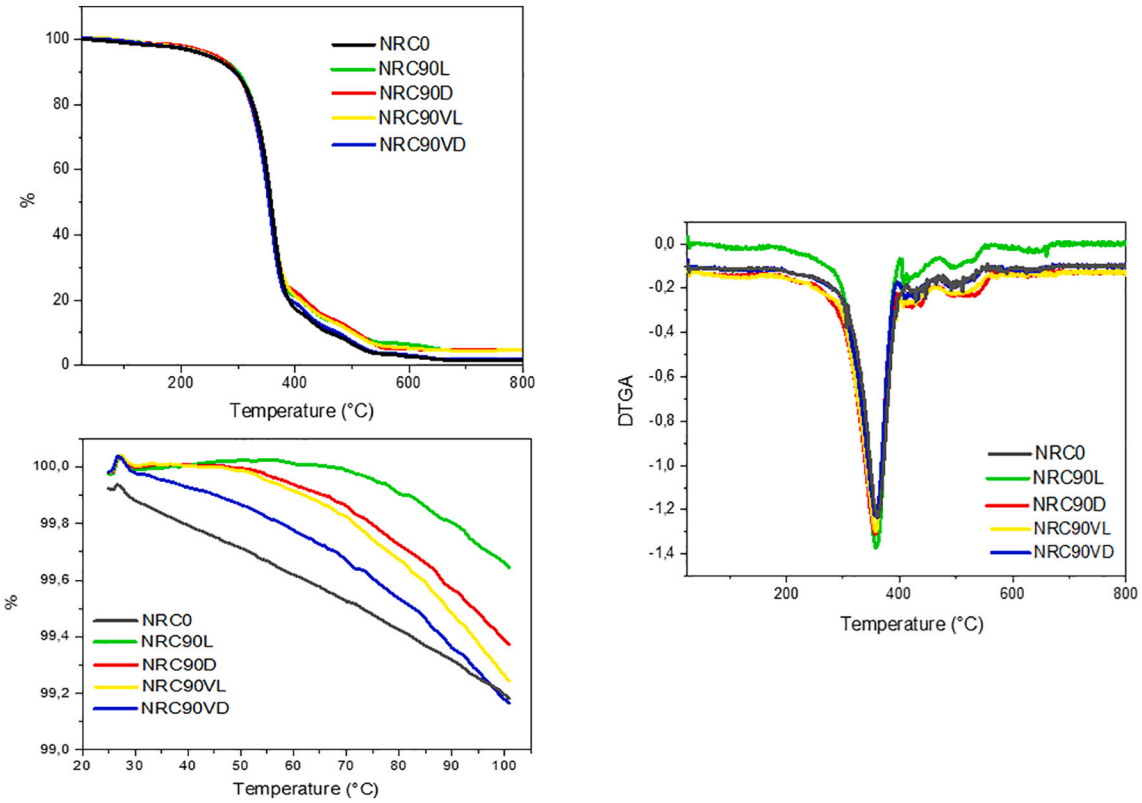

Fig. 3. A - Thermogravimetric analysis (TGA) of natural rubber membranes - copaiba (NRC0) at time zero and 90 days up to 800 °C. B - Thermogravimetric analysis (TGA) of natural rubber membranes - copaiba (NRC0) over time zero and 90 days up to 100 °C. C - DTGA of natural rubber-copaiba membranes (NRC) at zero and 90 days. NRC90VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC90D – natural rubber - copaiba membranes 90 days packaged in the absence of vacuum and stored in the absence of light; NRC90VL – natural rubber - copaiba membranes 90 days vacuum packed and stored in the presence of light; NRC90L - natural rubber - copaiba membranes 90 days packed in the absence of vacuum and stored in the presence of light.

Boscolli et al. [31], when obtaining composites of natural rubber with industrial residue of leather crosslinked with different peroxides, found values at 370 °C, which is related to the degradation of the polymeric matrix of natural rubber. At 400 °C, copaiba pyrolysis occurs [17], which corroborates data with those found in the thermogravimetric analysis, indicating the thermal stability of the NRC membrane, which did not change even with the incorporation of copaiba extract, as well as the temperature of degradation for the copaiba extract which was around 456 °C.

