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Experimental design approach applied to the development of chitosan coated poly(isobutylcyanoacrylate) nanocapsules encapsulating copaiba oil

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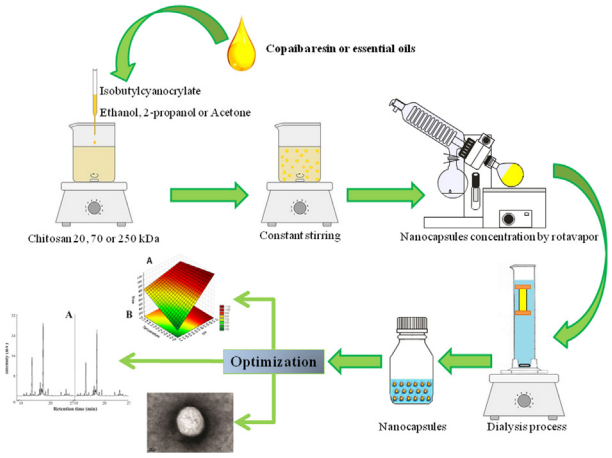
HIGHLIGHTS

- Copaiba oil-loaded chitosan decorated nanocapsules was produced.
- Nanocapsules size and zeta potential were optimized by experimental design.
- Chitosan was used as a stabilizer for the nanocapsules production.
- pH and the temperature of polymerization influenced both the size and zeta potential.
- Copaiba oil was efficiently encapsulated and showed all compounds of the parent oil.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this work was to develop, characterize and optimize the natural copaiba oil-loaded chitosan decorated poly(isobutylcyanoacrylate) nanocapsules. These innovatively obtained natural-based systems were developed by an original method of interfacial polymerization of isobutylcyanoacrylate using chitosan as a stabilizer for the nanocapsules. A preliminary study investigated the influence of the molecular weight of chitosan, the type of copaiba oil extract and the solvent phase. Nanocapsules could only be produced with copaiba resin oil, with size ranging from 300 to 1200 nm. Nanocapsule size and zeta potential were then optimized by two-level three-variable full-factorial experimental design. Samples showed

Abbreviations: $CO_{dispersed\text{-}phase}$, amount of copaiba oil found in the dispersed media of the nanocapsules; CO_{total}, total amount of copaiba oil used in the preparation; Adj R^2, adjusted determination coefficient; F_{model}, F-value of the model; F_{Tab}, tabulated F- value; $F_{Tabresidues}$, tabulated F- value of the residues; $F_{residues}$, F- value of the residues; R^2, coefficient of determination; x_1, pH of the polymerization medium; x_2, temperature of polymerization; x_3, concentration of chitosan in the polymerization medium; Y_1, predicted droplet size (nm).

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Experimental design approach

spherical objects when analyzed by transmission electron microscopy. The copaiba oil encapsulated in the nanocapsules showed all compounds of the parent oil. Nanocapsules with positive zeta potential were obtained consistently with the expected distribution of chitosan on the nanocapsule surface. Optimal nanocapsules showed a diameter of 473 nm, a zeta potential of +34 mV and an encapsulation efficiency of the oil of 74% including 55.5 μg of β- caryophyllene/mg of nanocapsules. The obtained nanocapsules can be suggested as oral delivery system for anticancer molecules including paclitaxel assuming a synergistic effect with anticancer active components of the oil.

1. Introducton

Nanoparticles composed of mucoadhesive polymers are promising systems for oral drug delivery applications [1,2]. Generally, nanoparticles are defined as solid colloidal particles that include both nanospheres and nanocapsules [3]. The latter are vesicular systems in which the drug is confined in a liquid/solid cavity surrounded by a polymer envelope.

Proposed to improve drug delivery, nanoparticle systems can modulate drug biodistribution and release in a controlled manner, increase intracellular uptake and improve the stability of active substances [4–6]. Many types of nanoparticles made of biodegradable polymers, including poly(isobutylcyanoacrylate), were considered to improve drug delivery by oral route. To this purpose, their surface property may be tuned to increase mucoadhesion [7,8]. Chitosan has been a widely used polysaccharide to formulate mucoadhesive systems [9,10]. In addition, this polysaccharide is biocompatible for oral administration. Its inherent mucoadhesive properties come from the amino groups included in the chemical structure that can interact with sialic acid groups of mucins composing the mucus via electrostatic interactions [11]. Besides, the positive charges of chitosan are also believed to play an essential role increasing the permeability of the intestinal epithelium thanks to its capacity to disturb the calcium concentration balance near the tight junction [12]. Although widely used to improve mucoadhesion of nanospheres, this polysaccharide was not yet used to improve mucoadhesion of polymeric nanocapsules, which are interesting as drug delivery systems for lipophilic drugs.

