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Preparation and characterization of chitosan/cashew gum beads loaded with *Lippia sidoides* essential oil

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ABSTRACT

Beads based on chitosan (CH) and cashew gum (CG), were prepared and loaded with an essential oil with larvicide activity (*Lippia sidoides* – Ls). CH and CH–CG beads were characterized by scanning electron microscopy (SEM), infrared and UV–VIS spectroscopy, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), as well as, regarding their larvicide loading, swelling, *in vitro* and *in vivo* release kinetics. The oil encapsulation was evidenced by FTIR analysis and LS loading ranges from 2.4% to 4.4%. CH beads duly showed swelling degree (Q) values from 4.0 to 6.7, reaching equilibrium after 30 min, whereas crosslinked CH–CG beads showed lower swelling values, from 0.4 to 3.8, exhibiting a longer equilibrium time. Liquid transport parameters have revealed diffusion coefficient for CH–CG beads, as low as 2×10^{-15} m^2/s. TGA and DSC revealed that CH:CG crosslinked beads are more thermally stable than CH beads. *In vitro* release follows a non-Fickian diffusion profile for both bead types, however, and a prolonged release being achieved only after beads crosslinking. *In vivo* release showed that both CH and CH–CG presented a prolonged larvicide effect. These aforesaid results, indicate that CH–CG beads loaded with LS are efficient for *A. aegypti* larval control.

1. Introduction

Natural polymers have been object of investigation by researchers all over the world, owing to their large field of applications, particularly as substitutes of synthetic polymers, whereby they present several advantages, such as low cost, low toxicity, ready availability and biodegradability [1]. Those biopolymers can be obtained from several sources such as seeds, algae, exudates from plants and microorganisms. The Brazilian biodiversity, is a very promising and yet quite unexplored research field which offers boundless possibilities to uncover new biopolymers with interesting properties for the pharmaceutical, medical and food industries, as well as for agriculture. In this sense, biopolymer exudates from trees of the Brazilian Northeastern region, such as cashew gum (CG) from *Anacardium occidentale* tree, have duly received a great deal of attention from academia, mainly due to its similarity to Arabic gum (regarding their molar masses, uronic acid content and same type of monosaccharides units, besides being exudated gums and have branched chains) as well as to its potential as a by-product of the cashew industry. Composition of cashew gum has been determined and reported as being a main chain of galactose (72%) units, having branches of arabinose (4.6%), glucose (14%) and rhamnose (3.2%). Uronic acid (4.7%) units, were also found to be present [2].

Chitosan (CH) is another biopolymer which is extensively employed in a myriad of applications in several research fields, largely due to its policationic nature, besides the above mentioned properties [3], CH microspheres crosslinked with politriphosphate have been used for textile industries, dye adsorption [4], as well as metals, such as aluminum, copper, iron [5,6] and nickel [7] from coal miner disposals. Recently, chitosan was coated with PVC for metal removal from water [8]. Alginate has also been used for beads production, such as for the controlled release of drugs such as ornidazole [9], and theophylline [10]. Despite the fact that microspheres and beads having been extensively used in controlled release formulations for pharmaceuticals, cosmetics, chemistry and in the agriculture, few papers dealt with their application as carriers for insecticides or pesticides [11–16]. Carriers developed for these purposes, must meet the needs for proficiency, efficiency and handling safety, regarding the user and the environment. In this sense, to the best of our knowledge, few authors, have been developing matrices for encapsulation of essential oil from plants, such as (*Azadirachta indica* A. Juss.), popularly known as "Neem" [16]. Alginate based matrices have been used as well as chitosan and cashew gum, nevertheless, the later has been employed for encapsulation of conventional pesticide [15].

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Lippia sidoides (Ls) is a native crop of the Brazilian Northeastern region, whereby its leaves contain an essential oil rich in thymol (54–75%), which has been shown to exhibit antimicrobial action against fungi and bacteria, as well as larvae of *Aedes aegypti*, the dengue vector [17,18]. Brazil, like most tropical countries, suffers from dengue epidemics in such a way, where 400,000 people all over the country, were infected during 2008. Dengue starts with chills, headache, pain upon moving the eyes, and low backache; the temperature rises quickly as high as 104 °F (40 °C). Classical dengue does not results in death, unlike the hemorrhagic type, which causes abdominal pain, hemorrhage (bleeding), and circulatory collapse (shock).

Fighting dengue, using conventional organophosphorated insecticides like temephos or pyretroids, has been proven unsatisfactory, mainly due to the fact that the dengue vector has developed resistance against those aforesaid substances [19]. Furthermore, environmental claims do not allow deleterious substances being thrown into the air or the water reservoirs.

This work reports on the preparation and characterization of CH and CH–CG beads, loaded with *L. sidoides* oil leaves, as well as *in vitro* and *in vivo* experiments, in pursuit of developing a new tool for dengue control.

