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Improvement in solubility and dissolution rate of flavonoids by complexation with β-cyclodextrin

Silvana Tommasini, Daniela Raneri, Rita Ficarra, Maria Luisa Calabrò*, Rosanna Stancanelli, Paola Ficarra

Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Messina, Viale Annunziata, 98168 Messina, Italy

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Abstract

The inclusion into the β-cyclodextrin is used to improve pharmacokinetic characteristics of hesperetin and naringenin. Solubility of hesperetin and naringenin with increasing concentrations of β-cyclodextrin grows as long as the temperature increased. Stability constants were determined by the solubility method by Higuchi and Connors at different temperatures, and the thermodynamic parameters were calculated for inclusion complex formation in aqueous solution. The solid complexes were obtained in a molar ratio of 1:1 and their dissolution behavior at different pH was examined.

Keywords: Hesperetin; Naringenin; β-Cyclodextrin; Phase solubility studies; Dissolution

1. Introduction

In the pharmaceutical field, cyclodextrins have been recognized as potent candidates to overcome the undesirable properties of drug molecules through the formation of inclusion complexes, in which each guest molecule is surrounded by the hydrophobic environment of the CD cavity. This can lead to the alteration of physicochemical properties of guest molecules.

The flavanones hesperetin and naringenin (Fig. 1) are widely spread in nature and easily extracted from a lot of different plants. Their protective effect against lipid peroxidation of membranes, involved in several physiological and pathological disorders, as aging, inflammation, atherosclerosis, ischemia, toxicity of oxygen and chemical substances has been largely studied [1–7].

Since a limiting factor of the use of flavonoids is their low water solubility, the aim of this study was to increase the solubility and dissolution rate of hesperetin and naringenin by inclusion complex formation with β-cyclodextrin (β-CD).

Inclusion with cyclodextrins is a convenient alternative to solve the problems encountered in the administration of hydrophobic drugs. Cyclodextrins are cyclic oligosaccharides able to include entirely or, at least partially, into their hydrophobic cavity, a lot of molecules with different structures. This inclusion process leads to changes in the physicochemical properties of the guest, such as solubility, dissolution rate and bioavailability [8–16].

* Corresponding author. Tel.: +39-090-6766409; fax: +39-090-355613.
E-mail address: mlcalabro@pharma.unime.it (M.L. Calabrò).

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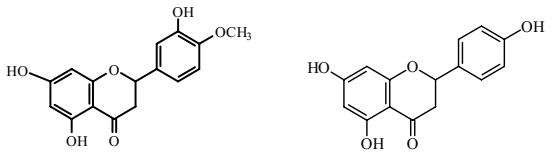

Fig. 1. Chemical structures of hesperetin (I) and naringenin (II).

In the practical application of cyclodextrins, attention should be directed towards the dissociation equilibrium and stoichiometry of the inclusion complex. When a CD complex is dissolved in water or introduced into body fluids, it dissociates rapidly to free components in equilibrium with the complex. The stability constant (K_c) is an useful index to estimate the binding strength of the complex and the changes in the physicochemical properties of a guest in the complex.

The degree of dissociation of the complexes depends on the magnitude of K_c and various environmental factors, such as dilution, temperature, pH, affect the K_c value.

The formation of complexes of hesperetin and naringenin with β-CD had been previously analyzed [17]; in this paper solubility phase studies of the flavonoids in presence of β-CD were performed at different temperatures, in order to calculate the stability constants of these complexes and to investigate the effect of temperature on the improvement of solubility.

Literature reports various studies on thermodinamyc properties of cyclodextrins [11–14,18].

The thermodynamic behavior of complexes β-CD/hesperetin and β-CD/naringenin was described and values of free energy, enthalpy and entropy were calculated.

The dissolution of a substance in a solvent depends on its structural characteristics, which condition solute–solvent interaction forces. Moreover, the dissolution of a substance is strictly linked to its solubility, to its crystalline status, to its particle size, to the presence of different kinds of excipients [16]. Our goal was to improve the dissolution properties of hesperetin and naringenin by β-CD complexation.

The dissolution properties of pure flavonoids and their complexes, in aqueous solution at different pH values, were evaluated by HPLC and compared.

2. Experimental

2.1. Materials

The flavonoids hesperetin and naringenin were supplied by Sigma-Aldrich S.r.l. (Milan, Italy).β-CD was purchased from Fluka Chemie (Buchs, Switzerland).Methanol of analytical grade was purchased from Merck (Darmstadt, Germany).

All solutions were filtered through 0.45-μm Gelman Sciences Acrodisc® LC 13 PVDF filters provided by Merck (Darmstadt, Germany).