In Fig. 5, the DSC data of the natural rubber membranes with copaiba (NRC) from time zero and 90 days are represented. In the membranes of the 90 days group, more evident exothermic peaks appear in the region between 383 °C and 388 °C, characteristic of the oxidation process, as well as the zero-time membrane of natural rubber with copaiba (NRC), and these peaks may be related to processes of oxidation and oxidative degradation of the natural rubber polymeric chain. These results are also related to the degradation of Copaiba compounds, being one more indication that the material, even with the incorporation of the extract, remains thermally resistant even over time, as well as the zero-time membrane. At temperatures lower than 25 °C to 100 °C we can observe these same characteristics in the membranes.

The thermogravimetric analyses TGA and DSC corroborates the hydrophilicity data found through the analysis of the contact angle of the membranes, where no differences related to membrane hydrophilicity were found in relation to the storage time and type of packaging used, as well as there was no difference in the thermal stability of the membranes under the same conditions analyzed.

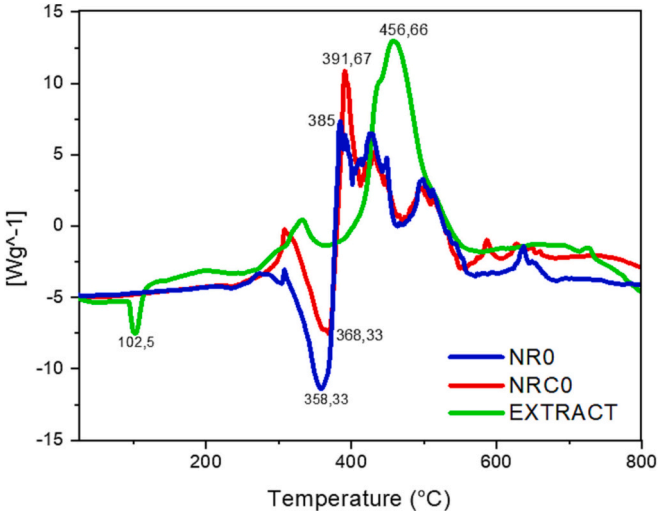

Fig. 4. Differential scanning calorimetry (DSC) of pure natural rubber (NR), natural rubber - copaiba (NRC0) membrane and pure copaiba extract at time zero.

3.3. Fourier transform infrared spectroscopy (FTIR)

Fig. 6, is represented the spectra of pure natural rubber, natural rubber with copaiba and copaiba extract. When analyzing the natural rubber spectra incorporated with copaiba extract, it was observed that the characteristic bands of poly(1,4-cis-isoprene) remain, presenting similarity with pure natural rubber and indicating that there is no formation of a new compound, only the interaction between these materials.

In the spectra of pure natural rubber there is a broadband between 3500 and 3200 cm^{-1} corresponding to the elongation of O—H (hydrogen bonds) by the presence of carboxylic groups. Between 2965 and 2850 cm^{-1} it is the result of the asymmetric stretch of CH_3 and CH, and symmetrical of CH_2, respectively. In 1658 cm^{-1} C═C binding characteristic of primary amide, assigned to proteins and polypeptides bound to natural rubber. The region of 1450 and 1380 cm^{-1} corresponds to the asymmetric angular deformation of CH_3 and CH_2 and the symmetrical of the methyl group, respectively. The band in the 840 cm^{-1}

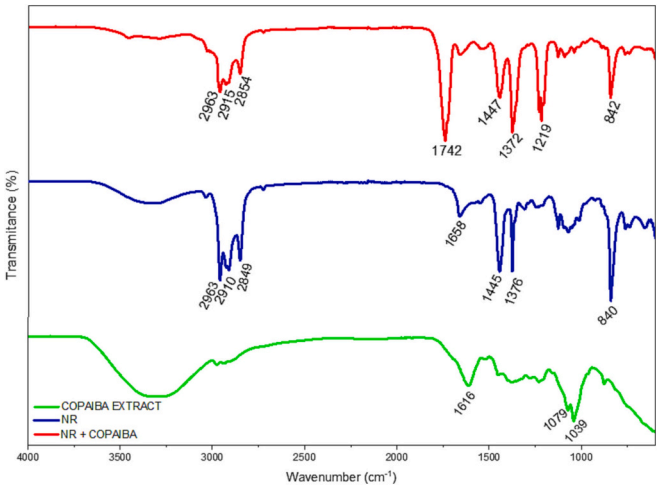

Fig. 6. Infrared Spectrum (FT-IR) pure natural rubber membrane, natural rubber-copaiba membranes from time zero. With 4 cm^{-1} resolution in the region between 4000 and 600 cm^{-1}.

