Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev Ciênc Farm Básica Apl., 2015;36(3):379-384
ISSN 1808-4532

Novel sustained-release of *Stryphnodendron obovatum* leaves extract using natural rubber latex as carrier

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ABSTRACT

Natural rubber latex biomembranes (NRL), obtained from rubber tree *Hevea brasiliensis* **(Willd. ex A. Juss.) Mull. Arg., have been used as sustained drug release of drugs and plant extracts with medicinal properties. The** *Stryphnodendron obovatum* **Bench (Fabaceae), popularly known as "barbatimão" has anti-inflammatory and healing properties already described in literature. Thus, the aim of this work were to study the release behavior of the hydroethanolic extract from the leaves of** *S. obovatum* **loaded in the NRL by ultraviolet–visible spectroscopy (UV-VIS). The release followed a bi-exponential pattern and the mechanism of release was Super Case II (n > 1). FTIR analyses did not show reaction between NRL and extract, only intermolecular interaction. From SEM was possible to observe the extract at the surface, responsible for the initial fast release, which the concentrations at 5.0 mg/mL released 2.4% and at 0.1 mg/mL released 96.8%; both reached the** *plateau* **in 7 days.**

Keywords: *Stryphnodendron obovatum*. *Hevea brasiliensis*. Sustained release. Barbatimão. Tannin. Natural rubber latex.

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INTRODUCTION

The sustained release systems are not conventional means of drug delivery, usually used when they cannot reach a specific target in an organism at concentrations necessary to cause the expected therapeutic effect; either by problems of local administration, or other obstacles whether anatomical, chemical or biological (Herculano *et al*., 2009). Among these systems, we highlight the drugs and plant extracts release through natural rubber latex (NRL) biomembranes from *Hevea brasiliensis* (Euphorbiaceae). The NRL biomembrane is biocompatible, low cost and has high mechanical strength (Herculano *et al*., 2010; Herculano *et al*., 2009), has good adhesion to tissues and acts as a physical barrier against infection (Ferreira *et al*., 2009; Neves-Junior *et al*., 2006). Same proteins in the latex stimulate angiogenesis, which accelerated wound healing and tissue repair (Alves *et al*., 2003; Herculano *et al*., 2010). These biomembranes did not show rejection or allergy reaction (Ferreira *et al*., 2009), and its healing properties could be potentiated with the addition of plant extracts and/or drugs.

The *Stryphnodendron* spp. (Fabaceae), known as "barbatimão" are commonly used in folk medicine to treat gonorrhea, hernia, diarrhea and bleeding wounds (Lopes *et al*., 2009; Maroni *et al*., 2006) among others. From *Stryphnodendron* genus barks has been described their antimicrobial (Sanches *et al*., 2005), healing (Lopes *et al*., 2005), antiulcer (Lopes *et al*., 2009), antioxidant (Lopes *et al*. 2005) and anti-inflammatory activities (Lima *et al*. 1998). In the *S. obovatum*, the secondary metabolites responsible for these activities are gallic acid, p-hydroxybenzoic acid, catechin derivatives and tannins isolated from leaf and bark extracts (Lopes *et al*., 2009 and 2005). Recent works have incorporated *Stryphnodendron* spp. extract in PVA, zein, chitosan nanoparticle and NRL, but only NRL and chitosan nanoparticle have realized its release.

NRL is extract from rubber tree (*Hevea brasiliensis*), and its main polymer chain is the cis-1,4-polyisoprene. Although same latex proteins are related with allergies,

a centrifugation can be applied to reduce those proteins related to allergy (Mrué *et al*., 2004). Thus, NRL have been used inclusive *in vivo* to bladder augmentation (Domingos *et al*., 2009), bone (Herculano 2009), skin ulcers healing (Mrué *et al*., 2004), among others, enhancing the healing and showing neoangiogenesis activity, but with no allergy reactions or rejection (Mrué *et al*., 2004). NRL has also been used as matrix for drug, protein (Herculano 2009) and inclusive plant extract release (Borges *et al*, 2014; Romeira *et al*., 2012).

Thus, the aim of this work was to study the interaction between the hydroethanolic extract from leaves of *Stryphnodendron obovatum* Benth and the natural rubber latex biomembrane and their behavior and mechanism of release.

MATERIALS AND METHODS

Latex from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.

The NRL was extracted at BDF Rubber Latex Co. Ltd (producer and distributor of concentrated rubber latex) Guarantã, Brazil. After extraction, it was centrifuged to reduce the proteins related to allergies reaction (Mrué *et al*., 2004.), concentrated at 60% of rubber and stabilized with ammonia to keep it liquid (pH 9-10).

