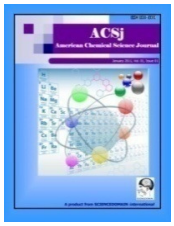

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Natural Latex Films as Carrier for *Casearia sylvestris* Swartz Extract Associated with Ciprofloxacin

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LFCB, FAB and JLFC perform the experiments. Authors LFCB, RGS and RDH wrote the draft of the manuscript, the author AGS provided the Casearia sylvestris swatrz extract and is the second advisor and the author RDH is the first advisor and the head of laboratory. All authors read and approved the final manuscript.

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ABSTRACT

In order to create a new sustained drug delivery system applied for tissue regeneration, this study beget a natural rubber latex (NRL) film with *Casearia sylvestris* extract and ciprofloxacin. Manipulated from the biomaterial latex obtained by the rubber tree *Hevea brasiliensis*, the NRL film has shown to be a great angiogenic compound and also has shown a great potential in being a possible carrier for sustained drug release. In order to increase the therapeutic spectrum of the film two substances was added to it: the *C. sylvestris* extract which has antiulcer, anti-inflammatory and

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wound healing effects due to its casearins and the antibiotic ciprofloxacin which is considered as one of the main drugs used for open wounds in the skin. The study occurred at State São Paulo University in Assis (UNESP-FCL), Brazil. The substances were mixed and a film was made by casting at ambient temperature creating circular films with 5cm of diameter. After that the film was placed in 1 liter of water to start the release study. The release behavior was analyzed by UV-VIS spectrophotometry being the casearins related to the wavelength of 235nm and the ciprofloxacin of 320nm. The film supported the release of compounds, releasing 93.73% of the extract and 56.53% of the drug, along 29 hours. In addition, the incorporation was proven by Scanning Electron Microscopy (SEM), which showed the morphology of pure and incorporated films and also by Fourier Transform Infrared Spectroscopy (FTIR) which has shown that no chemical change occurred during the process. Thereby, according to results, the material has a possible approach to biomedical application.

Keywords: Sustained release; latex; Casearia sylvestris; ciprofloxacin; biomaterials.

1. INTRODUCTION

Nowadays, some terms like “sustained release and the drug delivery systems” has come to evidence in the pharmacology field. The main reason of this is because pharmacological substances when applied in a conventional manner, as orally for example, take too much time to reach the site in which they are needed and hardly achieves it under the appropriate concentrations to cause the expected therapeutic effect [1,2]. This fact is justified because there are numerous obstacles in the body, such as anatomical and chemical barriers between the place of administration of the drug and its final target. For example, if we are dealing with a deep wound in an organism, only one part in ten thousand of a drug taken orally will be able to achieve its final target.

The latex from the rubber tree *Hevea brasiliensis* has shown to be a biocompatible material with good qualities to serve as a potential base for a drug delivery system. It has shown numerous biomedical applications, along with high mechanical strength and low cost, making it useful in medicine. Herculano et al. [3] has shown that is possible to do a NRL film to use it as a carier matrix and also that the amount of porous in the surface of the film can be controlled with different polymerization temperatures, a key factor in controlling the release of the compound. Furthermore, Alves et al. [4] showed that the latex has the property of accelerating angiogenesis, it means, the growth of blood vessels, important characteristic in a tissue regeneration field.

The *Casearia sylvestris* is a plant present in America, occurring from northern Mexico to south Brazil (where it is popularly known as "guaçatonga"). It belongs to the family *Salicaceae* and its leaves extract possesses antiulcer activity, wound healing activity, antivenin, anti-inflammatory, antiseptic and anticancer properties. This plant has compounds known as clerodane diterpenes, that are secondary metabolites derived from isoprene, among which are present in the A-X casearins with excellent cytotoxic and antitumor activity proven by numerous authors [5].

