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*Research Article*

# Natural Rubber Latex: Study of a Novel Carrier for *Casearia sylvestris* Swartz Delivery

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Natural rubber latex (NRL) from *Hevea brasiliensis* has showed interesting biomedical properties as improving wound healing, cell adherence, tissue formation, and angiogenesis. It is used for biosynthesis of nanoparticles, sensors and prosthesis and for drug delivery systems (for drugs, plant extracts, and nanoparticles). To enhance its wound healing properties was incorporated *Casearia sylvestris* Swartz extract, whose pharmacological activity includes anti-inflammatory, analgesic, antiseptic, antiulcer, and antitumor due to its casearins and phenols. Results showed the prolonged release of its compounds (35 days) and the mechanism of release is super case II ($n > 1$) by Korsmeyer-Peppas model. Although SEM shows different sizes of clusters at the surface, the release is homogeneous through the biomembrane. FTIR shows no interaction between the matrix and the extract, with computation of the presence of some casearins.

## 1. Introduction

Natural rubber latex (NRL) extracted from *Hevea brasiliensis* (rubber tree) is a colloidal system containing 50% of water, 4-5% of nonrubber (as proteins, lipids, etc.), and 30–45% of rubber particles (*cis*-1,4-polyisoprene) [1]. When in contact with air, the proteins that stabilize rubber particles get in degradation and lead to latex coagulation [1]. Latex is largely used in artefacts such as gloves, condoms, and baby bottle teats.

Proteins in latex also possess angiogenic properties [1], propitiate cell adhesion, and accelerate wound healing [2]. It has been used as prosthesis (vascular [3], tympanum [4], bladder [5], etc.) with no allergy or rejection, demonstrated to be biocompatible and suitable for biomedical application [2]. It has also been used for nanoparticles production [6], delivery system [7], and occlusive biomembrane [8], among others.

*Casearia sylvestris* Swartz (popularly known as "guaçatonga") is used traditionally to treat diarrhea, skin diseases, snakebite, antiulcer, and so forth [9]. Its bioactive properties are related to its secondary metabolites such as presence of casearins (A–X) and phenols. Related to its pharmacological applications to wound healing, it shows anti-inflammatory [10], antiseptic [11], and analgesic [12] properties; moreover,

casearins possess oxygenated backbone related to remarkable cytotoxic and antitumor action [9] and phenol is related to antioxidant activity [13].

Delivery system is important to avoid multiple doses, increasing the patient compliance to the therapy. One possible way to accelerate the tissue repair process is to incorporate the *Casearia sylvestris* Sw. extract in NRL biomembranes.

## 2. Materials and Methods

Natural rubber latex (NRL) from *Hevea brasiliensis* (clones RRIM 600 and PB 235) was obtained from BDF Latex Ltd. (Guarantã, São Paulo, Brazil). NRL is centrifuged at 8000 g to reduce protein content related to allergic reactions [14]. The latex has 60% of dry rubber content and was stabilized with ammonia.

*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.

To elaborate the biomembranes, extract was diluted at aqueous solution with 20% of ethanol (to avoid latex coagulation) at 0,25 mg/mL. Biomembranes were prepared by mixing 5 mL of latex with 3 mL of extract solution, casting it into Petri dishes (60 × 15 mm) and solvent evaporation at room temperature (RT), with no chemicals (such as carbamates or sulphur) related to allergic reactions [14].

The release was performed in 400 mL of aqueous solution at RT and measured by UV-VIS spectrophotometer LGS53, BEL Photonics. Measurement interval was at time $t$ (minutes): 0, 15, 30, 45, 60, 120, 180, 240, and 300 and then daily, for 40 days. To perform the homogeneity test, the biomembranes were prepared and released as mentioned before, and each quarter of it was released in 100 mL (to maintain the proportionality). The data was normalized by the size of the biomembrane.

Statistical analyses were persuaded by OriginPro SR4, from OriginLab Corporation, also used to plot graphics and fit the released functions. By the integral of the functions, the software shows the quantity of the extract released. All analyses were performed in triplicate for statistical purposes. Analyses of the mathematical models (First Order, Higuchi, Hixon-Crowell, Baker-Lonsdale and Korsmeyer-Peppas) of the mechanism of release were performed by Sigma Plot 12.5 (from Systat Software).

