Preparation and Characterization of Inclusions Complexes Between Propolis Ethanolic Extracts and 2-hydroxypropyl-β-cyclodextrin

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The ethanolic extracts of propolis consist mainly of polyphenolics (such as flavonoids and non-flavonoids), which are responsible for various pharmacological activities, but have poor aqueous solubility and are easily decomposed by oxidation under the action of air, heat, and light during the processing steps and storage. Complexation with cyclodextrins (CDs) is an effective pharmaceutical method to enhance the bioavailability of poor water soluble compounds and to improve their chemical stability. Three different propolis sorts and 60% (v/v) ethanolic solution as extraction solvent were used to obtain the propolis ethanolic extracts. The propolis ethanolic extract/2-hydroxypropyl-β-cyclodextrin inclusion complexes were prepared by kneading method and characterized by optical microscopy and differential scanning calorimetry (DSC). Experimental results confirmed the inclusion of polyphenolics from propolis ethanolic extracts in 2-hydroxypropyl-β-cyclodextrin (2HPβCD). Furthermore, the results of DSC studies demonstrated that the polyphenolics of the studied propolis extracts were included in different concentrations in the 2HPβCD cavity. The present study provides useful information for the potential applications of ethanolic propolis extracts/2HPβCD complexes, as stable, effective and easy to process raw materials.

Keywords: propolis ethanolic extracts; 2-hydroxypropyl-β-cyclodextrin; inclusion complexes; kneading method

Propolis, one of the by-products of the beehive, has been used by man since ancient times for its medicinal properties, and nowadays its constituents are used as major active ingredients in cosmetic, pharmaceutical and health products [1]. In the last two decades, many studies have proved that the beneficial effects of propolis on human health are mainly due to polyphenolic compounds, which are responsible of a wide range of biological activities, including antioxidant, anti-inflammatory, antibacterial, antiviral and antitumoral [2-7].

Propolis, also called bee glue, is a lipophilic, heterogeneous, resinous or wax-like mass, of solid consistency, sometimes compact, sometimes becoming malleable and adherent particles, and other times granular or friable, taking the appearance of powdery crumbs. It possesses a pleasant, aromatic smell, and varies in colour, depending on its source and age, from pale yellow to dark brown, even black [1, 8]. As for the propolis chemistry, there are numerous literature reports which have showed its complex chemical composition, indicating that the main groups of compounds are waxes, resins, balsams, aromatic and ethereal oils, pollen, and other organic matter [9-12].

Considering the physical characteristics of propolis, as a result of its complex structure, it is used only in the form of extracts prepared with a suitable solvent, such as water, ethanol, methanol, chloroform, acetone, dichloromethane, and ether.

The major components of ethanolic extracts of propolis include polyphenolics such as flavonoids (i.e. chrysin, rutin, apigenin, kaempferol, quercetin, pinocembrin, and acacetin) and non-flavonoids (i.e. caffeic acid), which are responsible for the propolis extract’s bioactivities [8, 13-15]. As a result, there is a growing interest in developing nutraceutical and pharmaceutical products based on propolis extracts. However, the application of propolis extracts in these products is severely restricted since some of the contained polyphenolic compounds have poor water solubility, poor bioavailability, and are easily decomposed by oxidation under the action of air, heat, and light during the processing steps and storage. Therefore, to maintain the structural integrity of the bioactive molecules, it is necessary to develop a formulation capable to deliver them to the physiological targets, keeping their bioactivity. One of the most widely used method to improve chemical stability and the water solubility of the polyphenolic agents is the preparation of cyclodextrin (CD) inclusion complexes [16-19]. As known, CDs presents the ability to encapsulate molecules, acting as host molecules in the formation of inclusion complexes with numerous hydrophobic guest molecules, which are entirely or partially included in the

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nonpolar cavity of CDs. With this property, CDs have mainly been used in the pharmaceutical industry to increase the hydro solubility of active compounds poorly soluble in water, in order to increase their bioavailability and to improve stability. In addition, CDs can be used to reduce or eliminate unpleasant smells or tastes, prevent drug-drug or drug-additive interactions within a formulation, based on reduction of the free drug in solution [20, 21].

