



## Characterization and Microbiological Application of Ciprofloxacin Loaded in Natural Rubber Latex Membranes

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors RDH and BCG designed the study. Authors FAB and NRB performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RES, KN, MCRM, IUM, PD, JLP, BHO, VMGN and AVL managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

Natural rubber latex (NRL) from *Hevea brasiliensis* presents interesting characteristics in biomedical research due to its ability to stimulate angiogenesis, cellular adhesion and formation of extracellular matrix. A broad spectrum antibacterial agent used in skin infection is Ciprofloxacin hydrochloride (CIP). It is a fluoroquinolone employed to treat different bacterial infections caused by gram-positive and gram-negative microorganism. The aim of the present study is to promote

the CIP incorporation on the natural material due to develop a biomaterial which could accelerate tissue repair and control wounds infection. The biomaterial characterization was made through several technics. The compound presence on the membrane was observed by Scanning Electron Microscopy technique, which shown crystals merged on the material surface. Through X-Ray Powder Diffraction technic was possible to notice the absence of crystallinity (amorphous structure) in the drug-loaded NRL membrane indicating that the drug is molecularly dispersed within the NRL. Besides that, there are no molecular interaction between drug and NRL showed by Fourier Transform Infrared Spectroscopy neither Raman spectroscopy. Additionally, there were no significant changes in the NRL pyrolysis profile when the drug was added, indicating weak interaction between both compounds, as presented by Derivative Thermogravimetric Analysis assay. Comparing the mechanical properties, both samples (with and without CIP) had a similar behavior. Thus, is possible to presume the material preserved enough elasticity to medical application. Furthermore, antimicrobial assay proved that CIP activity was preserved after its incorporation to the NRL. It allows us to conclude that the material has potential application in infected wound treatment.

**Keywords:** Natural rubber latex; biopolymers; drug delivery system; ciprofloxacin; biomaterials; membranes; antibacterial activity.

## 1. INTRODUCTION

### 1.1 Drug Delivery Systems

Pharmaceutical industry is constantly updating its technology due to provide a drug with high efficiency and more attractive properties. Among the characteristics which have been seeking are the decrease of side effect, a faster drug response and high specification. Conventional drug formulations are mostly made in higher concentration and with a short half-life, these shortcomings lead the patient to consume more drug than is necessary to cause the desired effect [1,2]. These can results in side effects increase, allergies, and antibiotic resistance.

Drug Delivery System (DDS) shows up as an alternative to overcome these issues. This method allows interaction between drug and the target organ/tissue in a more specific way. DDS consists on delivering the drug in a lower concentration, a controlled form along a previously established period of time [3,4]. It brings advantages as the reduction in the frequency of the drug dosages taken by the patient, more uniform drug effect, reduction of drug side-effects, and reduced fluctuation in circulating drug levels.

Biomaterials have been seen as an interesting tool for DDS. A widely number of materials has been explored to this application. Different sources has been used, such as metallic, ceramic and polymeric [4,5].

### 1.2 Natural Rubber Latex

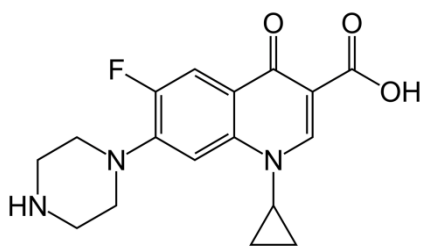
A polymeric material with interesting characteristics in DDS is the natural rubber latex (NRL). It consists in cis-1,4-polyisoprene, extracted from *Hevea brasiliensis*, widely employed as raw material. In the industry, the material is found in the manufacturing of gloves, condoms, balloons, and other medical and dental devices [1,3,6]. The mechanical properties are its strengths; its of easy manipulation, low cost product and presents high mechanical resistance.

The material has also increased biomedical interest due to its angiogenic properties [7-10]. Studies have presented NRL capacity to stimulate vascularization and ability to induce tissue regeneration [8,11]. Since then, the NRL has been used for various therapeutic purposes such as, palate reconstruction in dogs, stomach regeneration in rabbits and the treatment of ulcers of different etiologies [6-11].