Ciprofloxacin is an antibiotic which belongs the groups of fluoroquinolones. The antimicrobial action occurs in a biomolecular way acting on the inhibition of DNA gyrase and topoisomerase IV, important structures in the process of self-replicating DNA. Ciprofloxacin binds to DNA during opening and playing the spiraling DNA gyrase, a bacterial DNA binding prevents access of cellular machinery to their genetic information, causing cell death. Actually, ciprofloxacin is commonly used in treatments for skin bacterial infection. [6]

Therefore the study aimed to join these three substances, and the importance of that is because latex will act as the matrix of sustain along with its angiogenic potential, the extract of the plant will act as a potent natural anti-inflamatory and the ciprofloxacin will act as an antimicrobial, aiming, this way, to generate a new complex applied for the healing and regeneration tissue area. The idea is that the sustained release device when applied directly on the wound site will provide constantly the amount of drug necessary in a short period of time to help the cicatrization and the healing process. For this mean, first this study propose to evaluate the interaction of the extract of *C. sylvestris* along to the ciprofloxacin in NRL films, then understand

their sustained release behavior to analyze if the aggregation is possible.

2. MATERIALS AND METHODS

2.1 MATERIALS

This study followed the methodology proposed by Romeira et al. [7] and Aielo et al. [8] for the production of the NRL films. The NRL was extracted at BDF Rubber Latex Co. Ltd, (Producer and distributor of concentrated rubber latex) Guarantã, Brazil. The latex solution extracted from *Hevea brasiliensis* consisted in a mixture of different clones. After extraction, ammonia was used to keep the liquid latex, and this material was centrifuged at 8000rpm. The centrifugation was important because it reduced some proteins contained in natural latex that cause allergic reactions [8,9].

Casearia sylvestris Sw. extract was obtained by ethanol extraction of leaves at 40°C for seven days and concentrated by lyophilization. The material was collected at "Horto de Plantas Medicinais e Tóxicas da Faculdade de Ciências Farmacêuticas da UNESP" in May 2010. Voucher specimen is deposited with the Herbarium "Maria Eneida P. Kaufmann" (Instituto Botânico do Estado de São Paulo, São Paulo, Brazil) with the reference number AGS101 [5].

Ciprofloxacin ($C_{17}H_{18}FN_3O_3$) was obtained in gel capsules, without excipients. Each of them contained 100mg of ciprofloxacin in powder form. The capsules were purchased from *Callithea* Pharmaceutics Ltd., Brazil.

2.2 METHODS

To make the films, first a solution containing the extract and the ciprofloxacin was prepared. This solution was made by adding 20mg of extract and 100mg of ciprofloxacin in 20mL of pure water. After the complete homogenization the extract concentration obtained was 1mg/mL and the drug concentration was 5mg/mL. Then the films were fabricated by depositing 5mL natural latex plus 3mL of this solution previously done. After that, this mixture was deposited in some cylindrical shapes containing 5cm of diameter each, making that the amount of extract and ciprofloxacin loaded in the film be 1.018mg/cm² and 5.094mg/cm² respectively. Next the films were dried at room temperature (21°C) for 48h. Following that, the films were removed off the forms and placed each one in a beaker containing 1L of pure water.

The release behavior was observed by shaking the sample and periodically withdrawing aliquots of 1mL of the solution (where the film was present) for 300 hours [6]. The release was characterized using optical spectroscopy technique (UV-VIS). The aliquots were placed in quartz cuvettes and then placed into the spectrophotometer. The ciprofloxacin was characterized by a wavelength of 320nm and extract characterized by the wavelength of 235nm. After the realization of the test the withdrawal rate was returned to the beaker. In order to avoid and minimize errors, the films and samples were made in triplicates.

The surface morphology of the NRL film was observed using a Scanning Electron Microscopy (SEM) model Zeiss® EVO 50 (20 KV) and a take off angle of 35° [8]. Fourier transform infrared spectroscopy (FTIR) were obtained to prove the chemical integrity of the drug and the extract 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 films were characterized using a TENSOR 27 (Bruker, Germany) (4000-500cm^{-1}) with a resolution of 4cm^{-1}. To make the statistical analysis of the data, the software Origin Pro 8® was utilized.

3. RESULTS AND DISCUSSION

Initially, a UV-spectrum was made in order to localize which wavelength corresponds to which compounds in study (Fig. 1).

