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Kinetic and physical-chemical study of the inclusion complex of β-cyclodextrin containing carvacrol

Paula dos Passos Menezes [a], Mairim Russo Serafini [a], Yasmim Maria Barbosa Gomes de Carvalho [a], Dayanne Valéria Soares Santana [a], Bruno Santos Lima [a], Lucindo José Quintans-Júnior [b], Ricardo Neves Marreto [c], Thiago Mendonça de Aquino [d], Adilson Rodrigues Sabino [d], Luciana Scotti [e], Marcus Tullius Scotti [e], Severino Grangeiro-Júnior [f], Adriano Antunes de Souza Araújo [a, *]

[a] Department of Pharmacy, Federal University of Sergipe, São Cristóvão, Brazil
[b] Department of Physiology, Federal University of Sergipe, São Cristóvão, Brazil
[c] College of Pharmacy, Goiás University, Goiânia, Brazil
[d] Nuclear Magnetic Resonance Laboratory, Federal University of Alagoas, Maceió, Brazil
[e] Center for Applied Science and Education Campus, Federal University of Paraíba, João Pessoa, Paraíba, Brazil
[f] Federal University of Pernambuco, Department of Pharmacy, Recife, Pernambuco, Brazil

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ABSTRACT

Carvacrol is a good natural antimicrobial and antioxidant agent; however, its poor aqueous solubility and high volatility limit its application in food systems. Different methods of complexation have been used to preserve aromas in food products and complexation in cyclodextrins (CDs) is among the most efficient ways. In the present study, we investigated the complexation efficiency of carvacrol in β-CD using methods different from those already reported in the literature for this compound. The supramolecular structure of the carvacrol/β-CD complex was investigated by means of X-ray diffraction (XRD), Nuclear magnetic resonance (NMR), docking, complexation efficiency, thermogravimetry/derivate thermogravimetry (TG/DTG) and Karl Fischer titration. Results clearly showed the formation of a supramolecular complex in which the guest molecule, carvacrol, was entrapped inside the cavity of the host, β-CD mainly by slurry method. These results contribute to other studies involving this type of system.

1. Introduction

Phenolic monoterpenes such as carvacrol (2-methyl-5-(1-methylethyl)phenol) are common constituents of many essential oils (EOs) that have been extensively studied as antimicrobial agents for use in food [1]. Some studies have reported their pharmacological activities, such as the pronounced antioxidant effect against free radicals generated *in vitro*, the analgesic and anti-inflammatory profiles in different *in vivo* experimental protocols, including cancer pain model, and the cytokine production modulation, such as IL-10 [2,3].

Studies of Muller-Riebau, Berger & Yegen and Abbaszadeh, Sharifzadeh, Shokri & Khosravi demonstrated the *in vitro* antifungal effects of carvacrol in different levels of potency [4,5]. In addition, this monoterpene is generally considered safe for consumption and is approved by the Food and Drug Administration for food use as a GRAS (generally recognized as safe). Also, it has been included by the Council of Europe in the list of chemical flavorings that may be added to food-related compositions at the 2 ppm level in beverages, 5 ppm in food and 25 ppm in candies [6,7].

Some of the direct applications of carvacrol in the food industry are in baked goods, soft drinks and chewing gum. However, the direct application to foods, as a preservative, may be limited since it can be lost during storage due to its high volatility and reactivity with various food components [8–10]. Therefore, Higueras, Lopez-Carballo, Hernández-Muñoz, Catalá & Gavara studied the utilization of carvacrol complexed with hydroxypropyl-β-cyclodextrin as an alternative for antimicrobial activity in the active packaging of

* Corresponding author. Department of Pharmacy, Federal University of Sergipe, Av. Marechal Rondon, Jardim Rosa Elze, 49100-000 São Cristóvão, Sergipe, Brazil.
E-mail addresses: paula.dp.menezes@gmail.com (P.P. Menezes), adriasa2001@yahoo.com.br (A.A. de Souza Araújo).

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food products [11].

In this context, one of the best matrices for molecular complexation (host-guest supramolecular systems or inclusion compounds) are cyclodextrins (CDs), which are water-soluble cyclic oligomers composed by 6–8 U of glucopyranose bonded together by α-(1,4) linkages. CDs are non-toxic ingredients and the most common form is β-CD, which is composed by 7 U of glucopyranose; it has the shape of a hollow truncated cone with the capacity to enclose (partially or totally) small organic molecules. The presence of primary and secondary hydroxyl groups on the outer part increases the water solubility of these compounds and the corresponding host-guest complexes [12–14]. Of the parent CDs, β-CD is widely chosen because of its better complex forming ability, suitable cavity dimension, and ready availability and being economical. Many drugs have been complexed with β-CD leading to noteworthy improved properties [15]. Our group has recently demonstrated that the complexation with CD can bring benefits to the biological properties of monoterpenes such as carvacrol [16–19].