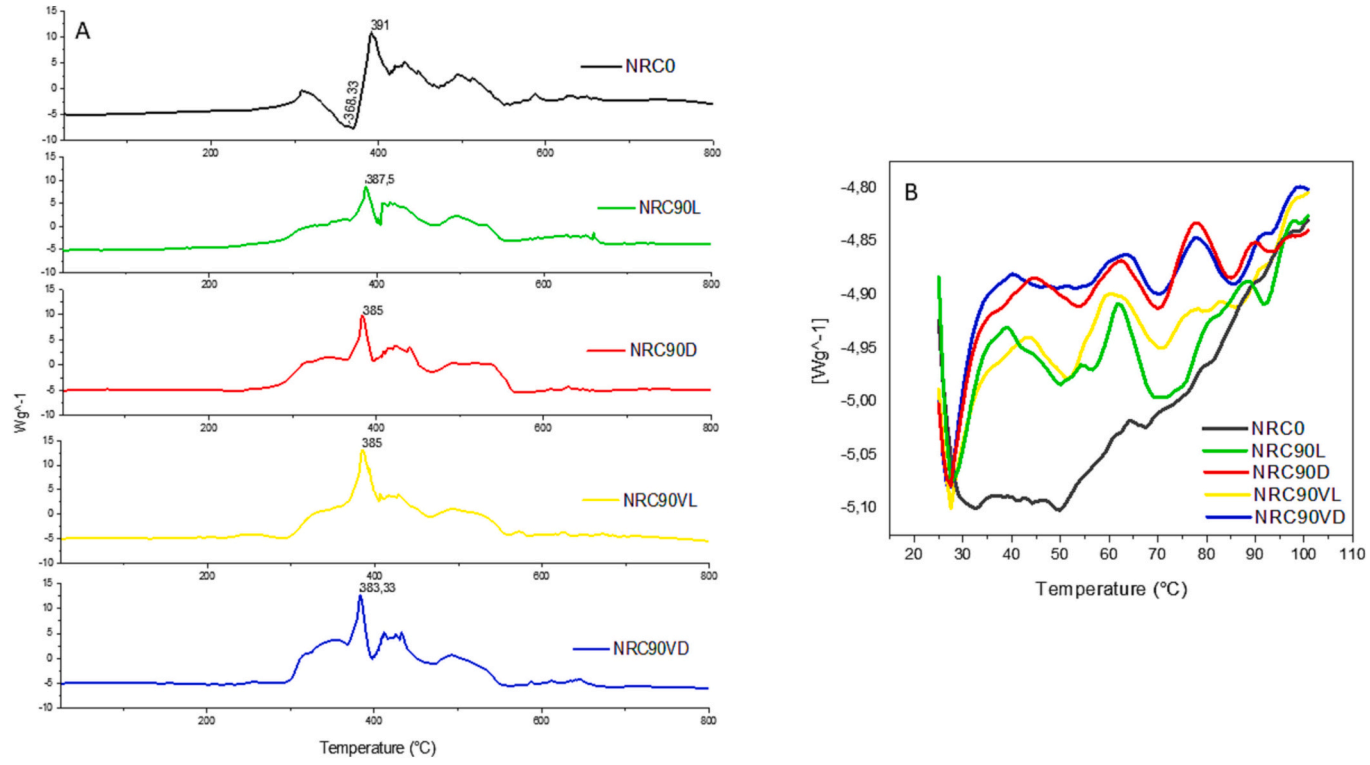

Fig. 5. A - Differential scanning calorimetry (DSC) of natural rubber - copaiba membrane (NRC0) Time zero and 90 days at 800 °C. B - Differential scanning calorimetry (DSC) of natural rubber - copaiba membrane (NRC0) Time zero and 90 days at 100 °C. NRC90VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC90D – natural rubber - copaiba membranes 90 days packaged in the absence of vacuum and stored in the absence of light; NRC90VL – natural rubber - copaiba membranes 90 days vacuum packed and stored in the presence of light; NRC90L - natural rubber - copaiba membranes 90 days packed in the absence of vacuum and stored in the presence of light.

region features C—H bond deformation (double bonding) features the R2C=CHR function (cis-1.4). Characteristic bands of poly-cis-1,4-isopropylene were found in regions close to those described in the literature, in the regions of 836, 1450, 2970 cm^{-1} [30,35,36,37,38].