### 1.3 Ciprofloxacin

Ciprofloxacin hydrochloride (CIP),  $C_{17}H_{18}FN_3O_3$  (Fig. 1), is a broad spectrum fluoroquinolone antibacterial agent. Fluoroquinolones inhibit DNA gyrase, which is an enzyme that plays a key role in metabolism and bacterial reproduction. There are two quinones generation, and the second-generation is the group which CIP is on [12,13]. Antibiotics from this generation have shown activity against Gram-negative and Gram-positive bacteria and also against certain

pathogens [14]. It makes CIP an antibiotic with potential application on wound infections.



**Fig. 1. Structural formula of ciprofloxacin hydrochloride**

The present study aims to develop a membrane of NRL loaded with CIP to accelerate tissue repair and control wounds infection. Furthermore, it is focus on the characterization of the new biomaterial as its structure, morphology and resistance and also assesses its microbiological activity.

## 2. MATERIALS AND METHODS

### 2.1 Membranes

#### 2.1.1 Natural rubber latex

Due to prepare the NRL membranes, it was used the commercial natural latex (BDF Rubber Latex Co. Ltd., Guarantã, Brazil). The material consists in about 60% dry rubber content (DRC), 4-5% weight of non-rubber constituents such as protein, lipids, carbohydrates, and sugar, and 35% of water [15]. After it been extracted, ammonia was used to maintain the latex liquid. A centrifugation processes was performed at 8,000 g [3,7] to reduce protein contents related with allergies.

#### 2.1.2 Ciprofloxacin

CIP powder obtained from gel capsules (Callithea Pharmaceuticals Ltd., Brazil). The encapsulated drug is excipient free. Only the powder was used to perform the experiment. CIP was solubilized in water to facilitate the membranes preparation and a concentration of 10 mg.mL<sup>-1</sup> was adopted.

#### 2.1.3 Membrane production

NRL loaded with CIP (10 mg.mL<sup>-1</sup>) membranes were prepared by casting method [1]. The membranes presented 30 mg of CIP and 1:1 of CIP solution to natural rubber latex solution.

Then, the resulting solution was poured into Petri dishes and maintained at room temperature (25°C) for 48 h until complete polymerization. The CIP in the solution was analyzed by measuring the UV-VIS spectra (Bel Engineering SF200 ADV spectrophotometer), as the drug has its maximum absorption at 275 nm.

### 2.2 Characterization

#### 2.2.1 Scanning electron microscopy (SEM)

To observe the NRL surface morphology, SEM (model Zeiss® EVO 50) was performed at an accelerating voltage of 20 KV and a take off angle of 35°.

#### 2.2.2 X-ray powder diffraction (XRD)

The materials crystal phase was identified by XRD (Siemens D5005) using X-ray diffractometer and a graphite crystal as monochromator to select Cu Kα1 radiation (1.5406 Å), in a step of 0.02° s<sup>-1</sup>.

#### 2.2.3 Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of CIP powder, NRL membrane and NRL + CIP membrane were obtained to prove the chemical integrity of the drug in the polymer. The samples were measured directly by Attenuated Total Reflection (ATR) method, which is an excellent method for obtaining infrared information for the powder sample surface. The membranes were characterized using a TENSOR T 27 (Bruker, Germany) (4000-500 cm<sup>-1</sup>) with a resolution of 4 cm<sup>-1</sup> with 16 scans.

#### 2.2.4 Raman spectroscopy

The FT-Raman spectrum has been recorded using 632.81 nm line of He-Ne laser as excitation wavelength in the region 50-3500 cm<sup>-1</sup> on a HORIBA JOBIN-YVON model LabRAM HR 800 spectrometer equipped with a CCD camera model DU420A-OE-325. It was analyzed samples of CIP (powder), NRL and NRL + CIP.

#### 2.2.5 Thermogravimetric and derivative thermogravimetric analysis

Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves were obtained from NRL membrane, NRL + CIP membrane and CIP powder. These samples were studied by TG

using SDT Q600 V8.3 Build 101 thermal analyzer instrument, ranging from 25 to 1000°C at a heating rate of 10°C min<sup>-1</sup>, using alumina pans under dynamic nitrogen atmosphere (100 mL min<sup>-1</sup>).