The copaiba oil-resin (*Copaifera langsdorffii*) is an oily plant extract used in folk medicine in its *in-natura* form [13]. Phytochemical studies on oil-resin reveal that it contains a complex mixture of diterpene and sesquiterpene hydrocarbons [14], giving this oil many interesting therapeutic activities. For instance, these include anti-inflammatory, antitumor, anti-tetanus, antimicrobial, antileishmania activities, among others [15–17]. Although used for years in folk medicine, it is believed that pharmacological activities of this oil may be increased by developing appropriate formulations.

Therefore, the aim of the present work was to develop an original formulation of nanocapsules coated with chitosan as mucoadhesive compound and including copaiba oil in the reservoir of the vesicle. The polymer composing the nanocapsule envelope was a critical choice. Poly(isobutylcyanoacrylate) was selected because of its capacity to formulate nanocapsules that resist well to the gastric medium and promote release in the intestinal medium [18]. The development of mucoadhesive copaiba oil-containing nanocapsules of poly(isobutylcyanoacrylate) have not been described before. The formulation process was based on the use of an experimental design approach that was never applied so far while developing new formulations of oil-containing nanocapsules prepared by interfacial polymerization of isobutylcyanoacrylate. It is noteworthy that experimental design approach was not so much applied for the development of nanocapsules while such an approach could be helpful to optimize formulations, limiting the number of experiments to perform hence the amount of reagent and time [19].

2. Materials and methods

2.1. Materials

Copaiba resin oil was purchased from Flores & Ervas (Piracicaba, SP, Brazil). Isobutylcyanoacrylate was provided by ORAPI engineered solutions worldwide (Vaulx-en-Velin, France). Water soluble chitosan (Mw 20,000 g/mol) was purchased from Amicogen (Jinju, South Gyeongsang, South Korea). Ethanol, acetone, 2-propanol, sodium hydroxide, ethyl acetate, nitric acid were provided by Fisher Scientific (Pittsburgh, PA, EUA). Diazomethane and β-caryophyllene were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Ultrapure water was obtained from a Millipore purification system (Milli-Q® plus, Millipore, St Quentin en Yvelines, France). All chemicals were reagent grade and used as received.

2.2. Copaiba essential oil extraction

Copaiba essential oil was obtained from the hydrodistillation of 400 mL of copaiba resin oil using a Clevenger-type apparatus for 3 h. The extracted essential oil was dried with sodium sulphate, filtered, stored in the refrigerator and protected from light until use.

2.3. Preparation of the nanocapsules

Copaiba oil-loaded chitosan-decorated poly(isobutylcyanoacrylate) nanocapsules were elaborated by the method of interfacial polymerization [20,21] that was adapted because of the use of chitosan. A preliminary study investigated the influence of the molecular weight of chitosan (20, 70 and 250 kDa), the type of copaiba oil (resin and essential oil) and the nature of the solvent phase (ethanol, 2-propanol and acetone) in order to identify the best substances to produce nanocapsules. Thus, 0.25 mL of copaiba oil and 0.032 mL of isobutylcyanoacrylate were solubilized in 6.25 mL of solvent to produce the organic phase. This phase was slowly injected dropwise in 12.5 mL of the polymerization medium prepared with 0.6% of chitosan at pH 6 and homogenized for 10 min at 250 rpm at 25 °C (Fisher- Bioblock Scientific AM 3001 K, Illkirch, France). The obtained colloidal dispersion was concentrated by rotary evaporator for 20 min at 35 °C/43 mBar (BÜCHI Rotavapor R-125, Heating Bath B-491, Vacuum pump V-700, recirculating Chiller F-108, Flawil, Switzerland) to eliminate the solvent. Then, the dispersion was filtered through a 5 μm minisart NML membrane (Sartorius GmbH, Goettingen, Germany). The obtained nanocapsule dispersions were purified by dialysis (Spectra/Por Biotech membranes, cellulose ester, 100,000 g/mol molecular weight, Rancho Dominquez, CA, USA) against ultrapure water three times for 60 min and once overnight to remove non-associated chitosan.

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