The surface morphology of the NRL biomembrane was observed using a scanning electron microscopy (SEM) model Zeiss EVO 50 (20 KV) and a take-off angle of 35°. The FT-IR spectra were measured directly by attenuated total reflection (ATR) method using a VERTEX 70 (Bruker, Germany) (4000–500 $cm^{-1}$) with a resolution of 4 $cm^{-1}$.

## 3. Results and Discussion

The goal of a drug delivery system is to release the content at the local site providing the desired concentration at the local site and avoiding systemic effects, which requires the characterization and modeling of the product. In this work was proposed a simple method for preparation and the release of *Casearia sylvestris* Sw. extract from its leaves by a natural rubber latex biomembrane. Figure 1 shows the SEM of the scaffold.

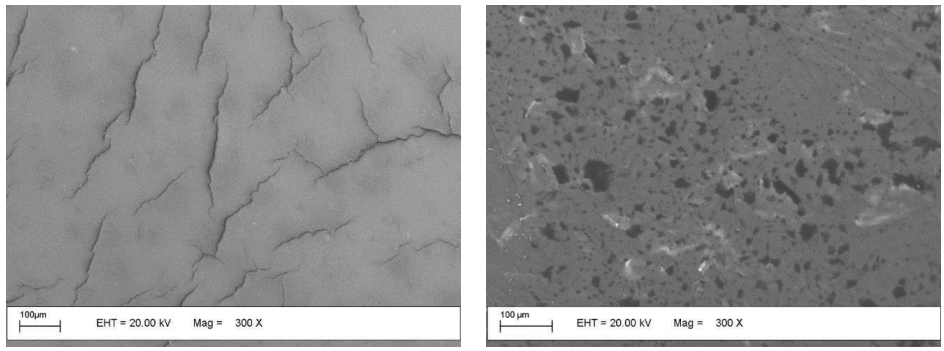
It was observed by UV-VIS spectrophotometer that the extract has two main absorption bands (datum not shown), at 235 nm related to casearins (A–X) [15] and at 269 nm related to phenols compounds [16]. The release was monitored at these wavelengths.

The release profiles (Figures 2 and 3) show that both releases behaved as biexponential function as before for NRL release [7], as $y(t) = y_0 + A_1 e^{(-t/\tau 1)} + A_2 e^{(-t/\tau 2)}$, where $y(t)$ is the amount of compound in the NRL at a given time, $t$, $y_0$ is the initial content, and $A_1$, $A_2$, $\tau_2$, and $\tau_2$ are constants. The fast initial release is due to the extract at the surface (showed by SEM), and the slower release is related to the diffusion of the compound through the biomembrane fractures, which work as a reservoir. As we can see, the plateau (time of saturation) reaches saturation at 35th day for casearins with release of 99,8% and at 40th for phenols with release of 82,7%. The parameters of fitted bi-exponential equation is at table of each graphic release.

Figure 4 shows the homogeneity of the release for casearins and phenols. The first step of the release presents more variation due to the considered irregular dispersion size at the surface evidenced by SEM, although these differences diminish after the first day, indicating homogeneous release of the compounds.

The physical parameter of the release depends on several mechanisms such as degradation of the polymeric matrix, diffusion through porous, swelling, and erosion. To understand the physical parameters of the kinect release, the same semi-empirical mathematical models were applied to assess information regarding the mechanisms that govern the release of the substance (Tables 1 and 2).

The first-order dissolution phenomenon had a good fit ($R^2$ = 0,9948); from this model, the matrix releases the compound proportionally to the remaining amount in the bulk so that the amount of drug released is decreased [17]. However, the best coefficient of correlation is for Korsmeyer-Peppas ($f(t) = kt^n$), where $f(t)$ is the fractional release of compound at elapsed time $t$, $n$ is the release exponent (which suggests the mechanism of release), and $k$ is kinetic constant (related to the structural and geometrical characteristics of the carrier). This model is applied when the release mechanism is unknown or there is more than one mechanism. If $n \leq 0,5$ indicates Fickian diffusion (also known as "Higuchi" equation); $0,5 < n < 1,0$ indicates anomalous transport mechanism; $n = 1$ indicates case II transport (also known as "zero order" equation, is due relaxation, erosion and/or swelling of the polymer); and $n > 1$ indicates super case II transport. In this work all $n$ values are higher than 1, which indicates super case II [18]. The kinect constant ($k$) is different for both compounds; for casearins $k$ = 1,135 $hour^{-1}$ and for phenols $k$ = 1,116 $hour^{-1}$; this may explain the fact




(a) (b)

Figure 1: SEM of the (a) NRL biomembrane; (b) NRL with *C. sylvestris* extract incorporation.