In the spectra of the pure extract of copaiba we can observe broad-band in the region 3600 to 3100 cm^{-1} corresponding to the OH stretch derived from phenol groups. At 2948 cm^{-1} typical asymmetric and symmetrical stretch of CH_2. In 1616 cm^{-1} angular deformation C=C is characteristic of monoterpenes. In region 1080 to 1030 cm^{-1} stretch C—O, C—C, CCO is derived from fatty acids.

In the spectrum of natural rubber with copaiba extract, we can observe the presence of characteristic bands of natural rubber, some bands only changing the intensity of these. At 1742 cm^{-1} we can identify stretch =C. What can be attributed to primary amine grouping. In the region between 1450 and 1375 cm^{-1} angular deformations of CH_2 and CH_3 may be associated with flavonoids and aromatic rings. In 1219 cm^{-1} related to c-o grouping related to polyols. In NRC membranes of the aging time of 60 and 90 days, an enlargement of the bands in the region 1620 cm^{-1} is observed, except for the NRC90VC membrane (vacuum-packed NRC membrane, maintained in the presence of light for 90 days). In the membranes of the 60-day group, one can observe a band in the region 1750 cm^{-1}, which can be attributed to functional groups C=O, however in the group 90 days this band does not appear. In the region between 3500 and 3200 cm^{-1} there is an intensification in the OH band in groups 60 and 90 days in relation to the 30-day group (Fig. 7).

In the FTIR analyses of the aging time, it can be observed that there were no changes in the bands of polycis-1,4-isoprene, occurring alterations in the bands of the non-rubber groups that are related to the oxygenated groups. These bands may be in overlap with bands of compounds in very close regions and during the aging time analyzed they became more evident.

3.4. Scanning Electron Microscopy (SEM)

The micrographs of the membranes at time zero and those of the groups 30, 60 and 90 days with the presence and absence of light packed in vacuum and absence of vacuum were analyzed and compared (Fig. 8).

Micrographs were taken to analyze the surface morphology of NRC membranes. Rubber particles from the latex coagulation process during the drying process in an oven, the presence of stable and coalesced rubber particles are observed. In addition, crystalline aggregates of copaiba dispersed in the membrane can be observed, and the copaiba particles are well dispersed in the matrix, indicating good sample processing conditions. The pores in the membrane were not observed, during the metallization process these may have been filled by the conductive material Au (gold) used in the metallization. Another factor that may be related to the absence of pores is the temperature used for the polymerization of the material.

The morphology of a polymeric compound depends on several factors, among them we can mention: interfacial adhesion, viscosity ratio, components and processing conditions. Being able to, through the morphological analysis of a material, observe the homogeneity, the crystallinity, as well as its phase changes of this material [39].

In a study carried out by Herculano et al. [40], when developing natural rubber membranes for the release of drugs that polymerized at