CDs are cyclic oligosaccharides with six (α-CD), seven (β-CD), eight (γ-CD) or more glucose residues linked by (α-1,4) glycosidic bonds [22]. 2-hydroxypropyl- β-cyclodextrin (2HPbCD) is one of the hydroxyalkylated β-CD derivatives, prepared through chemical modification of the natives’ β-CD hydroxyl group, that exhibits relatively higher water solubility and lower toxicity than v-CD, and a satisfactory inclusion ability [23]. At present, 2HPbCD is the most commonly applied CD to encapsulate plant bioactive compounds, compared to the other CDs derivatives, due to its above mentioned favorable properties. To the extent of our knowledge, there are only a few reports regarding the encapsulation of propolis extracts by β-CD and its hydrophilic derivatives, such as 2HPbCD [23-26]. On the other hand, numerous published studies on the preparation/standardization of propolis extracts reported ethanol of different concentrations as solvent for extraction of polyphenolics, because it allows obtaining higher extraction yields and is less toxic than other solvents [1, 15, 24, 27]. Therefore, alcohol was used for the obtaining of the propolis extracts studied in this work.

The objective of this work was the encapsulation of three different ethanolic propolis extracts within a 2HPbCD complex, aiming to obtain a stable, effective and easy to process material. Optical microscopy and differential scanning calorimetry (DSC) were used to ensure the inclusion of propolis ethanolic extracts within 2HPbCD.

**Experimental part**

**Materials and methods**

Propolis, as raw material, was obtained from three different apiaries located in the West region of Romania: an alpine apiary located in Barzava (46°07’ 00” N; 21°58’59” E; altitude 218 m), and two river meadow apiaries located in Timisoara (45°44’57” N; 21°13’37” E) and Minis (46°08’00” N; 21°36’00” E) respectively. The three sorts of propolis were codified P1-Timisoara (P1-Tm), P2-Barzava (P2-B) and P3-Minis (P3-M). Ethanol p.a. (Chimopar, Bucharest, Romania) and distilled water were used for propolis extraction. 2-Hydroxypropyl-β-cyclodextrin (with purity greater than 99%, an average Mw 1310 and an average degree of substitution of 4.6) was purchased from CycloLab R&D Ltd (Budapest, Hungary).

**Preparation of propolis ethanolic extracts**

The three propolis ethanolic extracts, corresponding to P1-Tm, P2-B and P3-M propolis sorts, were obtained using a 60% (v/v) ethanolic solution as extraction solvent, by the method described in our previous study [26]. Briefly, 5 g of each pulverized propolis sort was extracted for 96 h with 20-fold volume of 60% ethanol solution using a magnetic stirrer. Extraction was carried out at room temperature, in a sealed flask and protected from light. The obtained ethanolic extracts were filtered to remove insoluble materials, the residues were washed with 5 mL of the same solvent and the final propolis extracts were stored at 4°C until encapsulation within 2HPbCD. These propolis extracts (codified P1-Tm_60, P2-B_60 and P3-M_60) were physico-chemical characterized by HPLC method in our previous studies [26, 28].

**Preparation of propolis extract-2HPbCD inclusion complexes**

The inclusion complex between each propolis extract and 2HPbCD was prepared by kneading method as follows: in a preheated to 50°C glass mortar, 160 mL of each propolis ethanolic extract (P1-Tm_60, P2-B_60 or P3-M_60) were added over an accurately weighted amount of 2HPbCD (126.400 g, 126.420 g and 126.470 g respectively). The physical mixtures were kneaded thoroughly for an hour with a pestle to obtain a paste, which was then dried to constant mass in an oven at 25°C, for 6 h. The dried masses were further pulverized in a mortar, sieved through a 0.16 mm sieve and stored in a desiccator until further evaluation.

**Characterization of propolis ethanolic extract/2HPbCD inclusion complexes**

**Optical microscopy**

Morphological evaluation of the propolis ethanolic extracts/2HPbCD inclusion complexes was performed by optical microscopy, using Nikon Eclipse E8000 Confocal Microscope, connected to the Nikon Coolpix E4500 digital camera, with a 40-600x optical zoom (Nikon, Japan).

**Differential scanning calorimetry (DSC)**

The crystallinity of propolis ethanolic extracts/2HPbCD inclusion complexes was characterized by DSC on a Differential Scanning Calorimeter DSC 204 (Netzsch-Geratebau GmbH, Germany), provided with the specific software DSC Netzsch 204 – Acquisition Soft/2000 for data acquisition and the software Netzsch Proteus-Thermal Analysis version 4.0/2000 for data processing. Samples of approximately 10±2 g were weighed and sealed in aluminum pans. The samples were heated at a rate of 4°C/min, in a range of 20-500°C, the cooling being performed with liquid nitrogen.

**Results and discussions**

**Preparation of propolis extract-2HPbCD inclusion complexes**

The average yields of the propolis ethanolic extracts/2HPbCD inclusion complexes, codified as P1-Tm_60/2HPbCD, P2-B_60/2HPbCD and P3-M_60/2HPbCD, were 97.6% (w:w), 96.5% (w:w) and 98.2% (w:w) respectively.