Solutions of different pH values (1.5, 3.0, and 8.0) were prepared. The hydrogen ion concentration was obtained, according to Italian Pharmacopoeia (FU) [19], by using solution of HCl 0.1 M and KCl 0.1 M (pH 1.5); sodium phosphate dibasic 0.2 M and citric acid 0.1 M (pH 3.0 and 8.0), purchased from Carlo Erba (Milan, Italy).

The water used for solutions was distilled, deionized and filtered through 0.22 μm Millipore filters (Bedford, USA).

All other materials were analytical grade reagents.

2.2. Apparatus

The dissolution rate studies were carried out using the FU paddle method [19]. The dissolved amount of the drug was quantified by using a Perkin-Elmer series 410 liquid chromatograph equipped with a septumless injector (Rheodyne 7125-075) and a column heater (Perkin-Elmer TC 931). A variable wavelength diode array detector (Perkin-Elmer LC 235) was used. Peak area integration was performed using a chromatographic data system (Perkin-Elmer LCI 100 laboratory computing integrator). A Vydac reversed-phase C_{18} column (25 cm × 4.6 mm i.d., particle size 10 μm), thermostated at 25 °C, was the stationary phase.

The pH of the solutions was measured by a pH-meter Jeanway model 3310 with an accuracy of ±0.1.

Phase solubility studies were performed by using a spectrophotometer UV-Vis Perkin-Elmer, Model Lambda 45, with a range of wavelength of 190–1100 nm, connected to a Pentium III 800 MHz computer. Solutions were thermostated by using a Haake C25 bath equipped with a Haake F6 controller

which allows variation of temperature with an accuracy of ±0.01 °C.

Processing of data for the fitting was done using the Statistica® software by Statsoft.

2.3. Dissolution study

The inclusion complexes of hesperetin and naringenin with β-CD were prepared by the coprecipitation method [17].

The influence of β-CD on the dissolution of hesperetin and naringenin was studied.

The samples used for the dissolution study were: pure hesperetin (60 mg), hesperetin/β-CD inclusion complex (corresponding to 60 mg of pure flavonoid); pure naringenin (40 mg), naringenin/β-CD inclusion complex (corresponding to 40 mg of pure flavonoid).

The dissolution media were solutions at different pH values (1.5, 3.0, and 8.0), maintained at 37 ± 0.5 °C and stirred at 50 rpm. Aliquots of the dissolution medium (1 ml) were withdrawn at suitable time intervals and filtered. The withdrawn samples were replaced by equal volumes of the fresh dissolution medium maintained at the same temperature (according to the dissolution test FU) [19]. The samples were assayed by HPLC at the wavelength of 285 nm with an absorbance unit equal to 0.05, according to Perkin-Elmer Laboratory Computing Integrator (LCI-100). The presence of the cyclodextrin did not interfere with the chromatographic analysis. Each data point is the average of three determinations.

2.4. Phase solubility studies

The phase solubility studies were carried out according to the method of Higuchi and Connors [15]. Excess amounts of hesperetin or naringenin were added to 10 ml tubes containing aqueous solutions of increasing concentrations of β-CD (0–0.014 M) and shaked at 25 ± 0.5 °C, 35 ± 0.5 °C, 45 ± 0.5 °C, and 55 ± 0.5 °C.

At the equilibrium after 72 h, an aliquot from each vial was filtered by a syringe equipped with a Gellman Science Acrodisc® LC PVDF 45 μm filter. A portion of the sample was adequately diluted and analyzed by spectrophotometry at 289 nm to value the concentration of the hesperetin or naringenin dissolved. The experiments were carried out in triplicate at each temperature.

The stability constants, K_c, were calculated from the straight-line portion of the phase solubility diagram according to Higuchi–Connors equation (Eq. (1)):

$$K_c = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})}. \quad (1)$$

3. Results and discussion

Dissolution profiles of pure hesperetin, naringenin and their inclusion complexes in buffer solutions at different pH values are shown in Fig. 2 (a–f).

The graphics show the pH values influence on pure flavonoids solubility and point out a higher solubility at pH 8.0, due to the weak acid characteristics of both hesperetin and naringenin; in fact the pure flavonoids dissociate and are more soluble in basic ambient than in acid media [20]. The greater amount of pure substance in the aqueous basic media contributes to the dissolution increase of hesperetin and naringenin from the complexes (Fig. 2c and f). It can be observed that the dissolution of two flavonoids is significantly enhanced. At pH 1.5 the dissolved amount of both pure and complexed hesperetin rapidly increases within 30 min, followed by a slower dissolution until it reaches a plateau after about 1 h. The dissolved amounts were 24.6 and 28.0%, respectively (Fig. 2a). At pH 3.0 a similar behavior can be observed, the amount of pure hesperetin increases from 26.3% up to 34.0% when complexed (Fig. 2b). At pH 8.0 hesperetin has the greatest solubility (72.3%) due to its weak acid properties; moreover complexation yields the highest amount of dissolved drug (90.3%) (Fig. 2c).