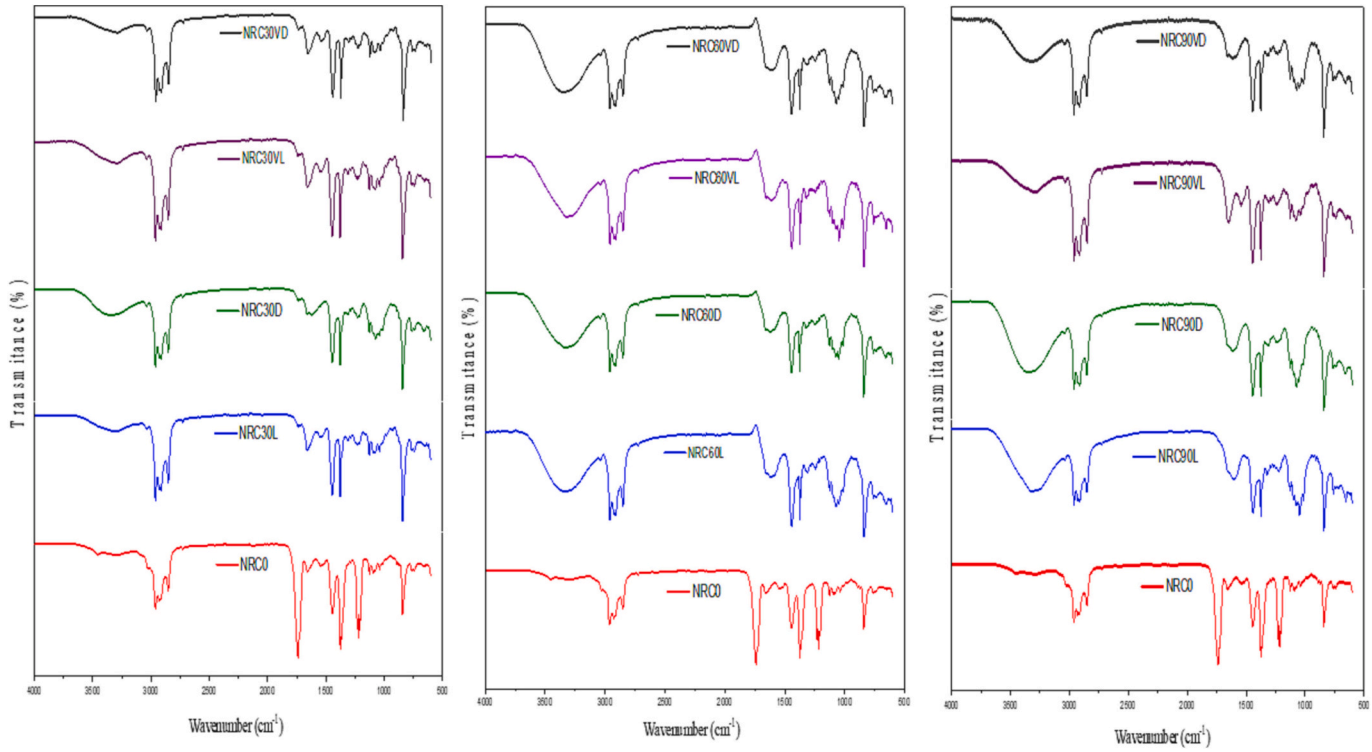

Fig. 7. FT-IR infrared analysis spectra of natural rubber - copaiba membranes. A - NRC group 30 days - NRC30 (NRC30VD – natural rubber - copaiba membranes 30 days vacuum packed and stored in the absence of light; NRC30D – natural rubber - copaiba membranes 30 days packaged in the absence of vacuum and stored in the absence of light; NRC30VL – natural rubber - copaiba membranes 30 days vacuum packed and stored in the presence of light; NRC30L - natural rubber - copaiba membranes 30 days packed in the absence of vacuum and stored in the presence of light), B - NRC group 60 days - NRC60 (NRC60VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC60D – natural rubber - copaiba membranes 60 days packaged in the absence of vacuum and stored in the absence of light; NRC60VL – natural rubber - copaiba membranes 60 days vacuum packed and stored in the presence of light; NRC60L - natural rubber - copaiba membranes 60 days packed in the absence of vacuum and stored in the presence of light) and C-NRC group 90 days - NRC90 (NRC90VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC90D – natural rubber - copaiba membranes 90 days packaged in the absence of vacuum and stored in the absence of light; NRC90VL – natural rubber - copaiba membranes 90 days vacuum packed and stored in the presence of light; NRC90L - natural rubber - copaiba membranes 90 days packed in the absence of vacuum and stored in the presence of light) compared to time zero membrane.