### 2.2.6 Mechanical assay

Tensile tests were carried out in a EMIC DL2000 fitted with 10 kgf load cell at a rate of 500 mm/min (according to ASTM D412). To perform the assay, NRL samples (44 × 15 × 1.0 mm, length × width × thickness), with and without CIP, were prepared. The membranes were submitted to elongation to failure. The tensile tests by pulling apart the specimen were performed at room temperature.

### 2.3 Antibacterial Activity

#### 2.3.1 Bacteria strain

In order to perform the antibacterial assay, *Burkholderia lata* (LBBIO-BL02) was isolated from a fungal culture contamination and identified by morphological and molecular methods (16S RNA) at Fundação André Tosello (FAT), Campinas - SP - Brazil [16].

#### 2.3.2 Broth dilution method

Flasks in triplicate containing 8 mL of LB (Luria Bertani) broth were incubated at 28°C for 48 h.

The control 1 (C1) was LB broth without inoculation, control 2 (C2) was LB broth inoculated with *B. lata*, control 3 (C3) was LB broth inoculated with bacteria and the NRL membrane. The Experiment (E1) had in its composition bacteria and NRL incorporate with CIP (Table 1).

## 3. RESULTS AND DISCUSSION

### 3.1 Scanning Electron Microscopy Analysis

SEM is a very versatile technique, which is mostly used to analyses microstructure of solids. The assay allows us to measure pore size and distribution, and the membranes composition, with and without CIP [17-19].

Fig. 2a brings CIP SEM image, where is possible to notice crystals of different sizes and shapes, closely united. The crystals formed a homogeneously matted surface of CIP. In Fig. 2b we can notice the presence of CIP crystals incorporated into the membrane. NRL when is not loaded with the drug, has a uniform surface morphology as showed in Fig. 2c, typical of amorphous material. It is possible to observe that the surface has been modified after CIP incorporation. As the drug merges with the NRL it forms a complete homogeneous coating of the drug crystals [1,20].

