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Formulation and *in vitro* evaluation of coconut oil-core cationic nanocapsules intended for vaginal delivery of clotrimazole

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ABSTRACT

The objective of this work was to propose coconut oil-core nanocapsules prepared from Eudragit® RS100, a cationic polymer, and to evaluate their potential for vaginal delivery of clotrimazole in candidiasis. Nanocapsule suspensions loaded with clotrimazole at 1.0 and 3.0 mg/mL were prepared by interfacial deposition of Eudragit® RS100. The physicochemical characterization showed average diameter lower than 200 nm, low polydispersity index, positive zeta potential (+10.94 to +14.57 mV), acid pH values (5.4–5.7) and encapsulation efficiencies close to 100%. After 60 days of storage at room temperature and protected from light, the nanocapsules were reasonably stable. Photodegradation studies showed that nanoencapsulation improved clotrimazole stability against UV radiation. The *in vitro* drug release at pH 4.5 was characterized by a prolonged release with no *burst* effect. The nanocapsules were more active than free clotrimazole against *Candida albicans* and *Candida glabrata* strains susceptible and resistant to fluconazole. Hence, clotrimazole-loaded coconut oil-core nanocapsules represent promising alternatives to the treatment of vulvovaginal candidiasis.

1. Introduction

Polymeric nanoparticles have been considered promising carriers of therapeutic drugs. The main advantages that these submicron devices have shown to have in recent years include site-specific targeting, controlled release of drugs, and improved protection of labile molecules against enzymatic hydrolysis, photodecomposition, chemical, and immunological degradation [1–3].

In general, polymeric nanoparticles may range from 100 to 500 nm [4] in size, and may be distinguished into two subtypes of nanostructures, namely nanospheres and nanocapsules [5]. Conceptually, nanospheres possess a matrix structure, while nanocapsules present a core-shell organization in which the polymer surrounds a liquid (lipophilic or hydrophilic) nucleus. In such nanocarriers, the active substances are usually entrapped or dissolved within or adsorbed on the particle surface [1]. When comparing these two types of nanostructures, one may note that oily-core nanocapsules feature some important advantages, such as higher drug loading capacity, better prevention from drug degradation, and reduced burst release [6]. Medium chain triglycerides, which make up a synthetic oil, are frequently employed in the preparation of nanocapsules because they present noteworthy qualities, such as biocompatibility and ability to solubilize a wide range of drugs [1,7]. Another important aspect to be taken into consideration in the choice of oil is the low solubility of the polymer in the respective oil and vice versa [8].

Recently, there has been an increasing interest in the preparation of polymeric nanocapsules with vegetable oils. Thus, some studies have reported the development of those nanocarriers using several vegetable oils such as linseed oil [9], sunflower [10], grape seed, almond kernel [11], Brazil nut, olive, rose hip, and carrot [12].

Among the oils of great economic interest, the coconut oil stands out for its high nutritional and pharmaceutical value [13]. Traditionally, coconut oil is used in the pharmaceutical industry as an emollient in bases of ointments, shampoos, soaps and liquid soaps [14]. Although there are few reports in the literature, some studies indicate that coconut oil has antifungal [15–17] and antioxidant activities [17–19].

Coconut oil is extracted from *Cocos nucifera* Linn (Palmae) and is described as a white paste with melting point between 23 and 26 °C [14]. On average, 75% of the fatty acids of virgin coconut oil are medium chain triglycerides and 90% of the fatty acids are saturated. Compared to other vegetable oils, coconut oil has a lower amount of unsaturated fatty acids (4%) than palm, peanut, corn, soy, and

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flaxseed oils, whose amount ranges from 53 to 90% [20]. The major fatty acids present in coconut oil are lauric (40–50%) and myristic acids (15–20%) [14].

Considering nanostructured systems, to the best of our knowledge, there is only one report on the preparation of polymeric nanocapsules containing this oil. Recently, the photostability of polymethine cyanine dye was improved due to its incorporation into poly(D,L-lactide) and polycaprolactone nanocapsules containing coconut oil core [21]. Regarding other types of nanocarriers, solid lipid nanoparticles of coconut oil were considered potential systems for the topical delivery of all-*trans*-retinoic acid [22] and lipoic acid polyethylene glycol ester [23]. In addition, the feasibility of nanoemulsions made up with coconut oil has also been demonstrated [24,25]. In spite of the antifungal activity of this vegetable oil, there is no study on the incorporation of an antimycotic drug into coconut oil nanocarriers.

Among antimycotics, clotrimazole, a broad-spectrum antifungal imidazole derivative, is a poor water soluble drug used topically in the treatment of vulvovaginitis caused by *Candida albicans.* However, this treatment is generally associated with mucosal irritation, leakage of the formulation, and low residence time at the vaginal cavity [26].

Taking all of this into account, this work was designed to prepare and characterize coconut oil-core nanocapsules from Eudragit® RS100, a cationic polymer, intended for the vaginal delivery of clotrimazole. The polymeric nanocapsules were evaluated for their stability at room temperature, photostability under UV light, *in vitro* drug release and *in vitro* antifungal activity against *Candida* species.