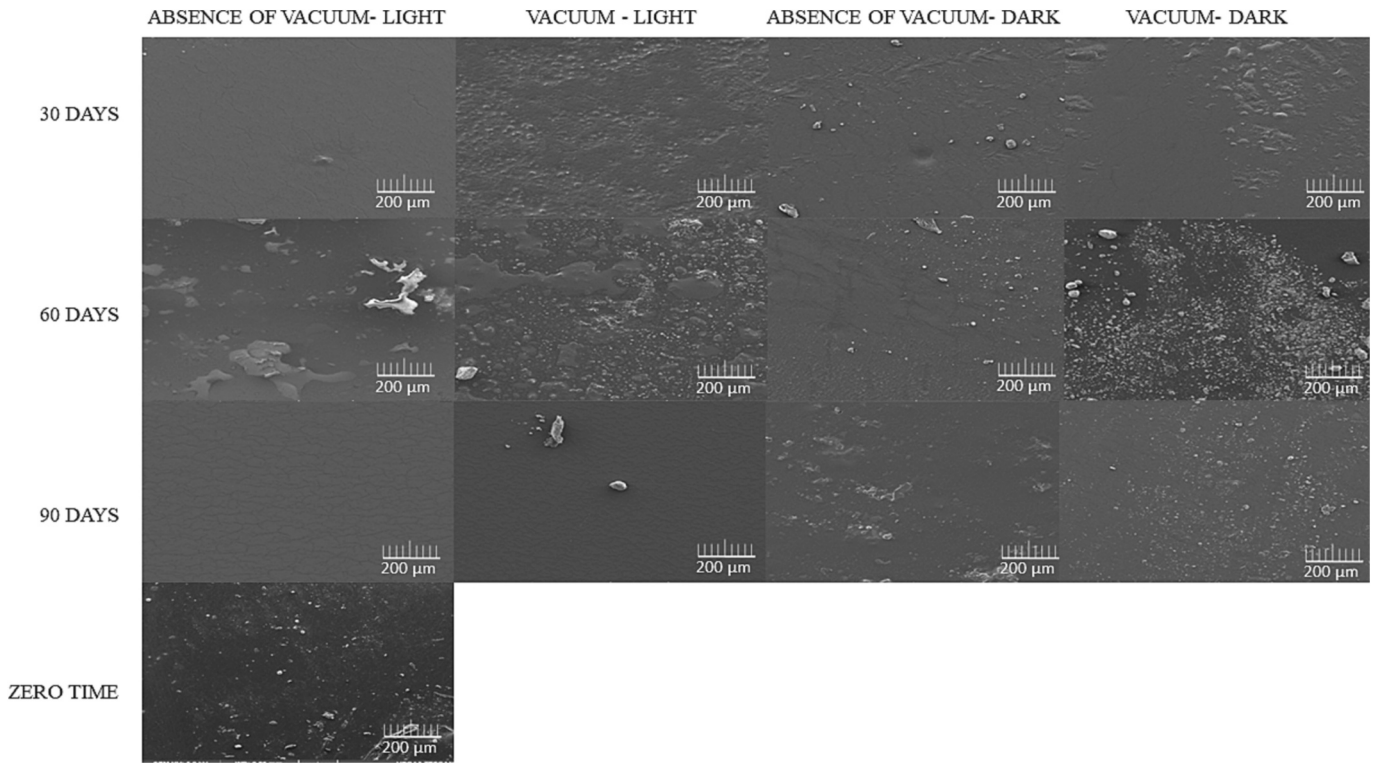

Fig. 8. Scanning electron microscopy of natural rubber - copaiba membranes packed in plastic packaging in the absence and presence of vacuum, in the presence or absence of light, at times zero, 30, 60 and 90 days using a voltage of 5.0 Kv and 10 Pa.

different temperatures (−10°, −1° and polymerized at room temperature), did not present pores in their structure when temperature environment was adopted. On the other hand, at other temperatures analyzed, the presence of pores was noticed, showing that the number of pores and the general dimensions of the pores in pure natural rubber membranes and those loaded with compounds of pharmacological interest are inversely proportional to the polymerization temperature. Likewise, Barros et al. [36] observed that natural rubber membranes loaded with alginate polymerized at room temperature also did not show pore formation on the surfaces of the membranes, which is yet another indication that the temperature used for the process of polymerization influences this characteristic.

In another study carried out by Morise et al. [30], pure natural rubber membranes incorporated with Scopolamine polymerized at 36 °C, no pores were found on the surface either.

Our results show, under the studied conditions, that the surfaces of the membranes of the 30, 60 and 90 days groups, in the presence or absence of light and vacuum packed or in the absence vacuum, remained with the same porosity characteristic, that is, regardless of the time of storage and the condition of this did not occur the appearance of pores during the analyzed period.

The NRC30C membranes (NRC membrane packed without vacuum, maintained in an environment with light for 30 days), NRC90C (NRC membrane packed absence of vacuum, maintained in the presence of light for 90 days) and NRC90E (NRC membrane packed with absence of vacuum, maintained in the absence of light for 90 days) presented a smoother surface when compared to the other membranes. The fact that surfaces appear smoother in certain groups may not be related to the conditions analyzed, but rather to the polymerization of these membranes during the kiln drying process, in which rubber particles and copaiba particles did not follow a pattern of organization on the surface of the material.

3.5. Antioxidant activity

The ability to scavenge free radicals in relation to the stable radical DPPH was chosen because it is a simple, fast and sensitive methodology [41] An analysis of the antioxidant activity of NRC membranes at time zero and 90 days was carried out. The membranes that were in the presence of light showed a slower oxidizing activity initially, with a gradual increase. The NCR membranes kept without the presence of light, in turn, initially showed a more pronounced capacity, but in the end the membranes showed a similar profile of antioxidant activity compared to the zero-time membrane. The NRC90C membrane showed a lower oxidizing capacity compared to the others (Fig. 9).

