IMPROVEMENT OF GALANGIN SOLUBILITY USING NATIVE AND DERIVATIVE CYCLODEXTRINS. AN UV–V_{is} AND NMR STUDY

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ABSTRACT

The slightly water-soluble flavonoid galangin (G) and its inclusion with either β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD) or Heptakis-2,6-*O*-di methyl-β-cyclodextrin (DMβCD) were investigated. The stoichiometric ratios and stability constants describing the extent of the formation of the complexes have been determined by phase–solubility measurements; in all cases type-A_L diagrams have been obtained (soluble 1:1 complexes). The results showed that the complex efficiency of βCD and its derivatives was the order: DMβCD > HPβCD > βCD. The NMR study indicate that the inclusion of galangin in the cyclodextrin nano-cavity is different depending on the type of cyclodextrin used.

Keyword: cyclodextrin, galangin, NMR.

INTRODUCTION

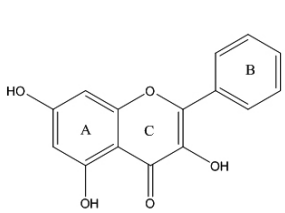
Among dietary compounds endowed with health-promoting properties, flavonoids have been described as disease-preventing dietary supplements, and have activity as cancer preventive agents[1]. Moreover, they are extremely safe and associated with low toxicity, making them excellent candidates for chemopreventive agents[1]. Flavonoids are part of a family of naturally occurring polyphenolic compounds and represent one of the most prevalent classes of compounds in vegetables, nuts, fruits and beverages such as coffee, tea, and red wine[2] as well as medical herbs.

Flavonols are polyphenol compounds possessing two benzene rings joined by a linear three carbon chain (C2, C3, C4), represented as the C6-C3-C6 system. The flavonol moiety (2,3-double bond in conjugation with a 4-oxo group and a 3-hydroxyl group) and the 5,7- dihydroxylation at the A-ring are important structural features for significant antioxidant activity[3]. In addition to –OH moieties in the structural arrangements of flavonols, the resonance of electrons between the A- and B-rings is very important for their antioxidant and biological activities[4]. The B-ring –OH moiety is the most significant determinant factor in the scavenging of reactive oxygen species[5]. Galangin (scheme 1) is present in *Alpinia officinarum,* honey, and propolis. The increasing interest of this type of flavonol lies in their broad pharmacological activities (e. g., antimicrobial, spasmolytic, antiallergic, anti-inflammatory, antiviral, anticarcinogenic)[6-9] Despite these applicable qualities, biological activities and therefore therapeutic usefulness of these substances are limited because of their unfavorable physicochemical properties, especially very poor water-solubility and low oxidative stability.

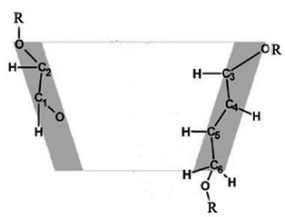
Scheme 1

(A) Molecular structures of Galangin. (B) Molecular structures of β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and Heptakis-2,6-*O*-di methyl-β-cyclodextrin.

A



B



β-cyclodextrin, R = H
2-Hydroxypropyl β-cyclodextrin, R = $CH_2CHOHCH_3$ or H
Heptakis (2,6 O di methyl)
β-cyclodextrin, $R_{2,\ 6} = CH_3$ $R_3 = H$

In pharmaceutical product development, β-cyclodextrins, a category of pharmaceutical excipients, have been widely used to improve solubility, chemical stability and bioavailability of a number of poorly soluble compounds. Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity[10].The cavity of CDs is relatively hydrophobic compared to water, while the external faces are hydrophilic[11]. The most extraordinary characteristic of a cyclodextrin is its ability to form inclusion complexes with a variety of compounds, i.e. caging foreign molecules (guest) in its cavity (host). Generally, hydrophobic molecules or some hydrophobic residues have the highest affinity with the CD's cavity in aqueous solution. It has been well established that the ability of β-cyclodextrin to enhance the stability and solubility of drugs is mediated through the formation of inclusion complexes[12]. The most widely used natural cyclodextrin, β-CD, is limited in its pharmaceutical applications due to its limited aqueous solubility (1.85 g/100 ml)[13]. Therefore, chemically modified β-CDs have been synthesized to overcome this problem. Examples include heptakis-(2,6-*O*-dimethyl) β-cyclodextrin (DMβCD) and hydroxypropyl-β-cyclodextrin (HPβCD).

Here we report the preparation of the inclusion complex of galangin with three different cyclodextrins, (HPβCD, DMβCD and βCD) in order to improve the aqueous solubility of the drug. Binding constant, estimated from phase-solubility studies, permit comparison between βCD, HPβCD and DMβCD with regard to their complex efficiency. We also present results of 2D-NMR studies which provide useful insights regarding the orientation of galangin in the cyclodextrin nano-cavity.