Extract obtention

Leaves of *S. obovatum* were collected in December (2009) in Assis (FCL-UNESP Assis) at the point (22º32'20''S and 50º22'60''W), State of Sao Paulo, Brazil. Dr. Antônio C.G. Melo identified the specimen and the voucher specimen (nº 40892) was deposited in the Herbarium D. Bento Pickel for future reference. Dried and crushed leaves (3.0 g) were extracted by dynamic maceration with ethanol: water 70:30 v/v (1:10 plant/solvent ratio, 3 x 2h), at room temperature. After filtration, the extract was concentrated under reduced pressure until complete organic solvent elimination providing hydroethanolic extract (SOHE) with 27.4% yield.

NRL biomembranes preparation

Natural rubber latex biomembranes loaded with extract were prepared by mixing 5.0 mL of liquid latex with 3.0 mL of a hydroethanolic solution EtOH:H2O 10% (v/v) of the extract at the concentrations of 0.1 and 5.0 mg/mL, and casting on a 5.0 cm of diameter glass Petri dishes, in triplicate. After dried at room temperature, the release were performed in 200 mL of distilled water and measured by ultraviolet–visible spectroscopy (UV-VIS) for 173 hours, at the maximum wavelength of 267 nm.

The FTIR 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}.

The micrographs were performed using a Scanning Electron Microscope (SEM) model Zeiss EVO 50 (20 kV) and a take-off angle of 35°.

The mathematical models (Higuchi, Hixon & Crowell, First order, Korsmeyer & Peppas and Baker & Lonsdale) were applied to analyses the mechanism of extract release from the carrier. The parameters calculated by means of kinetic models were applied to 60% of the released fraction and determined from the best coefficient of determination (r^2), employing the software Sigma Plot 12.5 (from Systat Software).

The kinetics releases (concentration versus time) were fitted to the linear regression for the best coefficient of determination (r^2) employing the software ORIGIN LAB®.

RESULTS AND DISCUSSION

The main advantage of using a carrier is its direct application to the site, which increases patient compliance, enhances efficacy and reduces toxicity. Extract from the leaves of 'barbatimão' (*S. obovatum*) have tannins, related to anti-inflammatory, antimicrobial and healing properties. In addition, NRL biomembrane have angiogenic activity. Both materials have already their efficiency tested *in vivo*, with sustainable exploitation of both crops.

In Figure 1 are shown the FTIR-ATR spectra of the *S. obovatum* Benth extract, NRL biomembrane and from NRL biomembrane containing the extract.

The genus *Stryphnodendron* spp. has been shown do possess tannins in its barks and leaves (Salgado *et al*., 2013). From FTIR analyses (Figure 1) are observed the absorption bands related with tannins at: 1606 and 1443 cm^{-1} (stretching of C=C bonds of the aromatic rings), 1539 cm^{-1} (stretching vibrations of the aromatic ring), 1338 cm^{-1} (Gonultas & Ucar, 2012) and 1199 and 1033 cm^{-1} (stretching of C-O) (Falcão & Araújo, 2013), 1146 cm^{-1} (aromatic O-H) (Gorinstein *et al*., 2009), 1103 cm^{-1}(C-O) and below 822 cm^{-1} (stretching C-H of the aromatic rings) (Souza *et al*., 2013). The broad band at 3200 cm^{-1} suggests hydrogen-bonding interaction.

Costa *et al*. (2013) have also observed these absorption bands in barbatimão-load chitosan nanoparticles, with respect to the tannins from de bark of *S. obovatum*. Although, it was observed a broadening and overlapping of the band related to hydrogen bonding between the components, and related to the 70% of not released plant extract.

In FTIR of NRL biomembranes are observed absorptions bands at 2960-2852 cm^{-1} (symmetric and asymmetric stretching of C-H and CH_2 e CH_3, respectively), 1446 and 1375 cm^{-1} (asymmetric deformation of CH_2 and CH_3, respectively), 835 cm^{-1} (out-of-plane wagging of =C-H) (Pichayakorn *et al*., 2012; Nallasamy & Mohan, 2004). According to Marques (2011), it is also observed a low intensity absorption at 1662cm^{-1} (C=C stretching). Besides the main absorption bands of the cis-1,4-polyisoprene, it is also noticed the absorbencies from proteins and phospholipids that stabilize the latex particles at 3537 cm^{-1} (OH and NH stretching), 1542 cm^{-1} (NH bending) and between 1130 to 1010 cm^{-1} indicating oxygenated

Figure 1: FTIR spectrum of NRL biomembrane plus *S. obovatum* extract (blue line), NRL biomembrane (red line), and *S. obovatum* extract (black line).