2. Materials and methods

2.1. Materials

Clotrimazole (99.22%, w/w) was purchased from Pharma Nostra (São Paulo, Brazil). Virgin Coconut oil was donated by TheraHerb (Niterói, Brazil). Eudragit® RS100 (Röhm Pharma, Germany) was a gift of Almapal (São Paulo, Brazil). Span 80® (sorbitan monooleate) was purchased from Sigma–Aldrich (São Paulo, Brazil) and Tween 80® (polysorbate 80) was supplied by Delaware (Porto Alegre, Brazil). HPLC-grade methanol was acquired from Tedia (Rio de Janeiro, Brazil). Other solvents and reagents were of analytical grade and used as received.

2.2. Analytical procedures

The experiments were performed on a LC-10A HPLC system (Shimadzu, Japan) equipped with a LC-20AT pump, an UV-VIS SPD-M20A detector, a CBM-20A system controller, and a Rheodyne manual sample injector valve with a 20 μL loop. Separation was achieved at room temperature using a RP C_{18} Phenomenex column (250 mm × 4.60 mm, 5 μm; 110 Å) coupled with a C_{18} guard column. The isocratic mobile phase consisted of methanol and water (90:10, v/v) at flow rate of 1 mL/min. Clotrimazole was detected at 229 nm with a retention time of about 5.1 min [27]. The method was validated according to the ICH guidelines to determine clotrimazole in coconut oil-core nanocapsules. The method was found to be linear ($r = 0.9993$), specific, accurate (100.51–101.88%), precise, and robust (relative standard deviation was <3% for all parameters) in the concentration range of 2–10 μg/ml.

2.3. Dissolution/swelling experiments of polymer films

Eudragit® RS100 films were prepared by dissolving 2 g polymer in acetone followed by the evaporation of the solvent at room temperature. About 100 mg film was accurately weighed and placed in contact with enough amount of coconut oil to cover it. In predetermined intervals for a period of 60 days, the films were removed from the contact with the oil and were carefully dried with the aid of an absorbing paper. Weight variation was determined using an analytical balance. The experiments were performed in triplicate.

2.4. Preparation of nanocapsules

Nanocapsule suspensions were prepared through the interfacial deposition of Eudragit® RS100. An organic phase constituted of polymer (0.100 g), acetone (27 mL), Span 80® (0.077 g), clotrimazole (0.01 or 0.03 g), and coconut oil (0.300 g) was kept for 60 min under moderate magnetic stirring at 40 °C. After the solubilization of all components, the acetone solution was poured to 53 mL of an aqueous dispersion of Tween 80® (0.077 g) and the magnetic stirring was maintained for 10 min. In the sequence, the organic solvent and part of the water were eliminated by evaporation under reduced pressure to achieve a final volume of 10 mL and clotrimazole concentration of 1.0 and 3.0 mg/mL (NC-1 and NC-3, respectively). For comparison, formulations without the drug (blank nanocapsules, NC-B) were also prepared. All samples were made in triplicate.

2.5. Characterization of nanoparticle suspensions

2.5.1. pH

The pH of nanoparticle suspensions was verified by directly immersing the electrode of a calibrated potentiometer (Model pH 21, Hanna Instruments, Brazil) in the formulations. Measurements were made at room temperature (25 ± 2 °C) in triplicate of batch.

2.5.2. Particle size analysis, polydispersity, index and zeta potential

Particle sizes and polydispersity indexes ($n = 3$) were determined through photon correlation spectroscopy (Zetasizer Nanoseries, Malvern Instruments, UK) after diluting the samples in ultrapure water (1:500). Zeta potentials were measured using the same instrument after the dilution of samples in 10 mM NaCl (1:500).

2.5.3. Drug content and encapsulation efficiency

Total content of clotrimazole in nanocapsule suspensions ($n = 3$) was assayed by diluting an aliquot of the sample in 10 mL methanol and submitting it to sonication for 10 min to extract the drug. Before injecting them into the HPLC system, the samples were filtered in a 0.45 μm membrane. To determine encapsulation efficiency, an aliquot of the samples was placed in a 10,000 MW centrifugal filter device (Amicon® Ultra, Millipore) and free drug was separated from the nanostructures using the ultrafiltration/centrifugation technique at 2.200 × *g* for 10 min. The encapsulation efficiency (%) was calculated as the difference between total and free concentrations of clotrimazole, determined in the nanostructures and ultrafiltrate, respectively.

2.5.4. Scanning electron microscopy

Nanocapsules were previously liofilized using trehalose (cryoprotectant) and the samples were gold sputtered on a Desk II Cold Sputter (Denton Vacuum, USA) and subsequently analyzed using an accelerating voltage of 15 kV (Scanning microscope JSM-6360, Jeol, Japan).

2.6. Stability studies

All formulations were monitored after preparation for 2 months in storage to check drug content, pH, particle size, polydispersity