EXPERIMENTAL

Materials

Galangin (3, 5, 7-trihydroxyflavone), was purchased from Sigma (USA). βCD (β-cyclodextrin), DMβCD (Heptakis-2,6-*O*-di methyl-β-cyclodextrin), HPβCD (2 Hydroxypropyl-β-cyclodextrin) [M.S. (average molar degree of substitution) = 1.0] was purchased from Sigma-Aldrich, Inc., St. Louis, MO. All solvents employed in the spectrophotometric analyses were of spectroscopic reagent grade, from Merck.

Phase-Solubility Measurements

Phase-Solubility measurements were carried out according to the method of Higuchi & Connors[14]. Excess amount of galangin (3mg) was added to 5 mL of deionized water containing increasing amounts of the different CD (ranging from 0 to 0.010 M). The resulting mixture was equilibrated in a Julabo thermostatic shaking water bath for 24 h at 30°C after which the equilibrium was reached. To minimize photochemical degradation amber flask were used. Then, suspensions were filtered through 0.45 μm cellulose acetate membrane filter to remove undissolved solid. An aliquot from each vial was adequately diluted and spectrophometrically analyzed at 374 nm on an UV_2 UNICAM spectrophotometer. The presence of cyclodextrin did not interfere in the spectrophotometric assay of galangin.

The apparent stability constant (K_a) of the complexes were calculated from the phase solubility diagrams according to the following equation:

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$$K_a = \frac{slope}{S_o(1-slope)} \qquad (1)$$

Where S_o is the solubility of galangin at 30°C in absence of cyclodextrin and slope means the corresponding slope of the phase-solubility diagrams, i.e., the slope of the drug molar concentration versus CDs molar concentration graph. The experiment was carried out in triplicate.

A very reliable method for evaluating the solubilizing potential of CD is to determine the complexation efficiency[15] (CE), which refers to the complexed/free cyclodextrin ratio and that can be obtained from the slope of their phase-solubility profile, according to equation 2

$$CE = S_o K_{11} = \frac{[G-CD]}{[CD]} = \frac{slope}{1-slope} \qquad (2)$$

where [G-CD] is the concentration of dissolved complex, [CD] is the concentration of dissolved free CD.

NMR

Rotating-frame Overhauser Effect SpectroscopY (ROESY) spectra were acquired in the phase sensitive mode using a Bruker Avance DRX operating at a proton NMR frequency of 300.13 MHz in unbuffered D_2O solutions (pulse program roesygpph19). Each spectrum consisted of a matrix of 16K (F2) by 8K (F1) points covering a spectral width of 3000 Hz. Spectra were obtained from the samples solutions prepared for the ^{1}H NMR studies, using a spin-lock mixing time of 400 ms, relaxation delay 2s, and 32 scans were recorded.

RESULTS

Fig. 1, show the effect of βCD concentrations on the absorption spectra of galangin in aqueous solutions. Increasing the concentration of all CDs from 1 to 10 mM resulted in an increase in the absorbance of galangin without any shifts of λ_{max}. The three cyclodextrin utilized enhanced the poor aqueous solubility of galangin, thus proving a certain degree of its inclusion complexation in aqueous solutions. Different results reported Bergonzi et al.[16] which found that α-, β-, and γ-cyclodextrin didn't enhance water solubility of galangin. Figure 2 depict the phase-solubility diagrams, of galangin with βCD, HPβCD and DMβCD within the concentration range studied, and they displayed a typical A_L type diagram (i.e., linear increase of galangin solubility with increasing CD concentration), consistent with a 1:1 molecular complex formation for each CD. The binding constant K_a of the complexes with this methodology are 1770, 12680 and 34000 M^{-1} for βCD, HPβCD and DMβCD, respectively, was calculated from the slopes of the linear phase-solubility plots according to the methodology described in the experimental part. A more accurate method for determination of the solubilizing efficiency of cyclodextrins is to determine their complexation efficiency CE, i.e. the concentration ratio between cyclodextrin in a complex and free cyclodextrin. CE is calculated from the slope of the phase-solubility diagrams and is independent of S_o. The CE values revealed that the solubilizing power of CDs towards the drug follows the order DMβCD (0.038) > HPβCD (0.014) > βCD (0.002). The highest CE value exhibited by DMβCD could be ascribed to the presence of methyl groups that expand the hydrophobic region of the CD cavity and thus increase its affinity towards galangin.

