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Alginate/cashew gum nanoparticles for essential oil encapsulation

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ABSTRACT

Alginate/cashew gum nanoparticles were prepared via spray-drying, aiming at the development of a biopolymer blend for encapsulation of an essential oil. Nanoparticles were characterized regarding to their hydrodynamic volume, surface charge, *Lippia sidoides* essential oil content and release profile, in addition to being analyzed by infrared spectroscopy (FT-IR), thermal analysis (TGA/DSC) and X-ray diffractometry. Nanoparticles in solution were found to have averaged sizes in the range 223–399 nm, and zeta potential values ranging from −30 to −36 mV. Encapsulated oil levels varied from 1.9 to 4.4% with an encapsulation efficiency of up to 55%. The *in vitro* release profile showed that between 45 and 95% of oil was released within 30–50 h. Kinetic studies revealed that release pattern follow a Korsmeyer–Peppas mechanism.

1. Introduction

Nanoscale biopolymer particles have attracted much interest of academia particularly for design and fabrication of new devices for drug delivery systems. Among many investigated biopolymers such as chitosan, starch, cellulose and dextran, alginate has unique and remarkable properties which accounts for its ability to form gels, spheres, micro- and nanoparticles [1–3]. This capability is due to the fact that alginate can interact with divalent cations that act as crosslinking agent, linking its functional groups, forming called "egg-box" complexes [4]. In addition to that, alginate has been proven to exhibit biocompatibility, biodegradability and non-toxicity [5]. It has been widely used to encapsulate active principles, such as pharmaceuticals and essential oils [6,7].

Regarding to its structure, alginate is a polyanionic linear biopolymer consisting of β-D-manuronic acid and α-L-guluronic acid blocks.

Cashew gum (CG) is a biopolymer extracted from the exudate of *Anacardium occidentale*, a common tree of Brazil's northeastern region. The gum main chain is composed of galactose (72%), with side-chains of arabinose (4.6%), glucose (14%), rhamnose (3.2%) and uronic acid (4.7%) [8]. CG properties were found to be similar to those of gum Arabic [9].

Essential oils are volatile, evaporating easily, and can decompose when exposed to light, heat and/or pressure [10]. The encapsulation of essential oils aims to preserve and protect their functional properties, in addition to provide a controlled release in a given medium. Some essential oils were encapsulated, such as the essential oil of *Zanthoxylum limonella* [11], *Cymbopogon winterianus* [12], *Lavandula hybrida* [13], and *Croton zehntneri* [14]. Moreover, active components of essential oils, such as carvacrol, were also encapsulated [15]. As matrices used as encapsulating agents, synthetic polymers such as polyethylene glycol and biopolymers such as chitosan, gelatin and gum Arabic were employed [13,16].

Lippia sidoides (LS) essential oil, primarily composed of thymol (50–70%) [17], has several biological applications, owing to its antibacterial and antimicrobial properties [18], being also used to fight nematodes found in ruminant animals [19,20] and dengue vector larvae [21,22]. Its pharmaceutical and medical properties, such as anti-inflammatory, sedative and pain reliever have also being investigated [23,24].

LS essential oil has being encapsulated employing different matrices such as chitosan/cashew gum [22] as well as chitosan/"angico" gum [25], however a low nanoparticle stability was reported, along with a low essential oil release profile.

Spray-drying is a technique that has been used for decades to encapsulate volatile substances in polymeric matrices [26]. Some essential oils have already been encapsulated by this technique, yielding micro- and nanoparticles [15,25,27].

This work aimed at the preparation and physicochemical characterization of nanoparticles (blends) of alginate/cashew gum as wall materials for encapsulation of *L. sidoides* essential oil, via spray-drying, as well as investigation of the effects of polymeric concentrations, alginate: gum and blend: oil ratios on nanoparticle properties. The *in vitro* release profiles for different alginate: gum ratios have also been taken into consideration.

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2. Experimental

2.1. Materials

Alginic acid sodium salt of low viscosity ($M_w = 5.4 \times 10^4$ g mol^{-1}) was purchased from Sigma. Cashew gum was extracted from native trees from Ceará ($M_v = 1.1 \times 10^5$ g mol^{-1}) and purified as described in a previous work [8]. *L. sidoide* oil (99% purity) was supplied by a local company (Produtos Naturais Ltda–Pronat Horizonte, CE). The reagents Tween 80 (Vetec) and calcium chloride (Synth) were used as received.

2.2. Preparation of alginate–cashew gum nanoparticles

Solutions of 0.2, 0.5 and 1.0% (w/v) of alginate and cashew gum were prepared using relative ratios of alginate:gum = 1:3, 1:1 and 3:1. The solutions were prepared by dissolving the desired amount of biopolymer in distillated water to the desired concentration. The cashew gum solution was added to the alginate solution by a peristaltic pump, using a flow rate of 1.0 mL min^{-1}. An emulsion was obtained by slowly adding LS oil and a surfactant (Tween 80) to the polymeric solution mixture using a blend: oil ratio of 10:1 (w/w). A $CaCl_2$ solution (0.5%, w/w) was slowly added to the former emulsion, under mechanical stirring at 18.000 rpm, and then spray dried in a Buchi equipment, model B290, operating at inlet temperature 170 °C, outlet temperature 65 °C, pump feed flow 5 mL min^{-1}, air volume flow 35 m^3 h^{-1} and aspirator flow meter 84 L h^{-1}.