The naringenin had almost the same behavior, as the greatest solubilization occurs within 30 min as well. At pH 1.5 the dissolution of pure naringenin was 14.0% and the amount of naringenin dissolved from the complex was 42.0% (Fig. 2d). At pH 3.0 and 8.0 the dissolution of the naringenin was 22.5 and 55.6% respectively, while the amounts dissolved from the complexes were 48.0 and 61.5% (Fig. 2e and f).

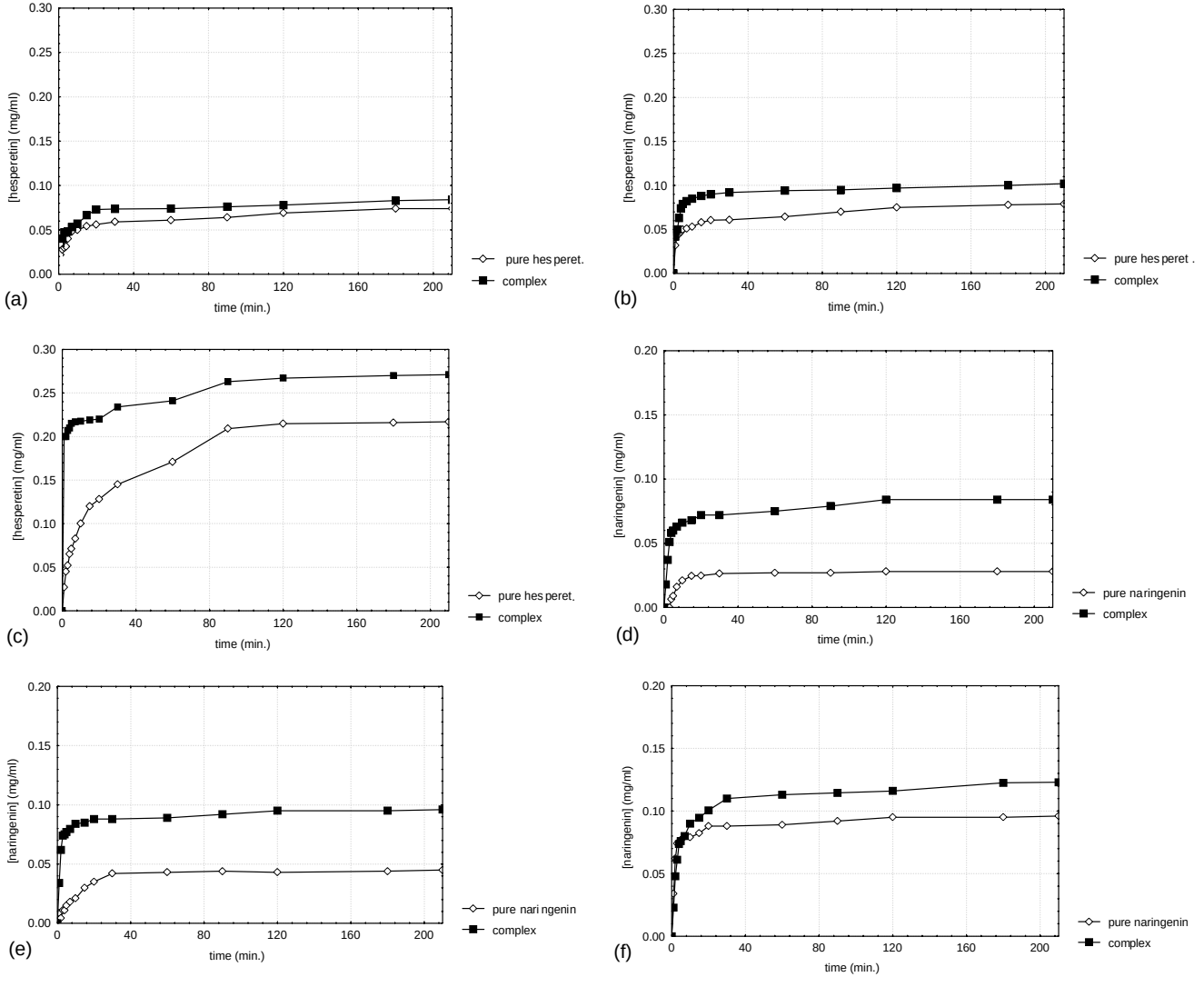

Fig. 2. Dissolution profile of hesperetin and its complex with β-cyclodextrin at 37 °C in buffer solution: pH 1.5 (a); pH 3.0 (b); pH 8.0 (c). Dissolution profile of naringenin and its complex with β-cyclodextrin at 37 °C in buffer solution: pH 1.5 (d); pH 3.0 (e); pH 8.0 (f).