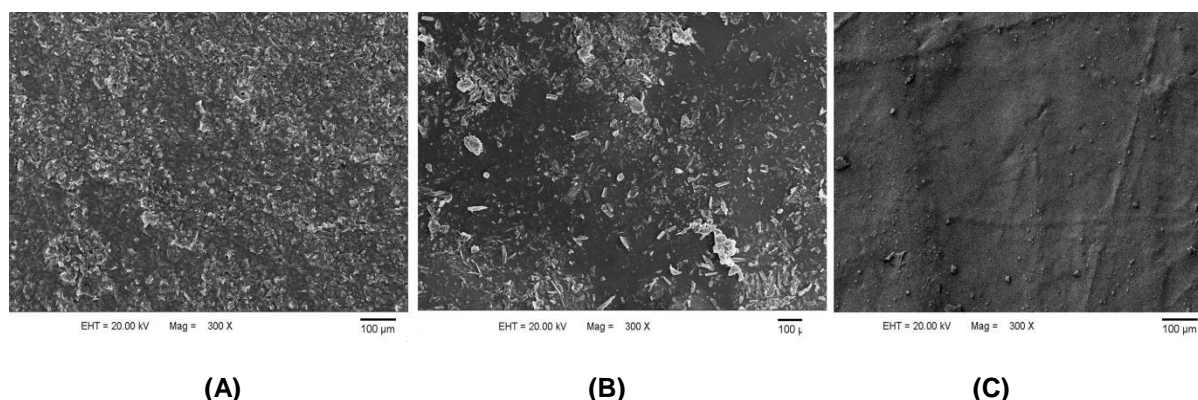


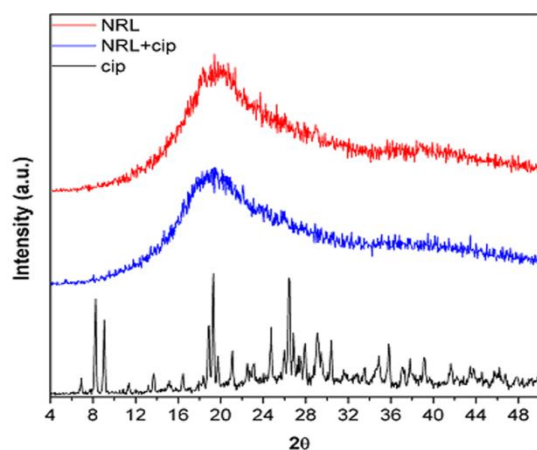
Fig. 2. 300x SEM morphology at an accelerating voltage of 20.000kv: a) CIP crystals, b-) NRL + CIP and c-) NRL membrane. Note the CIP presence on the NRL matrix (b)

Table 1. The cell growth inhibition assay

	C1	C2	C3	E1
Test tubes composition	LB broth	LB broth Bacterium NRL	LB broth Bacterium	LB broth Bacterium NRL + CIP

### 3.2 X-ray Diffraction Analysis

The main application of XRD refers to the identification of crystalline compounds, either organic or inorganic. This technique was used for structural characterization of NRL, NRL + CIP and CIP. Fig. 3 shows the diffractograms of NRL incorporated with the drug (NRL+CIP) and its components separately. The XRD patterns obtained for the NRL without CIP exhibits the amorphous structure of the material, as expected [21]. All peaks are related in accordance with the reference pattern obtained from the literature [22,23]. Due to CIP crystallinity, XRD has different pattern than an amorphous material, forming many peaks on the chart, as we can see in Fig. 3. After CIP had been incorporated into the membrane, peaks intensity have decreased. The absence of crystallinity in the drug-loaded NRL membrane indicates that the drug is molecularly dispersed within the NRL [24]. Studies have reported a reduction in peak intensity for drugs merged in polymers materials. Is is explained by the drug concentration found on the surface not being enough to provide peaks [25].



**Fig. 3. X-ray diffraction patterns of NRL+CIP (blue line), NRL (red line) and CIP powder (black line)**

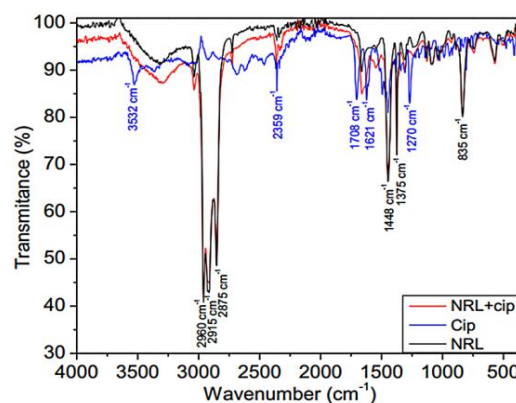
### 3.3 Fourier Transform Infrared Spectroscopy

FT-IR spectrum of NRL is presented in Fig. 4. The *cis*-1,4-polyisoprene absorption band of strong amplitude corresponding to =CH out of plane bending is observed at 835  $\text{cm}^{-1}$ . However, the *trans* isomer has no absorption at this wavelength [24,26,27]. The absorptions bands at 1375  $\text{cm}^{-1}$  and 1432  $\text{cm}^{-1}$  are characteristics of  $\text{CH}_2$  and  $\text{CH}_3$  asymmetric stretching, respectively. The  $\text{CH}_2$  symmetric stretching vibrations are observed at the region 2852  $\text{cm}^{-1}$ -

2925  $\text{cm}^{-1}$ . The  $\text{CH}_3$  asymmetric stretching in FT-IR of NRL is observed at 2961  $\text{cm}^{-1}$  [28]. In addition, Fig. 4 also shows a broad peak at approximately 3200-3500  $\text{cm}^{-1}$ . This absorption band might be related to the presence of a hydroxyl group, which appears to be generated after the hydrolytic ring opening of epoxy groups formed during the casting of NRL membranes.