As it can be noticed in the image, both compounds have a common point of absorption. The literature informs that *C. sylvestris* extract has two main points of absorption, the 235nm related the casearins and the 269nm related to phenols [5]. However the 269nm wavelength is also related to ciprofloxacin that has its maximum of absorption at 270nm [10]. Because of this, in this study, to avoid errors and over estimation of data, the quantifier methods were made by using the 235nm as the one related to the extract casearins and the 320nm wavelength as the one related to the ciprofloxacin. This choice was made because the drug also shows an absorbance peak at this spectrum.

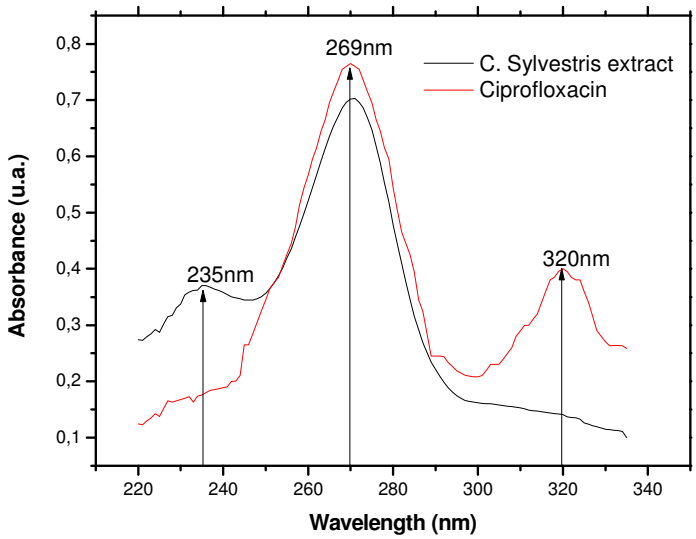

Fig. 1. UV-Spectrum of ciprofloxacin and of *C. sylvestris* extract

For correct interpretation of the data, two calibration curves were produced. One representing the casearins which followed the function Y(x) = 22.2677x + 0.03608 (Fig. 2a) indicated a Pearson regression coefficient (R^2) of 0.99527 and one representing the ciprofloxacin which followed that function Y(x) = 58.34074x + 0.04653 (Fig. 2b) indicating a R^2 of 0.99351.

Having these informations, the next step was to study the kinetics release for both the compounds in the film. (Fig. 3a) shows the extract kinetics release. From this curve it can be noticed a fast release of casearins (known as log phase) until the moment where the concentration passed to be constant due to the almost complete release of the substance present in the NRL film. This behavior can be explained because the extract present on the surface of the film was released much faster than the one present in the innermost part of film. It can also be observed that the release obeys a bi-exponential function. The release curve obtained was plotted using the function $Y(t) = y_0 + A_1e^{-t/\tau1} + A_2e^{-t/\tau2}$ which provide the better R^2 with the value of 0.99. In this equation Y (t) is the concentration of the extract released in the water in the interval t, y_0 is the initial amount of extract, A_1 and A_2 are constants, and τ_1 and τ_2 are characteristic times. On release, the values of A_1 and A_2 were -0.906178 and -1.8 respectively, and the values of τ_1 and τ_2 were 29.188 and 459.515, respectively. The release was done for 300 hours to be sure that even with a huge time in contact with water; nothing else would be released by the film.

However, if we considered that the release stopped at 1740 minutes, point in which the concentration starts to be constant, another inference can be made. Analyzing the data from 0 to 1740 minutes, (Fig. 3b), the release obeys a potential equation. The release curve obtained was plotted using the function $Y(t) = at^b$ which provide a R^2 of 0.958. In this equation Y (t) is the concentration of the extract released in the water in the interval t, “a” and “b” are constants which values are 0.2269 and 0.344 respectively.