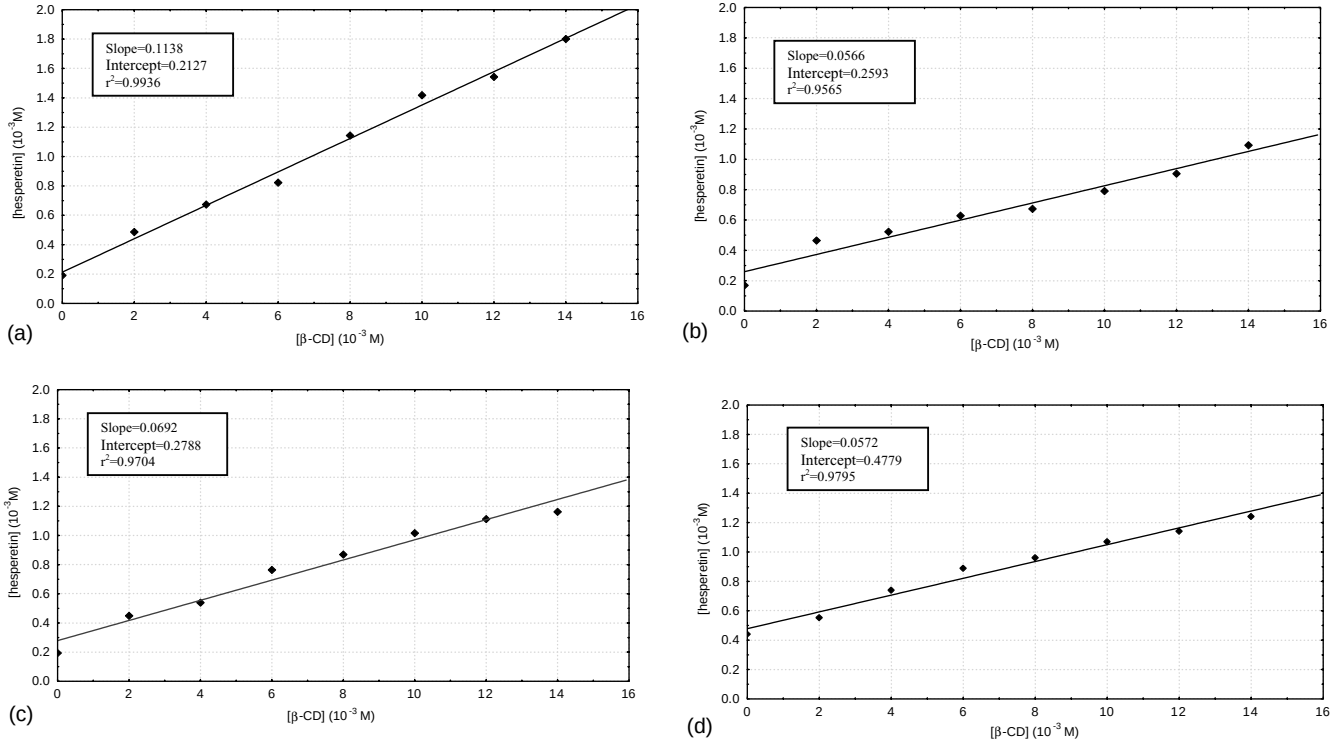

Fig. 3. Phase solubility study of hesperetin with β-cyclodextrin in water at 15 °C (a); 25 °C (b); 35° C (c); 45 °C (d).