In a previous study of our group [19], a natural rubber membrane with Copaíba at the concentration of 75 mg/mL presented values close to 177 %, presenting a similar profile with the results found, being another indication of biomaterial resistance, reinforcing the great potential for the formulation of dressings with biological activities for biomedical applications.

Economic interest in substances with antioxidant activity has been increasing in recent years since natural antioxidants are safer and as efficient as synthetic ones. The bioactive compounds present in biomaterials are known to have therapeutic effects, reparative and, mainly, by antioxidant properties, since they are able to fight free radicals produced during oxidation processes [23].

Biomaterials that exhibit antioxidant properties can help manage the oxidative stress of injured tissues and thus accelerate wound healing and tissue repair [42]. In view of the results found, the natural rubber-copaiba membrane is a promising material for the development of dressings for wound treatments.

4. Conclusion

Aging assays showed that membranes incorporated with copaiba extract remained hydrophilic regardless of storage conditions.

When thermally analyzing the membranes, it was observed that they

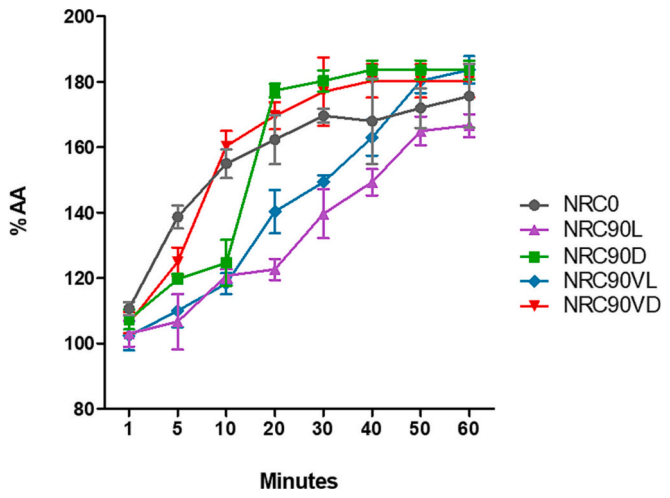

Fig. 9. Percentages of antioxidant activity of natural rubber - copaiba membranes packed in plastic packaging in the absence of vacuum and vacuum with presence and absence of light at zero and 90 days. NRC90VD – natural rubber - copaiba membranes 90 days vacuum packed and stored in the absence of light; NRC90D – natural rubber - copaiba membranes 90 days packaged in the absence of vacuum and stored in the absence of light; NRC90VL – natural rubber - copaiba membranes 90 days vacuum packed and stored in the presence of light; NRC90L - natural rubber - copaiba membranes 90 days packed in the absence of vacuum and stored in the presence of light.

remained stable throughout the study period. Regarding the chemical structure, there was no appearance of chemical compounds that could change the chemical structure of the material. Scanning microscopy analyzes showed that there were no changes in membrane morphology. In addition, membranes packaged for 90 days still showed favorable antioxidant activity.

Funding

This work was supported by the Coordination of Higher Education Personnel Improvement (CAPES), Brazil.

CRediT authorship contribution statement

Luana Caroline Hochberger: Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Jair Marques Junior:** Methodology, Formal analysis. **Loyane Almeida Gama:** Methodology, Validation, Formal analysis, Visualization. **Wagner Welber Arrais-Silva:** Writing – review & editing. **Nara Cristina de Souza:** Resources, Writing – review & editing. **Paula Cristina de Souza Souto:** Conceptualization, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Luana Caroline Hochberger reports financial support was provided by Coordination of Higher Education Personnel Improvement. Loyane Almeida Gama reports financial support was provided by Foundation for Research Support of Mato Grosso State. Paula Cristina de Souza Souto reports a relationship with Federal University of Mato Grosso that includes: employment. Wagner Welber Arrais-Silva reports a relationship with Federal University of Sergipe that includes: employment. Nara Cristina de Souza reports a relationship with Federal University of Goias that includes: employment.

Acknowledgements

This research received grants from the Coordination for the Coordination of Higher Education Personnel Improvement (CAPES). Thanks to the Nanostructured Materials Laboratory and the Araguaia Multiuser Research Laboratory (CPMUA).

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