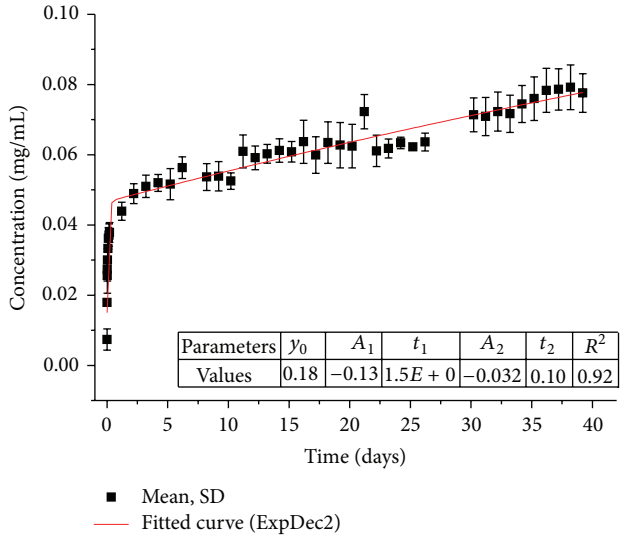



Figure 2: *C. sylvestris* extract release of casearins (235 nm) from NRL biomembrane.

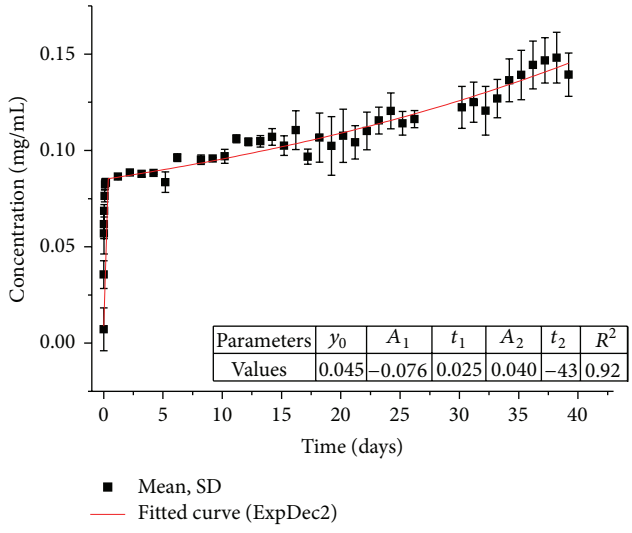



Figure 3: *C. sylvestris* extract release of phenols (269 nm) from NRL biomembrane.

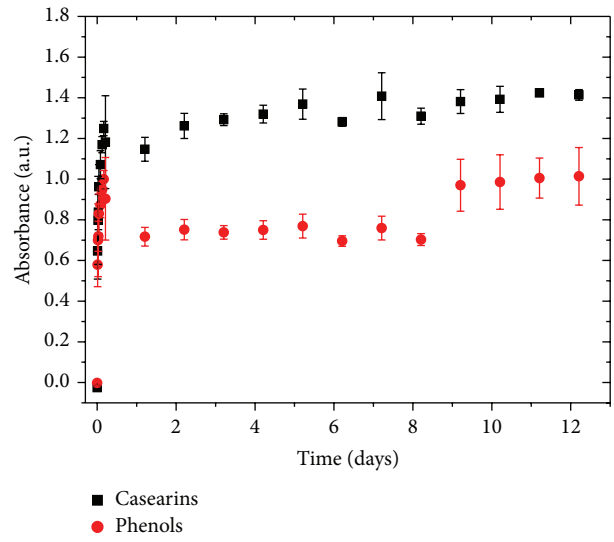



Figure 4: Release homogeneity for casearins and phenols from NRL biomembrane.