In the FT-IR spectrum of the CIP (Fig. 4) the absorption band observed at 1708  $\text{cm}^{-1}$  corresponds to carbonyl group. Moreover, one prominent characteristic peak, between 3500 and 3450  $\text{cm}^{-1}$ , was assigned to OH stretching vibration (intermolecular hydrogen bonding). The bands at 1750 to 1700  $\text{cm}^{-1}$  represent carbonyl C=O stretching, i.e., C=O while the peak at 1650 to 1600  $\text{cm}^{-1}$  are assigned to quinolones. The bands at the 1450 to 1400  $\text{cm}^{-1}$  are attributed to uC-O and the ones at 1300 to 1250  $\text{cm}^{-1}$  suggest bending vibration of O-H group which indicated the presence of carboxylic acid [1,29-31].

In the FT-IR spectrum of the polymer and drug (Fig. 4) was not altered after immobilization in the latex membrane. Due to the low drug concentration, some of the CIP peaks are missing in the NRL + CIP spectrum. However, the FTIR spectrum of NRL + CIP showed most of the characteristic bands of CIP and NRL, indicating that the stability of the drug after its incorporation were maintained.



**Fig. 4. FT-IR spectra of NRL membrane incorporated with CIP; NRL; and CIP powder**

### 3.4 Raman Spectroscopy

Raman spectroscopy was performed due to identify the molecular vibrations of CIP powder, NRL and NRL+CIP membranes. According to Skoulika et al. [32], bands of CIP can be observed at 1624, 1575, 1384, 752  $\text{cm}^{-1}$ . In addition, bands of NRL membrane were identified at 1001, 1452, 1666 and 2914  $\text{cm}^{-1}$ , in

agreement with Agostini et al. [33] and Job et al. [34]. Finally, we observed bands of CIP loaded NRL membrane at 1001, 1452, 1666  $\text{cm}^{-1}$  (Fig. 5).

Raman bands of CIP spectrum (Fig. 5) at 1624, 1575, and 1378  $\text{cm}^{-1}$  are attributed to the  $n(\text{COOH})$ ,  $n(\text{C}_5\text{O})$ , and also to antisymmetric and symmetric  $n(\text{O}-\text{C}-\text{O})$  vibrations, respectively. Lee et al. [35] assigned the band around 1624  $\text{cm}^{-1}$  to the  $\text{C}_5\text{O}$  stretch of different modes belonging to in-phase and out-of-phase vibrations. Bands around 752  $\text{cm}^{-1}$  are characteristic of the substituted benzene ring deformation modes.

The 1024  $\text{cm}^{-1}$  band is characteristic of the  $n(\text{CC})$  skeletal conformation stretching modes, while the 752  $\text{cm}^{-1}$  band is attributed to methylene rocking modes [32,35,36]. The band at 805  $\text{cm}^{-1}$  is attributed to the CH out-of-plane bending mode [36].

Raman bands of the NRL membrane spectrum at 1001, 1666, and 2914  $\text{cm}^{-1}$  are attributed to  $=\text{C}-\text{H}$ ,  $\text{cis}-(\text{C}=\text{C})$  and stretching CH, respectively [37].

### 3.5 Thermogravimetric and Derivative Thermogravimetric Analysis

The TG analysis showed that the NRL had higher mass loss than the others samples, due to

the absence of the drug on the polymer. Similar effects have been reported in thermal analysis of NRL membrane without additives (carbon black, sulfur, antioxidant) added the vulcanization process [38].

The TG of NRL + CIP was decomposed in a single step. Thus, NRL had higher pyrolysis (357°C) among all samples, while NRL+CIP and CIP powder were about 380°C and 358°C, respectively. Comparing to the plain NRL the onset decomposition temperature decreased with the addition of CIP into the NRL. This may be occurred due to the crystallinity of the drug molecules [40]. Similar behavior of onset decomposition temperature was reported by Unnithan et al. [39], in this study they added dextran in the polyurethane-dextran blended nanofibers and polyurethane-dextran-ciprofloxacin loaded nanofibers. The data obtained in this work demonstrated a significant difference in the thermal stability between the samples, as shown in Fig. 6.