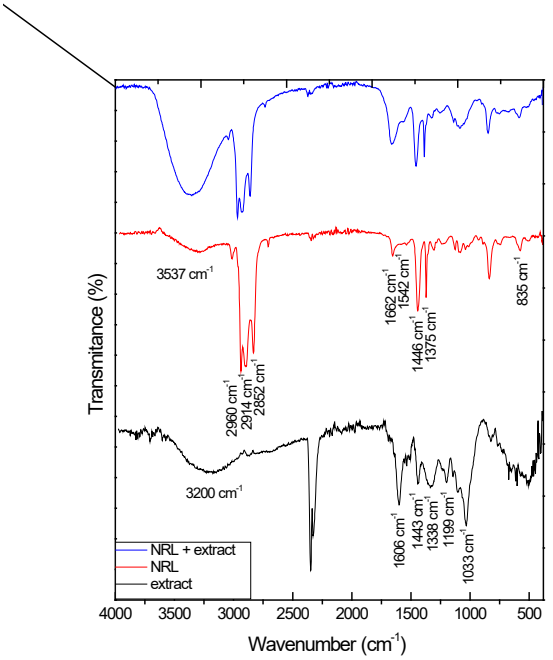
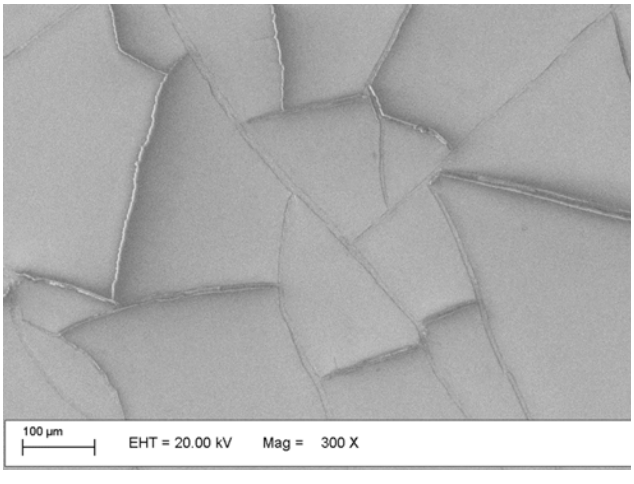


Figure 2: SEM of the (a) NRL biomembrane; (b) NRL with *S. obovatum* extract incorporation.



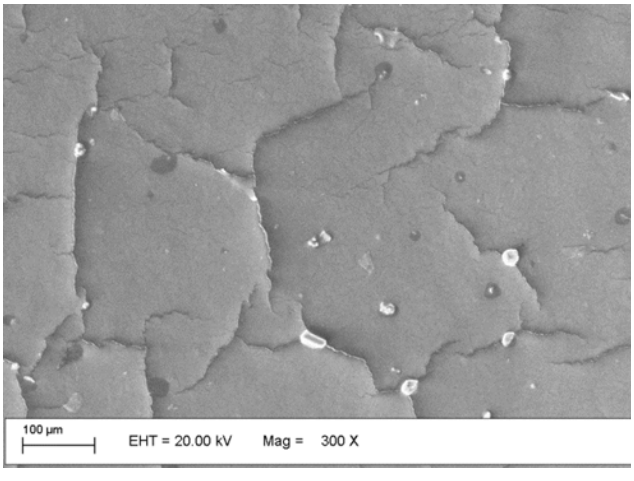
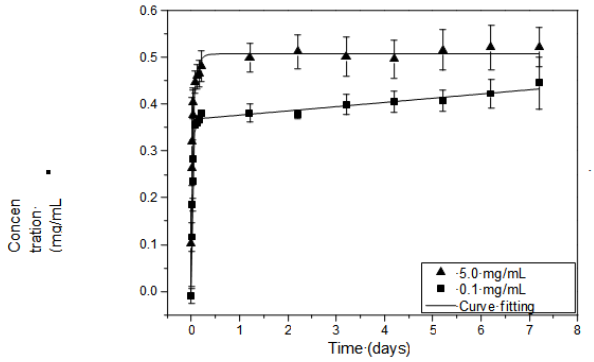


Figure 3: *S. obovatum* extract release at 5.0 mg/mL and 0.1 mg/mL (λmax= 267nm).



compounds (C–O and –O–O–) (Rippel *et al*., 2003; Pichayakorn *et al*., 2012)

It is possible to notice that there is no reaction between NRL biomembrane and the extract since there are no appearance, disappearance or shift of the bands of both compounds, there are only the intensification of the common groups as at 3400-3200 cm^{-1} (intermolecular hydrogen bond) and at 1600 cm^{-1} (C=C).