Furthermore, the maximum concentration obtained of the extract in water was 2.812µg/mL. Knowing that the liberation occurred at 1 liter of water and that each film contained 3mg of extract, this data indicated that approximately 93.73% of the extract presented in the NRL film was liberated

Performing the same analysis for ciprofloxacin, its kinetics release could be traced in (Fig. 4a). From this curve it can be noticed the same behavior of the Casearins release, also following a bi-exponential function. The release curve obtained was plotted using the function $Y(t) = y_0 + A_1e^{-t/\tau1} + A_2e^{-t/\tau2}$ which provide a R^2 of 0.989. On release, the values of A_1 and A_2 were -4.7039 and -3.5401 respectively, and the values of τ_1 and τ_2 were 1330.727 and 103.885 respectively.

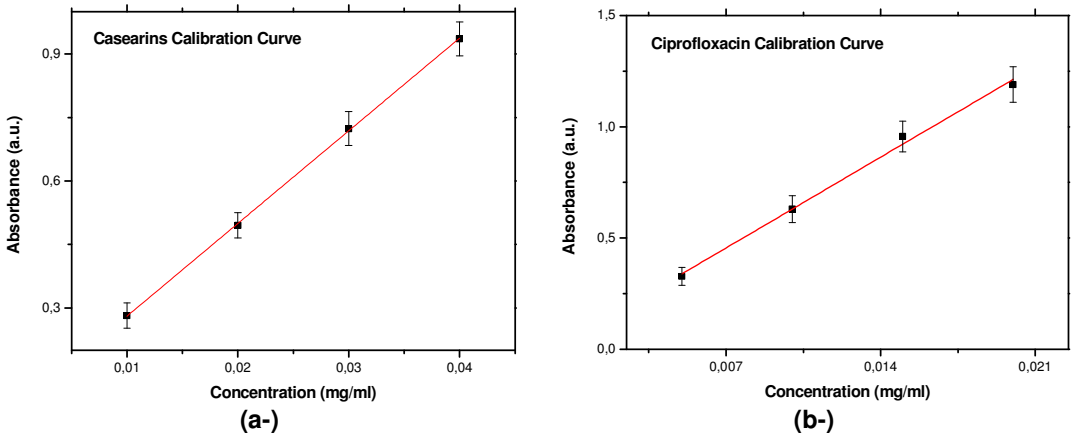

Fig. 2. Absorbance intensity as a function: of extract concentration in solution (a); of drug concentration in solution (b)

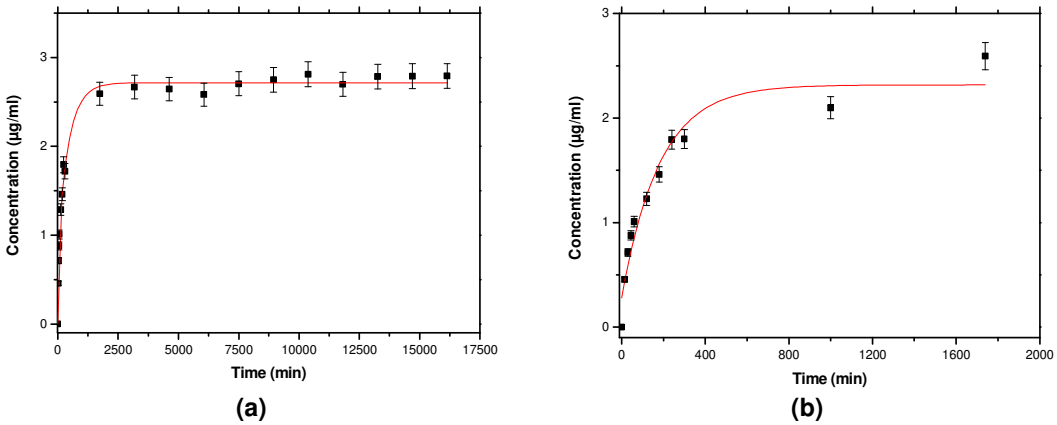

Fig. 3. Casearins release from NRL film from: 0 to 16140 minutes (a); 0 to 1740 minutes (b)

As in the other case, the maximum release is noted at 1740 minutes. Fitting the data from 0 to 1740min a graph following the potential method is obtained, showed in (Fig. 4b). The release curve obtained was plotted using the function $Y(t) = at^b$ which indicate a R^2 of 0.99. In this equation Y (t) is the concentration of the extract in the water in the interval t; "a" and "b" are constants which values are 0.2931 and 0.4512 respectively.