2. Experimental

2.1. Materials

Chitosan (75% deacetylation degree, MW = 1.8×10^5 g/mol), was supplied by a local industry in CE, Brazil. Cashew gum extracted from native trees of Ceará (1.1×10^5 g/mol), was purified as described in a previous work [2,20]. Glycerin (Synth), *L. sidoides* (Ls) oil (Produtos Naturais LTDA – Pronat Horizonte, CE), emulsifier Tween 80 (Vetec), Sodium Hydroxide (Synth) and Glutaraldehyde (Sigma), were used as received.

2.2. Preparation of CH and CH–CG beads

CH beads were prepared in the following manner: Ls essential oil (0.1% to 4.0%, w/w), glycerin (Ls: glycerin = 10:2, v/v) and Tween 80 surfactant (Ls:Tween = 10:1, v/v) were mixed with a 4% chitosan solution in 1% of an acetic acid solution. Ls oil was added in excess, using a Ls/CH ratio from 1/1 to 5.5/1. This mixture was stirred (600 rpm) for 15 min, in order to form a stable emulsion. Later on, the emulsion was added to a 5% NaOH solution through a syringe 22G connected to a peristaltic pump with a flow rate of 1.6 ml/min and kept for curing during 5 min, at stirring speed of 50 rpm. Beads were washed with deionized water and dried in a desiccator. CH beads coated with CG were prepared in a similar format, however, after washing, CH beads were placed in a 10% CG aqueous solution for 30 min, removed and further dipped in a 5% glutaraldehyde solution for 30 min. CH–CG beads were then washed with deionized water and stored in a desiccator.

2.3. Beads characterization

Beads had their average diameter determined, using a micrometer (Mitutoyo), and their average mass was weighted in an analytical balance (Micronal). The Beads structure was elucidated by analyzing their main functional groups through infrared spectroscopy (FTIR), in a Perkin Elmer instrument and their morphologies were determined by scanning electron microscopy, in a Philips model XL-30 Holland microscope, using a voltage acceleration of 20 kV. Thermal stability of the beads, was evaluated by thermogravimetric analysis (TGA) in a Shimadzu equipment model TGA-50, using N_2 atmosphere and a heating rate of 10 °C/min from 25 °C to 900 °C, as well as by differential scanning calorimetry (DSC) in a model 2910 from TA instruments, with a heating rate of 10 °C/min, at a temperature range from 25 °C to 400 °C.

2.4. Swelling degree

Swelling degree (Q) was determined by weighting in an analytical balance (Micronal), a determined mass of dried beads, then placing them in a vessel, containing distilled water. Swollen beads were then withdrawn at regular time intervals, having the excess water removed by means of a soft paper and weighted accordingly. Q was obtained in relation to Eq. (1):

$$Q = (M_t - M_o)/M_o \tag{1}$$

Where M_o is the mass of the dried beads and M_t the mass of the beads swollen in water, for a determined time interval.

2.5. Loading

Ls loading in the CH and CH–CG beads were evaluated through absorbance in an UV spectrometer at 260 nm, using a calibration curve of thymol (molar absorption coefficient 4.06×10^{-7} mol^{-1} m^2), the Ls essential oil main component. Thymol samples of different concentrations (1–7 ppm) had their absorbance read by UV spectroscopy, yielding a calibration curve given by Eq. (2). Bead samples were further smashed in ethanol and Ls concentrations were determined by means of Eq. (2).

$$Abs = 0.06064 + 0.00347\ Conc. \qquad R^2 = 0.9897 \tag{2}$$

Whereby, *Abs* is the absorbance and *Conc.* the concentration of Ls in ppm. The analyses were conducted in triplicate.

2.6. In vitro release

Ls *in vitro* release was evaluated by placing a determined mass of beads (10 mg), in a cell inside a beaker with 20 ml of distilled water. The system was kept at a temperature of 25 °C, under stirring. An aliquot of the duly released medium (0.5 mL), was withdrawn at predetermined time intervals and an equivalent amount of water, was replaced to the aforementioned solution. Aliquots of 0.5 ml were then diluted in total volume of 3 ml, as well as analyzed using a UV spectrophotometer at 260 nm, thus calculating the concentration of the oil present in the medium using Eq. (2). All measurements were made in triplicate and data were calculated with a 95% confidence level.

2.7. Bioassays

In vivo experiments were conducted as follows: Beads (9, 13, or 18 mg) were placed in a Becker containing 50 mL of water and 20 second and third instar *A. aegypti* larvae, and their death was monitored through a function of time. At determined time intervals, the number of dead larvae was registered and killed specimens were withdrawn. Data obtained were averaged, with mean values and standard deviation calculated with a 95% confidence level. Experiments were run in duplicate, and a control sample was obtained by the larvae being kept in the water under the same conditions, however, with unloaded beads, as described in Paula et al. [15]. Mean values were considered statistically different at the 0.05 significance level.

3. Results and discussion

Initially, CH beads were prepared according to previous work [15] with some modification such as the varying CH:Ls relative ratio in order to optimize the beads form, size and loading efficiency values.