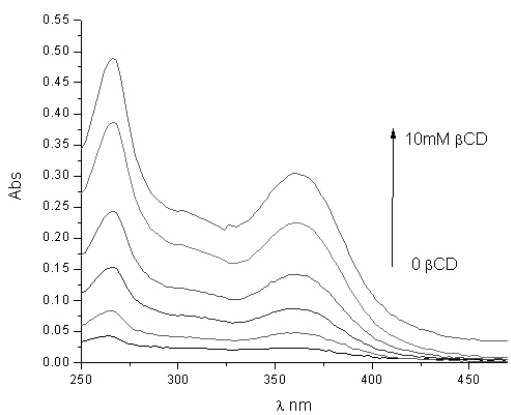

Figure 1.- Absorption spectra of galangin in aqueous solutions with different concentration of βCD.

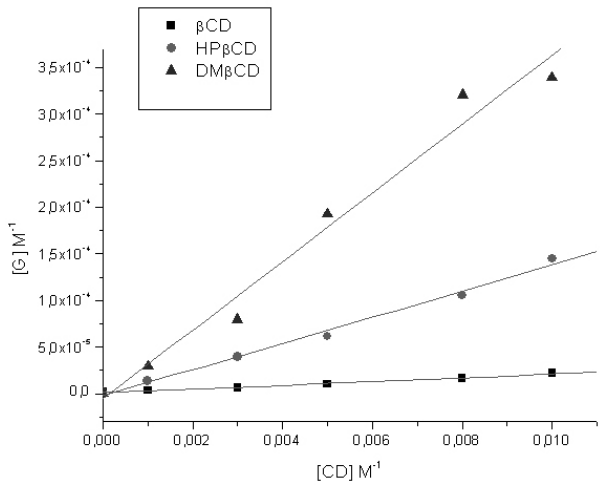

Figure 2.- Phase–solubility diagrams of G–βCD, G–HPβCD and G–DMβCD system in water at 30 ◦C.

On the other hand, according to the continuous variation method, if a physical parameter directly related to the concentration of the complex can be measured for a set of samples with continuously varying molar fraction of its components. The maximum concentration of the complex will be present in the sample where the molar ratio R corresponds to the complexation stoichiometry. In Figure 3, the maximum absorbance variation for galangin with native and modified CD was observed for R = 0.5, which might indicate that the main stoichiometry is 1:1, in agreement with the stoichiometry suggested from the phase-solubility study.

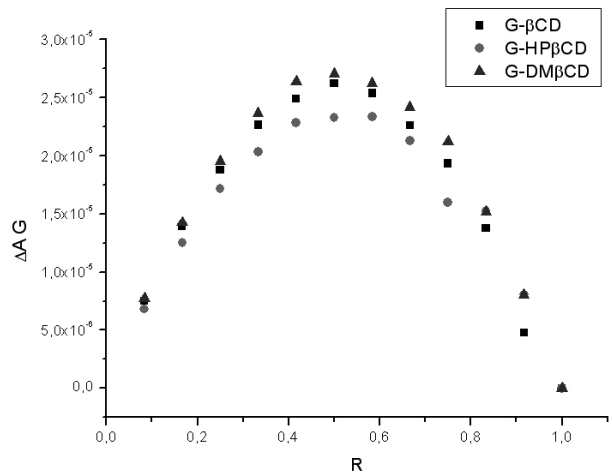

Figure 3.- Continuous variation plot for the G-βCD, G-HPβCD and G-DMβCD system from absorbance measurements.

Since ^{1}H-NMR spectroscopy was first introduced for the study of complex formation in aqueous solutions[17], there have been numerous studies involving aromatic compounds. The method relies on changes in chemical shifts caused by the guest and the host on each other. In case of aromatic compounds, some of the most important spectral changes that occur upon complexation come from the diamagnetic shielding of the aromatic host on the nearby spins of the guest. In the structure β-cyclodextrin, only hydrogens H-3 and H-5 are located inside the cavity (scheme 1). H-3 are located near the wider rim of the cyclodextrin cavity while the H-5 hydrogen form a ring near the narrower rim of the methylene (H-6) bearing the primary hydroxyl groups. All other hydrogens (H-1, H-2 and H-4) are located on the exterior of the cavity. In general ^{1}H-NMR spectroscopy provides evidence for the inclusion of the drug inside the cyclodextrin cavity, as well as to gain information about the geometry and orientation of the incorporated drug molecule. Examination of cycloamylose induced chemical shifts in the ^{1}H-NMR spectra of the aromatic substrate provides a convenient method of determining the substrate

penetration in the cavity. Due to the low solubility of galangin, it was difficult to obtain the detailed variation of the chemical shifts of the complexes before and after forming inclusion complex. Instead 2D-NMR spectroscopy is an effective method for studying spatial conformations of cyclodextrin inclusions. Two dimensional rotating-frame noe spectroscopy experiments have often been successfully applied to prove through-space intermolecular interactions in cyclodextrin complexes[18]. Indeed, in the ROESY experiments (Rotational nuclear Overhauser Effect Spectroscopy), dipolar interactions between protons at a distance less than 3-4 Å are detected as cross-peaks in a bi-dimensional map, indicating the portion of the guest situated in the torus cavity. In this study, ROESY spectra were collected to gain additional insights. The effects were only qualitatively used.