Among the models applied, the model with the best coefficient of determination (r^2) was Korsmeyers & Peppas for both concentrations used (Table 1). According to equation $f(t) = k*tn$, where f(t) is the fractional release of compound at elapsed time t, k is kinetic constant (related to the structural and geometrical characteristics of the carrier) and n is the release exponent (which suggests the mechanism of release). The interpretation of the value of n varies depending on the carrier, so that to thin films, $n < 0.5$ indicates Fickian diffusion, $0.5 < n < 1.0$ indicates anomalous transport mechanism, $n = 1.0$ indicates Case II transport, $n > 1.0$ indicates Super Case II transport (Steingräber *et al*., 2008).

The applied models indicated that the release mechanism was Super Case II, as observed in the release of casearins and phenolic compounds by NRL carrier (Borges *et al*., 2014). This mechanism assumes a high rate of permeation through the matrix, occurring diffusion, erosion, relaxation and swelling. However, because latex is hydrophobic, its swelling is irrelevant.

From the k values, the release at 0.1 mg/mL is faster than at 5.0 mg/mL. Borges *et al*. (2013) also observed a slower release with the increase of concentration. This may be due to increase in hydrogen bonding between the tannins and proteins from latex by increasing crosslinking and hindering the penetration of the medium.

Both releases can be fitted as bi-exponential decay (Figure 3) $y(t) = y0+A1e^{-t/\tau1}+A2e^{-t/\tau2}$, where y(t) corresponds to the amount released at the time t, y0 is the

Table 1: Equation parameters from kinetic release mechanism for 5.0 mg/mL and 0.1 mg/mL.

	5.0 mg/mL			0.1 mg/mL		
	r^2	k	n	r^2	k	n
Korsmeyers & Peppas	0.9999	3.164*10-3	1.0256	0.9999	1.217*10-1	1.044
Baker & Lonsdale	0.9998	1.102*10-5	-	0.8943	7.596*10-7	-
Hixon & Corwell	0.9125	7.216*10-3	-	0.9995	4.283*10-4	-
Higuchi	0.9998	3.306*10-5	-	0.8945	2.133*10-1	-
First order	0.9124	8.676*10-10	-	0.9995	1.286*10-3	-

Table 2: Bi-exponential release parameters.

	y0	A1	τ1	A2	τ2
0.1 mg/mL	641.5418	-641.173	70778.99	-0.37604	0.02861
5.0 mg/mL	0.50744	-0.17858	4.02e(-04)	-0.22793	0.07227

initial content, A1, τ1, A2, and τ2 are constants (Table 2). The concentrations show two stages of release: initially is fast due to extract on the surface as can be observed in micrographs (Figure 2b); then becomes slower and gradual until its stabilization, due the fractures at the surface, which may releases the extract in the bulk. The control biomembrane showed no extract at the surface (Figure 2a).

Although both biomembranes had different amounts of extract, is possible to notice that they stabilize at similar concentrations. The biomembrane with 0.1 mg/ml released 96.8 % and with 5.0 mg/ml, only 2.4 %, both in 7 days. In spite of the biomembrane at 5.0 mg/mL still had extract to be released, the biomembrane and the solution come into equilibrium, which can be shown by both biomembranes stabilizing the release at the same concentration.

The bi-exponential model has been observed in the release of drugs and plant extracts. From bark extract of *Stryphnodendron* sp., Romeira *et al*. (2012) have also obtained a bi-exponential release, however with a release of 49.89% in 15 days. Costa *et al*. (2013) have released 30% in 7 days from barbatimão-loaded chitosan nanoparticles.

Extracts from *Stryphnodendron* polyphyllum, *Stryphnodendron obovatum* and *Stryphnodendron adstringens* have already been tested *in vivo* in healing models, however few studies have used them with carriers (Lopes *et al*., 2005; Hernandes *et al*., 2010). In additional, Costa *et al*. (2013) and Mori *et al*. (2014) have incorporated S. adstringens extract in PVA/pineapple nanofibers and zein respectively, without studying their releases. Additionally, by methodology employed, all extract was incorporated in the NRL biomembrane, in contrast to the 38.23% of incorporation obtained by Costa *et al*. (2013) with chitosan nanoparticles.

CONCLUSIONS

From SEM it is possible to notice the extract of *S. obovatum* at the surface of NRL biomembrane. The releases followed a bi-exponential model with a fast initial release (due to extract the surface) and then slower. The release mechanism that best fit for both was to Korsmeyer & Peppas and the phenomenon of release was Super Case II (n > 1). FTIR analysis showed no observable reaction between the carrier and the extract, only intermolecular interactions. The release depended on the final concentration of the extract solution, at 5.0 mg/mL released 2.4% and at 0.1 mg/mL released 96.8% in 7 days.

ACKNOWLEDGMENTS

This work was supported by CNPq, FUNDUNESP and FAPESP (Process 2011/17411-8).

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Recebido em 23 de outubro de 2014

Aceito em 6 de novembro de 2015