The maximum concentration observed for the ciprofloxacin was of 8.48µg/mL. Knowing the context of the liberation and that each film contained 15mg of ciprofloxacin, this data indicates that 56.53% of the drug presented in the NRL film was liberated.

To analyze and demonstrate that both the drug and the extract were added to the film, SEM images were made illustrated at (Fig. 5). As it can be seen, all the compounds were successfully aggregated.

By analyzing the (Fig. 5), it can be noticed that NRL films when made by casting at ambient temperature present porous in its surface, indicating that the film can act as a scaffold for controlled release system, as the porous at the surface can be the responsible for the release of the substances presents at the film bulk. (Figs. 5b, 5c and 5d) confirmed that both extract and film could be incorporated into the NRL film.

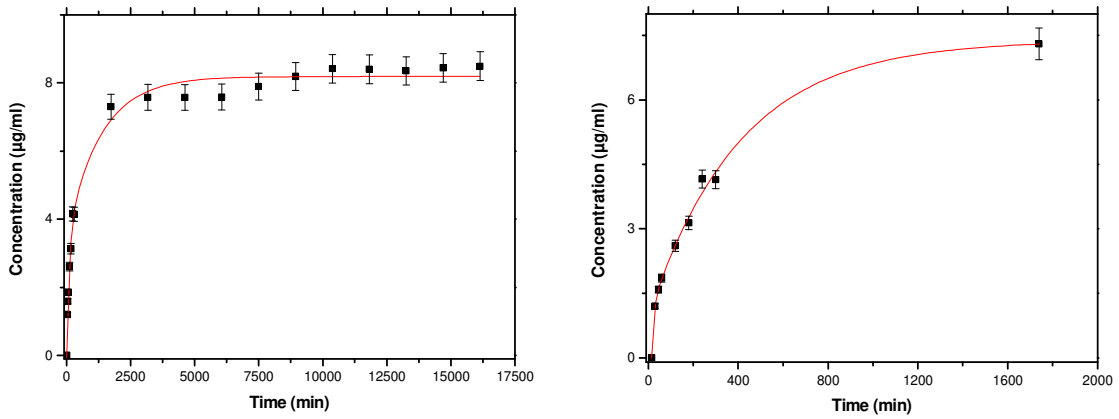

Fig. 4. Ciprofloxacin release from NRL film from: 0 to 16140 minutes (a); 0 to 1740 minutes (b)