2.3. Loading and encapsulation efficiency

Essential oil loading was determined by crushing a 10 mg sample in ethanol, leaving it resting for 4 h and calculating the resulting concentration using a calibration curve (equation 1), after absorbance readings by UV/VIS spectroscopy, at 260 nm, in a MICRONAL spectrometer (model B582, Brazil). All analysis were carried out in triplicate ($n = 3$) and data were averaged within a 95% confidence level. Data obtained were compared with those obtained from gas chromatography (GC) analysis.

$$Abs = 0.0036 - 0.0387\, conc \quad (1)$$

where *Abs* is the absorbance and *conc* is the LS concentration, given in ppm. Correlation coefficient was $r^2 = 0.998$. LS encapsulation efficiency (EE) was determined using the following equation:

$$EE = \frac{M}{Mo} \times 100 \quad (2)$$

where *M* is the amount of LS in the loaded sample (mg), as determined from equation 1 and *Mo* is the initial LS amount (mg) added to the emulsion.

2.4. Nanoparticle characterization

Nanoparticle morphologies were analyzed by Scanning Electron Microscopy–SEM, by placing powdered samples on carbon stickers on aluminum stubs, drying and coated with gold. Nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR) using KBr pellets, in a Shimadzu IR spectrophotometer (model 8300), operating between 400 and 4000 cm^{-1}. The particle size distribution and zeta potential were determined in a Malvern Nano Zetasizer, model Zen 3500 by dissolving powdered samples in deionized water and analyzing their size and charge distributions. The hydrodynamic diameter was measured by dynamic light scattering with laser with wavelength of 633 nm and a fixed scattering angle of 173°. Thermal analysis of the samples was carried out by thermogravimetric analysis (TGA) in a Shimadzu analyzer, model TG-50, in nitrogen atmosphere applying a heating rate of 10 °C min^{-1}, from 25 to 900 °C, and by differential scanning calorimetry (DSC) in a Shimadzu DSC-50, with a heating rate of 10 °C min^{-1}, from 25 to 400 °C. Wide-angle X-ray diffraction (WAXD) measurements were made on a PANalytical X'Pert Pro MPD instrument, where powder forms of the samples were exposed to Cu radiation, with increments of 1° min^{-1} and scanned over a 2θ range from 3° to 40°.

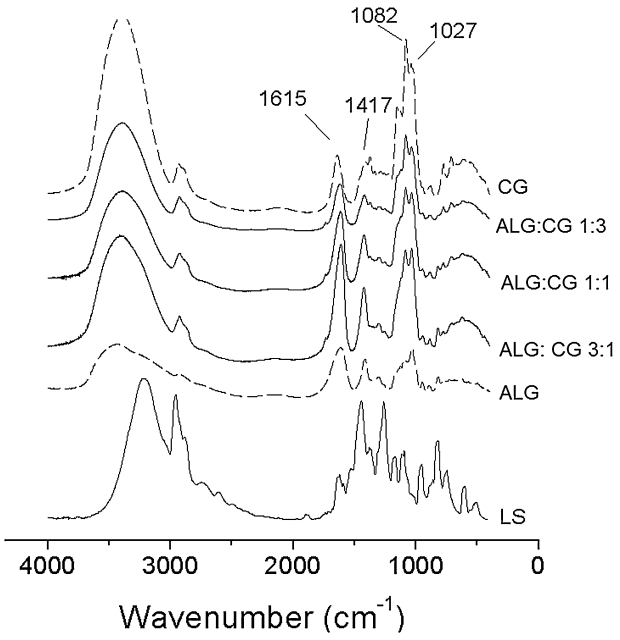

Fig. 1. FTIR of ALG, CG, LS essential oil and ALG–CG nanoparticles at different ratios.

2.5. In vitro release

Aiming at a likely application as a larvicide for *Aedes aegypti* larvae control, which usually occurs in aqueous environment, *in vitro* release study was conducted by dissolving 100 mg sample in 10 mL distilled water and the resulting solution placed in a dialysis bag (pore size: 14 kDa) which was kept in a beaker containing 200 mL distilled water, under stirring, at room temperature. An aliquot of the release medium was withdrawn at pre-determined time intervals and analyzed using a UV spectrophotometer at 260 nm. The concentration of the oil present in the medium was calculated using Eq. (1). All measurements were replicated twice ($n = 3$) and data were averaged, using a 95% confidence level.

3. Results and discussion

Preliminary experiments focused on the improvement of emulsion properties by using different blend:oil ratios; they had their opalescence, aggregation potentials and solution stabilities observed and compared. The best result was obtained for blend:oil ratio = 10:1 and this formulation was employed throughout this work.

3.1. Nanoparticle characterization

3.1.1. FT-IR spectroscopy

Infrared spectra of ALG, CG, LS samples and ALG: CG nanoparticles can be seen in Fig. 1. ALG: CG nanoparticles spectrum revealed the presence of the main absorbance of ALG-CG blend as following. Bands at 3440 cm^{-1} are assigned to –OH stretching group while those at 1082 and 1027 cm^{-1} are due to asymmetric stretching