The DTG curves of thermal decomposition of NRL, NRL loaded CIP and CIP powder, at heating rate 10°C  $\text{min}^{-1}$  under nitrogen atmosphere are shown in Fig. 7. DTG profile NRL + CIP membrane indicated that the number of degradation stages was modified by incorporation of CIP into the NRL matrix evidencing drug-polymer interactions during thermal decomposition. Macocinschi et al. [40]

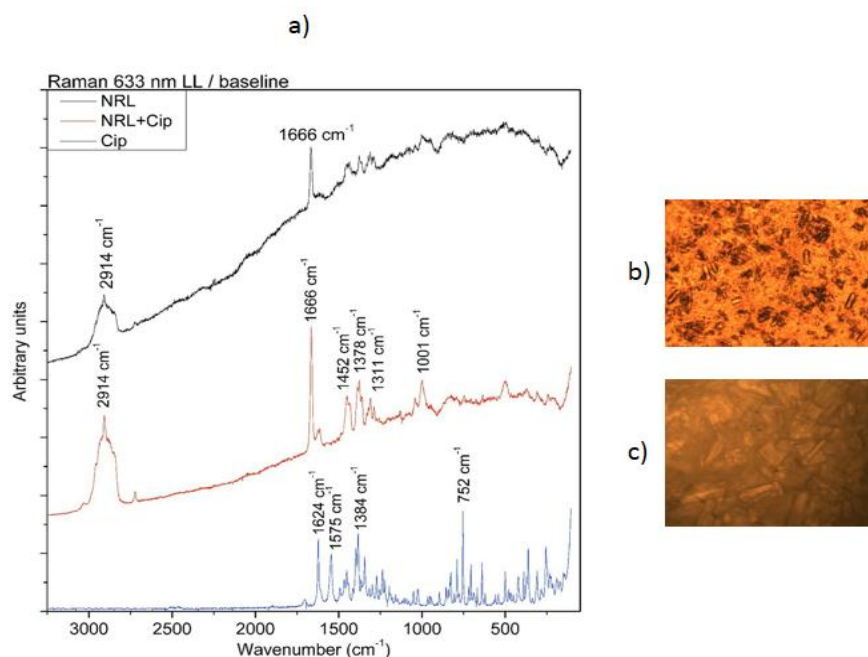


Fig. 5. Raman spectra (a). The area where Raman spectra were collected showed in a microscopy image: NRL + CIP membrane (b) and CIP crystals powder (c)

reported similar behavior of DTG, once that the main degradation stage was around 400°C with mass loss between 69.8% and 79.0%. Its higher temperature is similar with the temperature in present study (376.54°C), although mass loss (52.95%) is lower. It occurs because a different compound (CIP) was associated with the polymer, which was composed of degradation step in the range of 202.00 – 530.74°C. There were no significant changes in the NRL pyrolysis profile when the drug was added, indicating weak interaction between both compounds.

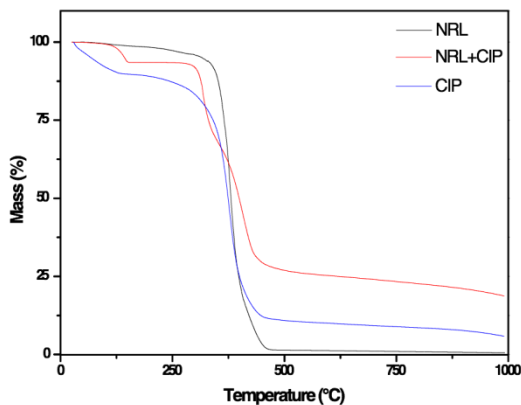


Fig. 6. TG curves for NRL, NRL + CIP and CIP

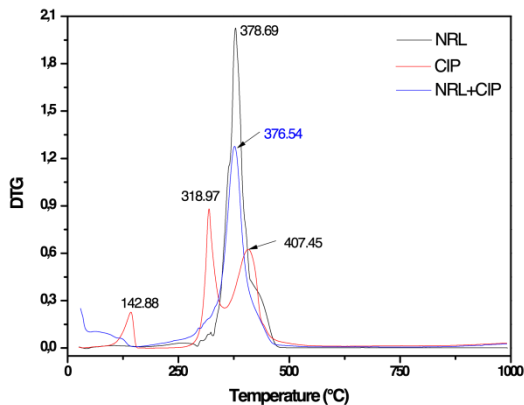


Fig. 7. DTG curves for NRL; NRL + CIP; and CIP (red line)