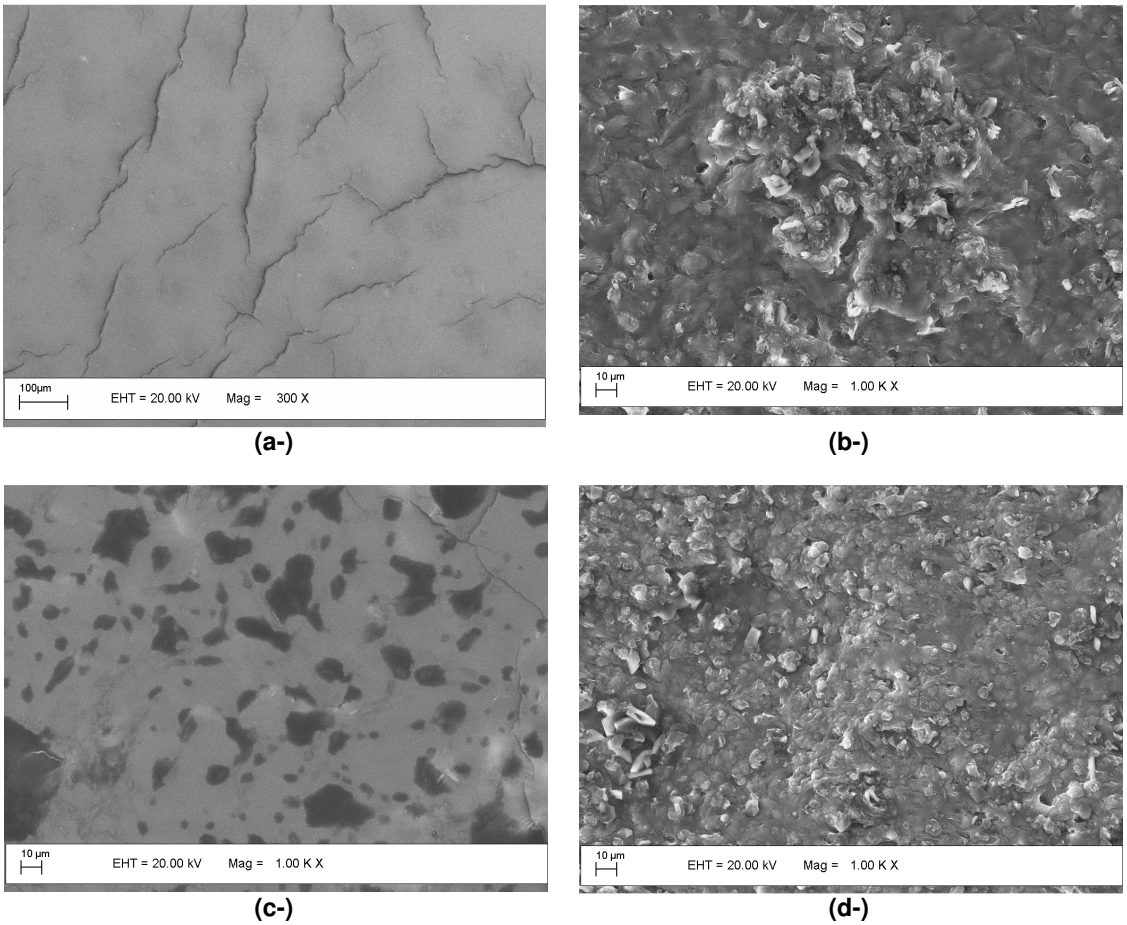

Fig. 5. SEM image of: NRL film 300x (a); NRL film incorporated with ciprofloxacin 1000x (b); NRL film incorporated with *C. sylvestris* extract 1000x (c); NRL film incorporated with ciprofloxacin and *C. sylvestris* extract 1000x (d)

The FTIR was performed to identify if there were any chemical interactions between the compounds presented in study. NRL films showed peaks at 2961cm^{-1} (CH_3 asymmetric stretching), 2859 cm^{-1} (CH_2 symmetric stretching), 1500cm^{-1} (NO links due to proteins presence), 1445cm^{-1}(CH_2 deformation), and at 1373cm^{-1} (CH_3 asymmetric deformation)[11]. The extract showed peaks around 1740 cm−1 (casearins F, U, and V and caseargrewiin F), 1457 cm^{-1} (casearins B, D, V, U, and X), 1375 cm^{-1} (casearins A, B, C, F, U, V, X and caseargrewiin F) and 1005 cm−1 (casearins D and V); the peaks around 2920cm^{-1} and 2845cm^{-1} are not related to characterized casearins [12-14]. The Ciprofloxacin showed peaks around 1200cm^{-1} (secondary amines), 1550cm-1 (aliphatic nitro compounds), 1715cm^{-1} (aliphatic ketones or aldehydes as cyclic chains with more than 6 members), 3400cm^{-1} (hydroxyl groups present in the chain) [15].

Comparing all the data is possible to infer that the compounds do not interact chemically with each other because there is no significant change on any kind of bond and there is no apparition of any different groups as the ones seen in the first curves (Fig. 6). The main change noted is in the intensity of the peaks. This is explained because the drug and extract were incorporated in the film, so the tests identify more chemical elements and bounds in the film. The major increase happened on the band of 3400 cm^{-1} and is attributed to the great distribution of the hydroxyl groups, indicating intermolecular interaction much probably between the ciprofloxacin and the latex, explaining the lower release of the drug [15].