Table 1: Parameters of equations for kinect release from casearins (235 nm).

| Phenols (269 nm) | $n$ | $k$ (hours$^{-1}$) | $R^2$ |
|---|---|---|---|
| Baker-Lonsdale | | 1,64$E$ − 07 | 0,8544 |
| Korsmeyer-Peppas | 1,116 | 1,35$E$ − 02 | 0,9997 |
| Hixon-Crowell | | 6,67$E$ − 05 | 0,9960 |
| Higuchi | | 9,92$E$ − 02 | 0,8547 |
| First Order | | 2,00$E$ − 04 | 0,9959 |

that casearins reach *plateau* faster than phenols [19]. Baker-Lonsdale model is more related to spherical matrixes and Hixon-Crowell model is applied when dissolution occurs in planes that are parallel to the surface, where the dimensions diminish proportionally.

FTIR (Figure 5) analysis was performed to assess the interaction between the extract and the polymer. The extract shows peaks around 1740 cm$^{-1}$ (casearins F, U, and V and caseargrewiin F), 1457 cm$^{-1}$ (casearins B, D, V, U, and X),

Table 2: Parameters of equations for kinect release from phenol (269 nm).

| Casearins (235 nm) | $n$ | $k$ (hours$^{-1}$) | $R^2$ |
|---|---|---|---|
| Baker-Lonsdale | | 2,92$E-07$ | 0,8487 |
| Korsmeyer-Peppas | 1,135 | 1,69$E-02$ | 0,9999 |
| Hixon-Crowell | | 8,91$E-05$ | 0,9949 |
| Higuchi | | 1,32$E-01$ | 0,8491 |
| First Order | | 2,67$E-04$ | 0,9948 |

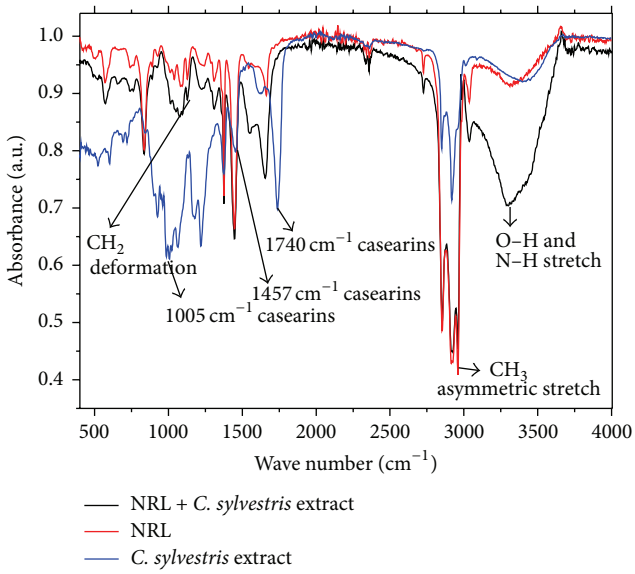



Figure 5: FTIR-ATR spectra of NRL biomembrane, pure extract, and NRL biomembrane loaded with extract.

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 2920 cm$^{-1}$ and 2845 cm$^{-1}$ are not related to characterized casearins [20–23]. The spectrum of the extract and the NRL are very similar. NRL presents peaks at 2961 cm$^{-1}$ related to $CH_3$ asymmetric stretching, at 2859 cm$^{-1}$ is $CH_2$ symmetric stretching, at 1445 cm$^{-1}$ is $CH_2$ deformation, and at 1373 cm$^{-1}$ is $CH_3$ asymmetric deformation [24]. The change in the intensity is due to the incorporation of the extract. The increase of the broadband centered at 3400 cm$^{-1}$ and isattributed to the wide distribution of the hydrogen and amines groups, indicating interaction with NRL molecules in the scaffold presumably through hydrogen bounding, explaining the slower release of the phenols [25].

## 4. Conclusions

A new way to administrate the extract of *C. sylvestris* was developed, which guaranties 100% of the incorporation due to the methodology of preparing the NRL biomembrane (casting and solvent evaporation). The release is gradual, homogeneous, and suitable for prolonged release. The amount of released compounds is higher than 80%, whose mechanism is super case II for both compounds. FTIR shows the presence of some casearins and those do not react with the matrix polymer.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Authors' Contribution

The authors confirm that Matheus Carlos Romeiro Miranda has contributed to the paper. He has contributed to the analyses of the mathematical model of the mechanism of release.

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