### 3.6 Tensile Stress-strength Strain

The CIP addition to the latex membrane had influence on the mechanical behavior of NRL (Fig. 8). We observed that the addition of CIP increased 1.03 times the elongation at break and had the tensile strength reduced in 1.07 times. The elongation at break, tensile strength and Young's Modulus of NRL membrane were 853.614%, 1.555 MPa and 1.203 MPa, respectively. Furthermore, the elongation at

break, tensile strength and Young's Modulus of CIP loaded NRL membrane were 876.001%, 1.456 MPa and 1.181 MPa, respectively. We found that the mechanical properties of the drug-loaded polymer is similar to NRL membrane [20,39,40,41].

### 3.7 Antibacterial Activity

#### 3.7.1 Broth dilution method

In the present assay, the turbidity of the culture medium is related to cell growth. It was observed that NRL by itself has no bactericidal effect (C2), once it had no inhibition action under the bacteria growth. However, when NRL membrane is loaded with CIP (E1), we observed the complete bacteria inhibition by the material (Fig. 9).

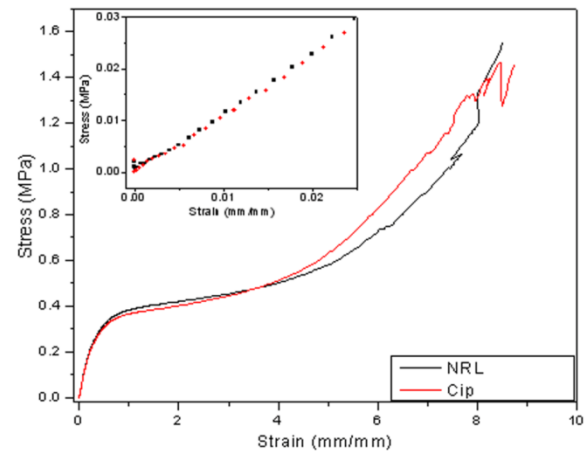


Fig. 8. Tensile stress-strength strain curves of NRL and NRL + CIP

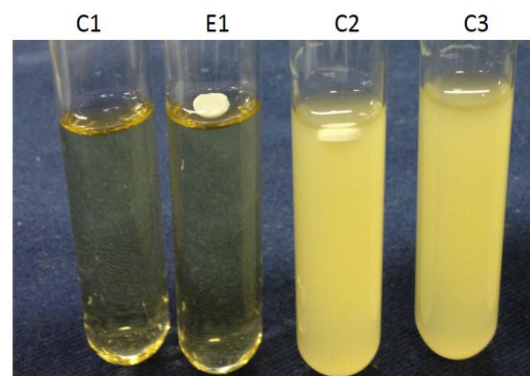


Fig. 9. Photography shows the inhibition of *B. lata* by NRL + CIP membrane (E1); C1: LB broth; C2: NRL (without CIP) in contact with LB broth inoculated with *B. lata*; C3: LB broth inoculated with *B. lata*

#### 4. CONCLUSION

A new biomembrane made by NRL+CIP has been characterized through different methods. The CIP presence on the membrane surface was showed by SEM, which allowed us to see crystals of the drug merged on the rubber membrane. XRD technique presented the absence of crystallinity in the drug-loaded NRL membrane indicating that the drug is molecularly dispersed within the NRL. FT-IR and Raman spectroscopy showed that there is no molecular-level interaction between drug and NRL. The TG assay demonstrated a significant difference between the samples in the thermal stability, but no significant changes in the NRL pyrolysis profile was observed. It indicates a weak interaction between both compounds, as presented by DTG assay. Mechanical properties of the NRL+CIP are similar to NRL. Although the addition of CIP increased 1.03 times the elongation at break and 1.07 times reduced the tensile strength, it still has enough elasticity to be used as a biomaterial for medical application. Moreover, antimicrobial assay showed that NRL+CIP membrane presents antibacterial activity to *Burkholderia cepacia* complex (Bcc), where the control LB grown broth (C1) and the NRL+CIP (E1) are quite the similar in terms of cell concentration.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENT

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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