Indeed, other studies involving carvacrol complexed in CDs with a focus on food have been investigated [20–22]. However, our research group has been presented in this study, different methods of low cost for successful inclusion complexes of carvacrol/β-CD as an alternative to the improvement the treatment of various pathological conditions, such as cancer pain [18], antibacterial and antioxidant applications [21]. Our aim was to evaluate the complexation efficiency of carvacrol in β-CD, and to investigate the physicochemical properties of carvacrol/CD inclusion complexes through different analytical techniques. Those included Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), entrapment efficiency, thermogravimetry/derivate thermogravimetry (TG/DTG) and Karl Fischer titration. Furthermore, ^{1}H and two-dimensional (2D) ROESY NMR analyses were performed to characterize the complex in terms of geometry. Molecular modeling was also used to elucidate the supramolecular structure of the complex.

2. Material and methods

Carvacrol (lot: W224502, purity ≥ 98%) and β-CD (lot: #041M1759V; purity ≥ 97%) were purchased from Sigma (St. Louis, USA). All other chemical reagents were of at least reagent grade and all materials were used as supplied.

2.1. Preparation of inclusion complexes

Inclusion complexes of β-CD/carvacrol were prepared through three different methods: physical mixture (PM), paste complexation (PC) and slurry complexation (SC). The PM was prepared by adding carvacrol (150 mg) in an agate mortar containing the powder of β-CD (1135 mg) under manual stirring. The carvacrol/β-CD molar ratio was maintained as described for the inclusion complex preparation (1:1) and was then stored in sealed glass containers. PC was performed through the homogenization of β-CD (1135 mg) and carvacrol (150 mg) in water (2 mL) (1.2:4 w/v) directly in an agate mortar. After that, the mixture paste was kept under constant manual agitation (during 5 min). Afterwards, the material was dried at room temperature (in a desiccator) until a glass film was formed, which was removed by manual trituration and stored in airtight glass containers. Finally, the SC was carried out by adding water (20 mL) to a beaker containing carvacrol (150 mg) and β-CD (1135 mg, 3:4 v/w), which is equal to about a 1:1 M guest:host ratio under magnetic stirring at 400 rpm for 36 h constantly (Quimis Q 261A21, Brazil). Then, the material was dried in a desiccator and removed by manual trituration as described in a PC method according to Marreto et al. [23].

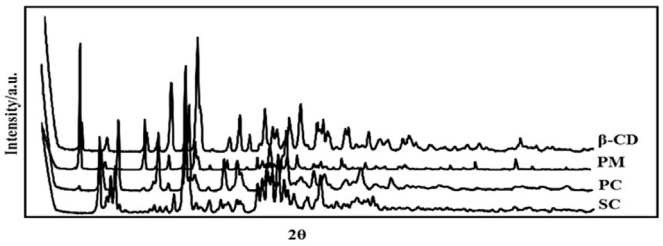

Fig. 1. Diffractograms of β-CD, physical mixture (PM), and paste complex (PC) and slurry complex (SC).

2.2. X-ray diffractometry (XRD)

XRD patterns were obtained on a Siemens D5000 X-ray diffractometer equipped with tube of Cu K-alpha, in the interval of 3–65 θ (2 h) and 1 s of pass time, using the powder XRD method.

2.3. Molecular docking study

The docking simulations were performed on the Auto-Dock 4.2 software [24]. Receptor and ligand preparation was carried out using VEGA ZZ 3.0.1 [25].

Initially, the structures of ligand and receptor were saved in pqbqt format to be used for docking calculations. PyRx 0.8 software [26] was used to aid the steps of work submission and analysis of the results. The grid maps were calculated with AutoGrid. The three-dimensional grid box with 50 Å grid size (x, y, z) with a spacing of 0.375 Å, was created. Each ligand was docked into this grid with the Lamarckian algorithm as implemented in AutoDock. The genetic-based algorithm ran 10 simulations per substrate with 2,500,000 energy evaluations and a maximum number of generations of 54,000. The crossover rate was increased to 0.8, rate of gene mutation was 0.02 and the number of individuals in each population was 200. All other parameters were kept at the AutoDock default settings [24].

2.4. Nuclear magnetic resonance (NMR)

^{1}H and H-H 2D NMR spectra were recorded for carvacrol, β-CD and inclusion complex (prepared through SC and PC methods) dissolved in D_2O using Bruker Ultrashield 400 MHz spectrometer. The spectra were acquired at 298 K in 5 mm tubes. Chemical shifts were measured relative to the peak at 4.80 ppm, due to the solvent (D_2O). The ROESY spectrum was recorded applying a mixing time of 150 ms under the spin lock condition, 256 increments were collected with 32 repetitions and the data matrix measured was processed as a matrix of 2 k (F2) by 1 k (F1) data points.

2.5. Entrapment efficiency (EE%)

The amount of carvacrol entrapped in the inclusion complex was determined by HPLC-UV at 254 nm. For each type of inclusion complex (SC 1:1, PC 1:1 and PM 1:1), 5 mg of sample was dissolved in 5 mL of acetonitrile and left for 24 h after being well mixed (250 rpm) to allow enough time for all entrapped active compound to be in solution. After this procedure, the solutions were centrifuged at 3200 rpm for 15 min to remove any β-CD from the solution, leaving only the active compound (carvacrol) [27]. 2 mL of the supernatant was collected, filtered on a 0.45 μm membrane filter (PTFE) and analyzed by HPLC-UV (254 nm) in triplicate. The EE% was calculated as follow: