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CHARACTERIZATION OF RESVERATROL/HYDROXIPROPYL-β-CYCLODEXTRIN INCLUSION COMPLEX FOR SUBSEQUENT APPLICATION IN HYPERGLICEMIC RATS[1]

CARACTERIZAÇÃO DO RESVERATROL COMPLEXADO À HIDROXIPROPIL-β-CICLODEXTRINA PARA POSTERIOR APLICAÇÃO EM RATOS HIPERGLICÊMICOS

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ABSTRACT

Resveratrol (RSV) belongs to a group of naturally polyphenol compounds identified in more than 70 species of plants. It has already been described to prevent and suppress some types of diseases. However, the poor water solubility and low stability may constitute a serious problem for its bioavailability. Therefore, the aim of this work was to improve RSV stability and water solubility by complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD) for subsequent application in diabetes treatment. The complexes were characterized by Fourier Transform Infrared Spectroscopy (FT-IR) and Ultraviolet (UV) Spectroscopy. The results of this study indicated that it was possible to prepare RSV complexes through HP-β-CD with adequate physico-chemical characteristics.

Keywords: Infrared spectroscopy, ultra-violet spectrophotometric, nanotechnology.

RESUMO

O resveratrol (RSV) faz parte do grupo de compostos polifenólicos, sendo encontrado em mais de 70 espécies de plantas. Já foi descrito na prevenção e supressão de alguns tipos de doenças. Porém, sua baixa solubilidade em água e a pouca estabilidade podem constituir um grave problema para sua biodisponibilidade. Deste modo, o objetivo deste trabalho foi aumentar a estabilidade e a solubilidade em água do RSV por meio da complexação com a hidroxipropil-β-ciclodextrina (HP-β-CD) para posterior aplicação no tratamento da diabetes. Os complexos foram caracterizados através de Espectroscopia na Região do Infra Vermelho com Transformada de Fourier (FT-IR) e Espectroscopia na região do Ultravioleta (UV). Os resultados deste estudo indicaram que foi possível realizar a complexação do RSV com a HP-β-CD e as análises químicas efetuadas confirmam adequadas características dos complexos obtidos.

Palavras-chave: *Espectroscopia no Infravermelho, espectrofotometria no ultravioleta, nanotecnologia.*

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INTRODUCTION

Resveratrol (*trans* 3, 5, 4´-trihydroxystilbene; RSV) is a phytoalexin present in a number of plant species (SOLEAS et al., 1997). RSV has been extensively studied due to its antioxidant and anti-inflammatory properties, a consequence of its estrogenic effects as well as the prevention and treatment of various metabolic disorders including diabetes (SZKUDELSKA; SZKUDELSKI, 2010). RSV is easily absorbed and metabolized in the gastrointestinal tract, and its excretion is very high, but its oral bioavailability is very limited due to oxidation and rapid turnover (PETYAEV et al., 2012). This limitation could be overcome by the formation of an inclusion complex with cyclodextrin (CD) (WENZEL; SOMOZA, 2005). The cyclodextrins are nanoscale structures used in the development of drug delivery systems. They also increase the solubility of hydrophobic substances in water and protect against hydrolysis, oxidation and photodecomposition, increasing its stability and bioavailability (LOFTSSON; DUCHÊNE, 2007).

The natural β-CD shows poor water solubility whereas 2-hydroxypropyl-β-CD (HP-β-CD) is widely used in the pharmaceutical field due to the modification of physico-chemical properties such as stability and solubility of the guest molecule (LATROFA et al., 2001; RAJEWSKI; STELLA, 1996; DUCHÊNE; WOUESSIDJEWE; PONCHEL, 1999).

Recent evidence has demonstrated that increased oxidative stress and decreased antioxidant protection are associated with various metabolic disorders (STYSKAL et al., 2011; HALLIWELL, 2012). Diabetes mellitus (DM) is a complex metabolic disease, which is subdivided into types 1 and 2, and both are characterized by an abnormal increase in the blood glucose level. The aim of this study was to prepare and characterize inclusion complexes of RSV through HP-β-CD in order to obtain new delivery systems to be used in the treatment of diabetes.

EXPERIMENTAL PROCEDURES

Materials: Resveratrol ($C_{14}H_{12}O_3$; molecular weight = 228.25 g/mol; purity > 98%) and 2-hydroxypropyl-β-cyclodextrin were obtained from Sigma Aldrich (St. Louis, MO, USA).

Preparation of the inclusion complex: 0.1 mM HP-β-CD solution in water (8 mL) at 40 °C was prepared by vigorous stirring in Ultra-Turrax® (3.2 rpm) and an equimolar amount of RSV (0.1 mM) was directly added to the suspension. RSV was previously suspended in ethanol HPLC grade (2 mL). After stirring it for one minute, the suspension was filtered through a 0.45µm cellulose acetate membrane filter in order to remove undissolved particles. The solvent was removed using rotary evaporation at 40 °C for about 10 min and the water was evaporated under vacuum for 8 h. This protocol was adapted from Bertacche et al. (2006) e Lu et al., (2009).

Characterization of the inclusion complex: Fourier Transformed Infra-Red Spectroscopy analyses were performed in Perkin Elmer (Spectrum One), RSV, HP-β-CD and the complex spectra were collected using an FT-IR in a spectral region between 4000 and 450 cm^{-1}. Samples were mixed in a crucible with potassium bromide (KBr) (1:100) and pressed in a hydraulic press (10 tons for 2 min) to obtain small tablets, which were placed in the infrared beam. Ultraviolet spectra were performed in UV-vis Spectrophotometer 1650 (SHIMADZU, Japan). The same samples were dissolved in ethanol from 200 to 400 nm of wavelength. Samples were analyzed in triplicate.

RESULTS

It was possible to obtain inclusion complexes by using RSV and HP-β-CD. Figure 1 shows infrared spectra of RSV, HP-β-CD and an inclusion complex. The spectrum of RSV presented the following band characteristics at 3293 cm^{-1} (Free O-H stretching vibration), 1463, 1513, 1586 and 1606 cm^{-1} (benzene skeleton vibrations), 965 and 988 cm^{-1} (bending vibration of C=C-H, the typical *trans*olefinic band). FT-IR spectrum of HP-β-CD indicated absorption bands at 3405 cm^{-1} (for O-H stretching vibrations), 2931 cm^{-1} (for C-H stretching vibration) and 1374 cm^{-1}, 1035 cm^{-1} (C-H, C-O stretching vibration). After analyzing the FT-IR spectrum of an inclusion complex, it showed changes in the spectral features of the guest molecule, mainly in the band intensities at 1384 and 965 cm^{-1}, while the bands at 1606 decreased and 1587 cm^{-1} disappeared. These results were compared with the data analyzed by Calabro et al. (2004), and indicate the formation of an inclusion complex.

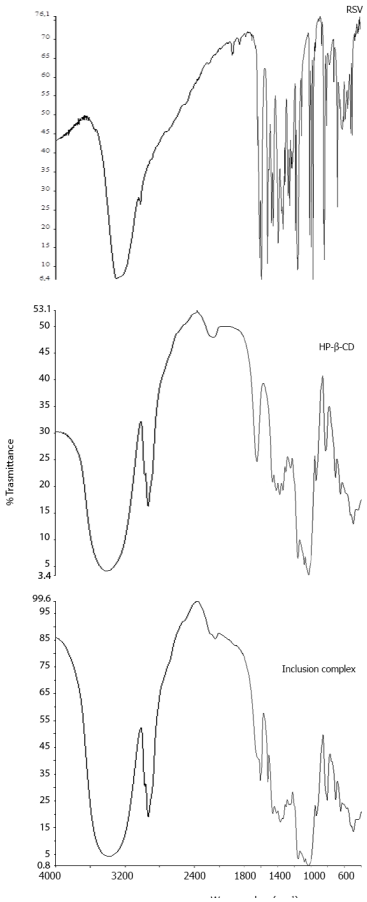

Figure 1 - FT-IR spectra of RSV, HP-β-CD and inclusion complex.

Our results are in agreement with studies developed by Kolouchová-Hanzlíková et al. (2004), who also report an inclusion complex in the same spectrums, where the typical trans-RSV UV spectrum maximum ranged from 306-320 nm (Fig. 2). Moreover, several works have reported that the presence (or not) of an isobestic point may determine the formation of a CD/guest complex (GUOMEI et al., 2003; ZHANG et al., 2008). As shown in Figure 2, the isobestic points appear at 253 and 326 nm, which again suggests the formation of inclusion complexes.

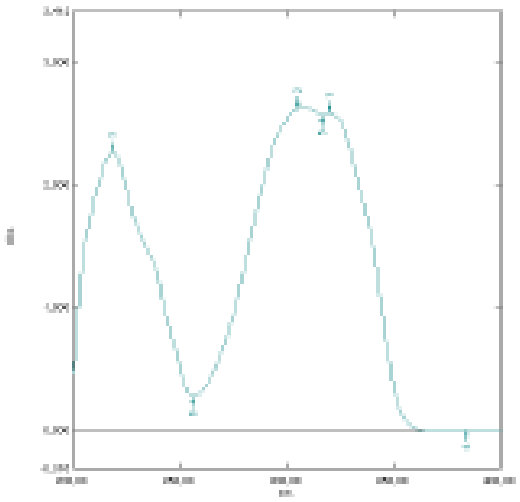

Figure 2 - UV spectrum of *trans*-RSV (left) and inclusion complex (right) with wavelength **(**1) 320 nm, (2) 306 nm.

CONCLUSIONS

Various studies in diabetic rats have shown the anti-hyperglycemic action of resveratrol (BAUR et al., 2006; SU et al., 2006; THIRUNAVUKKARASU et al., 2007), but the low solubility of RSV in an aqueous medium such as biological fluids makes its bioavailability limited. The results of this study indicated that it was possible to obtain the RSV complexation through HP-β-CD, and the desired chemical analyzes performed confirmed the characterization of the complex obtained. It is expected that the use of inclusion complex in animal models of diabetes in order to improve bioavailability and circumvent the rapid metabolism in vivo could be adopted in the treatment of this disease.

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