Figure 4 shows a partial contour plot of 2D-ROESY spectra of the inclusion complex of galangin and DMβCD. The cross-peak between the H-3 proton of the CD and the H-2' and H-6' protons of B-ring demonstrates the inclusion of galangin into the CD cavity. These indicate that galangin is inserted in the cyclodextrin cavity with the B-ring oriented towards the secondary hydroxyl group. No more interaction was observed between galangin and cyclodextrin indicating that the rest of the chromene remains outside the cavity.

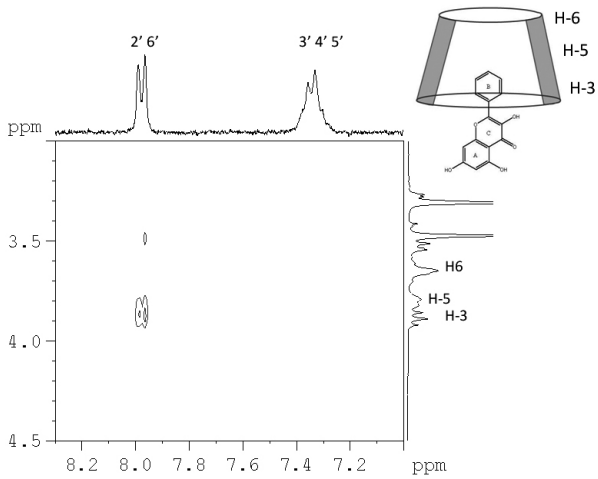

Figure 4.- Partial contour plot of the two-dimensional ROESY spectrum of galangin in the presence of DMβCD in D_2O.

In order to assign unambiguously H-3, H-5 and H-6 of the 2-hydroxypropyl-β-cyclodextrin region, an HSQC (Heteronuclear Single Quantum Correlation) spectrum of G-HPβCD system was performed in the same conditions as those used for the ROESY spectrum (data not shown). The interaction between the aromatic proton of B-ring of galangin with H-5 and H-6 protons of 2-hydroxypropyl-β-cyclodextrin, as depicted in Figure 5, indicates that galangin is inserted in the cyclodextrin cavity by the narrow end, with the rest of the flavonol protruding from the primary rim.

This indicates that we have different form of inclusion for G-DMβCD and G-HPβCD which means that both cyclodextrins offers different microenvironment for galangin. Moreover, due to the low association constant of the G-βCD complex, no cross-peak between galangin and cyclodextrin was observed, but in a previous work[19] we determine the inclusion geometry by molecular modeling and we find that the B-ring of galangin is inserted in the cavity by the secondary rim. Maybe the microenvironment of βCD and DMβCD are very similar.

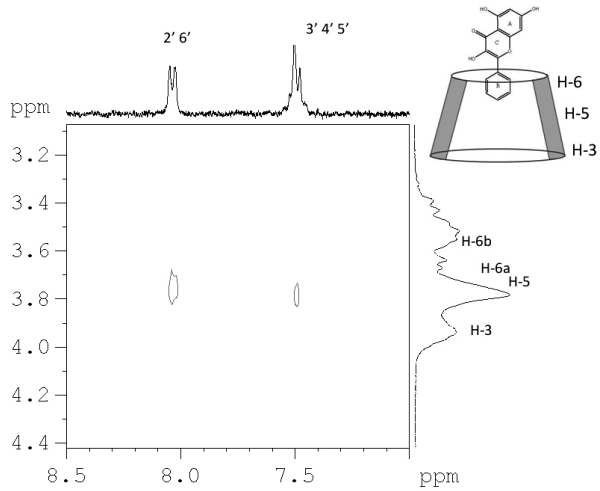

Figure 5.- Partial contour plot of the two-dimensional ROESY spectrum of galangin in the presence of HPβCD in D_2O.

CONCLUSION

A combination of the solubility diagrams method and 2D-NMR spectroscopy was used to demonstrate complexation between the cyclodextrins βCD, HPβCD and DMβCD and galangin. The findings of the solubility diagrams study indicate that all the cyclodextrin studied increase the apparent water solubility of galangin. The increase in solubility was greater for DMβCD than for HPβCD and βCD. Analysis of the continuous variation method confirms the formation of a 1:1 stoichiometric complex for galangin and the different CDs in aqueous medium. Different form of inclusion was found for galangin in DMβCD and HPβCD.

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