**Characterization of propolis ethanolic extract/2HPbCD inclusion complexes**

**Macroscopic examination**

In figure 1 is shown, as example, the image of P2-B_60/2HPbCD in solid state.

**The optical microscopy analysis**

The results of optical microscopy analysis of inclusion complexes obtained after the complexation of propolis ethanolic extract from P1-Tm, P2-B and P3-M sorts with 2HPbCD are presented in figure 2a, 2b and 2c.
The DSC thermograms of the prepared inclusion complexes between propolis ethanolic extracts and 2HPbCD are shown in figure 3.

**Preparation of propolis extract-2HPbCD inclusion complexes**

The obtaining of propolis extract/2HPbCD inclusion complexes through the kneading method was performed with massive yields of 97-98%, losses being caused mainly by the preparation technology.

**Characterization of propolis ethanolic extract/2HPbCD inclusion complexes**

**Macroscopic examination**

The inclusion complexes between propolis ethanolic extracts and 2HPbCD, obtained through the kneading method were amorphous yellow powders, with a pleasant flavoured taste and odour, characteristic of that of propolis.
The optical microscopy analysis of inclusion complexes obtained after the complexation of propolis ethanolic extract from P1-Tm, P2-B and P3-M sorts with 2HPbCD revealed in all cases a parallelepipedic crystal product, with micrometric-sized particles (fig. 2a, 2b and 2c).

**DSC analysis**

There are two endothermic peaks at 90 °C ($\Delta H = -27.29$ J/g) and 135 °C ($\Delta H = -577.2$ J/g) in the DSC thermogram of P1-Tm 60/2HPbCD inclusion complex (fig. 3a), corresponding to the dehydration/dissociation process, most probably due to the release of the water crystallization molecules. Moreover, in the release area of polyphenolics (up to ~ 280°C), the endothermic effect of dissociation was only 13 J/g and after that temperature (above 290°C) the decomposition of the complex occurred. This results indicated the relatively low content of polyphenolic bioactive compounds incorporated into the cavity of 2HPbCD.

The DSC curves of the P2-B 60/2HPbCD and P3-M 60/2HPbCD inclusion complexes showed significant endothermic effects between 150 and 280°C, corresponding to the inclusion complex dissociation (fig. 3b and 3c). Thus, in the case of the P2-B 60/2HPbCD inclusion complex the loss by dehydration up to about 135-140°C was of 676 J/g, while the dissociation of the inclusion complex was indicated by a loss of 75.24 J/g between 150 and 280°C (fig. 3b).

In the thermogram of the P3-M 60/2HPbCD inclusion complex the two endothermic peaks, indicating its dehydration and dissociation, were slightly shifted to lower temperatures (82.6 and 145.9 °C) and their corresponding areas were higher (~881.7 J/g and ~102.7 J/g) than that of P2-B 60/2HPbCD inclusion complex (fig. 3c). Although the absolute dissociation effect was greater for the P3-M 60/2HPbCD complex, its high water content makes this product unsuitable for pharmaceutical processing, compared with P2-B 60/2HPbCD complex, for which the molecular inclusion of compounds from extract is more effective (much lower content of water of crystallization in the finite complex).

**Conclusions**

In this study, three propolis/2HPbCD inclusion complexes were successfully prepared from three ethanolic extracts of propolis and 2HPbCD by kneading method. After the complexation with 2HPbCD, the three propolis sorts extracts of propolis and 2HPbCD by kneading method. After the complexation with 2HPbCD, the three propolis sorts were transformed from solid, heterogeneous, resinous and sticky masses to amorphous, parallelipipedic forms with micrometric-sized particles, as characterized by optical microscopy.

The DSC analysis revealed that the polyphenolics of the studied propolis extracts were included in different concentrations in the 2HPbCD cavity to form the corresponding propolis extract/2HPbCD inclusion complex, the lowest content of the bioactive compounds was found in the case of P1-Tm 60/2HPbCD complex. Also, the differential scanning calorimetry indicated the P2-B 60/2HPbCD inclusion complex is the most suitable for pharmaceutical processing, due to the lowest content of crystallization water and highest efficient molecular inclusion. However, in order to confirm the enhanced water-solubility and to explain in detail the inclusion mode of polyphenolics from propolis extracts in the 2HPbCD cavity further studies using phase solubility measurement, proton nuclear magnetic resonance (1H-NMR), and X-ray diffractometry (XRD) need to be performed.

**References**


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