Several studies had been utilized biopolymers as drug delivery systems. Borges et al. [5] made a drug delivery system using NRL films and *C. sylvestris* and obtained a measure of 99.5% of liberation of the casearins compounds in about 35 hours. This rate is proximal of the rate obtained in this present study, as almost all of the extract incorporated in the film is liberated in 29 hours, proving that the ciprofloxacin did not interfered in the extract liberation. Romeira et al. [7] also made a drug delivery system using NRL films but with *Stryphnodendron sp* and obtained a liberation tax of 49.89% in about 400 hours. This study indicated that extracts can be used in drug delivery systems with a high rate of liberation, and also that the *C. sylvestris* is a potential extract that can be aggregated and liberated by the NRL films.

Herculano et al. [11] studied a drug delivery system for metronidazole using NRL films liberating in 300h 53.15% of the drug. The rate of liberation shown is proximal of the rate observed in this study, which indicates that crystalline drugs have some difficulty to be liberated from NRL films, probably because of the intramolecular interaction between the latex and the drug.

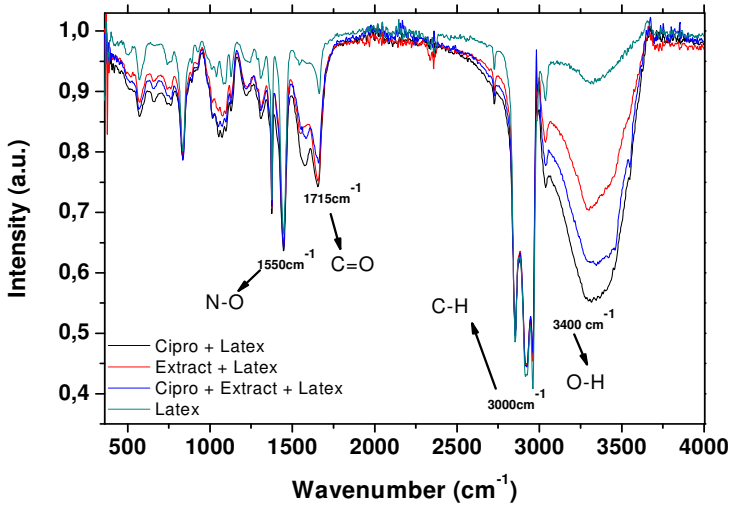

Fig. 6. FTIR-ATR spectra of latex natural film, latex film loaded with extract, latex film with ciprofloxacin and latex film loaded with extract and ciprofloxacin

Several studies has been done in the drug delivery area using different materials, like Lober et al. [16] that created a device based on polyhydroxyalkanoates for implantation of a glaucoma drainage system. Wang et al. [17] prepared uniform chitosan microspheres by film emulsification technique to work as drug delivery system of a protein called BSA. Pichayakorn et al. [18] has made a nicotin drug delivery system with NRL films blended with polyvinyl alcohol and tested on pig skin reaching really good rates of liberation aiming the creation of transdermal patches for teraphy. Verma et al. [19] wanted to explore the potential of low molecular mass chitosan (LMCH) as carrier for sustained delivery of water soluble drug ciprofloxacin. The results suggest that LMCH alone or in combination with Xantan Gum is an excellent material for stomach specific sustained delivery. So, all these studies show that the drug delivery systems area is growing really fast, and has a great potential to become a promising future pharmaceutical industry, justifying why the research for new biomaterial capable to act as drug delivery systems is important.

Therefore, along with the literature and the results of this research, it can be inferred that NRL film is a substance that can work as a matrix for sustained release for different substances, making itself useful in this study for more than 29 hours, and that the rate of release can be controlled by different factors of the local where it is placed.

4. CONCLUSION

The film characterization results showed that the substances were able to be aggregated by the NRL film by the casting technique, demonstrating a successful merger. Moreover, tests have shown that before and after incorporation of substances to the NRL film the substances properties were maintained and also that the materials did not interact chemically with each other in a way that could have altered their molecular structure. The release tax shown for extract was of 93.73% and for ciprofloxacin of 56.53%. In addition SEM has shown that both compounds could be adhered to the film and that porous was formed in the NRL surface. FTIR has shown that the compounds did not interact chemically with each other, so the chemical proprieties of them were preserved. Therefore the hypothesis of the work was corroborated, developing a new application of an alternative biomaterials, allowing more studies to be done for a future use in different cases and treatments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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