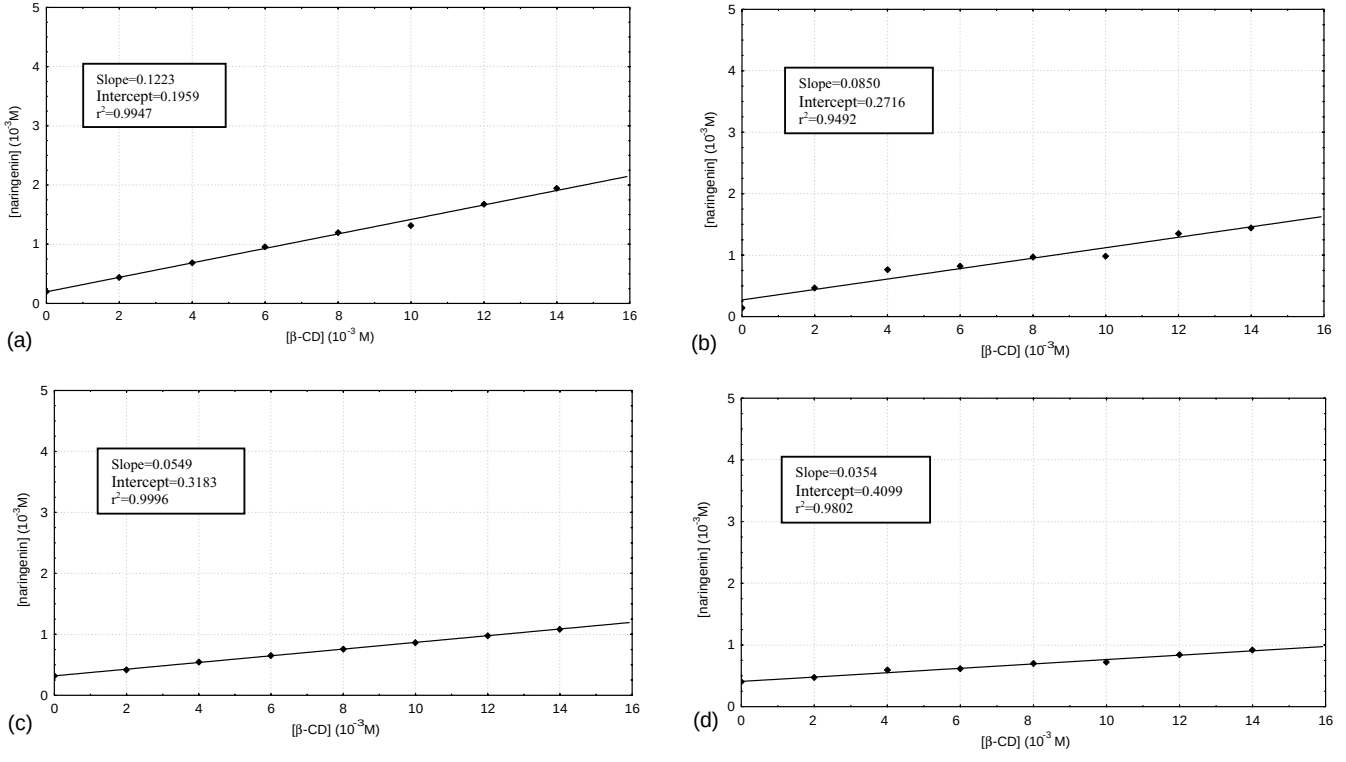

Fig. 4. Phase solubility study of naringenin with β-cyclodextrin in water at 15 °C (a); 25 °C (b); 35 °C (c); 45 °C (d).

Complexation with CDs in all cases led to an increased dissolution rate, even though to a variable extent.

The effect of complexation on the dissolution degree is particularly evident for naringenin at pH 1.5 and 3.0; in both cases this is less pronounced at pH 8.0 as complexes of ionized substances are weaker [21].

Phase solubility study showed that the solubility of both hesperetin and naringenin is a function of the apparent stability constant of the complex, K_c, and of the solubility of the drug in water. The solubility of these flavonoids increases with temperature, and in the same way their total concentration in aqueous medium containing cyclodextrins increases at higher temperatures too.

On the other hand, the affinity of the cyclodextrin for the substance and, consequently, the stability constant decrease with increasing temperatures, giving a negative contribution to the stability of the complex and to the final degree of solubility (S_t).

The final effect of temperature on solubility depends on the prevalence of one effect rather than the other.

The phase solubility diagrams of both hesperetin and naringenin at different temperatures are shown, respectively, in Figs. 3(a–d) and 4(a–d). The plots show linear trend at each of the temperatures studied, with slopes smaller than 1, so they all can be consequently considered as A_L-type diagrams, according to what previously assessed by Higuchi and Connors [15].

The stability constants of the complexes of hesperetin and naringenin at the different temperatures were also calculated, by using Eq. (1).

Table 1
Association constants (K_c) of complexes hesperetin/β-CD and naringenin/β-CD at different temperatures

Temperature (°C)	K_c	
	Hesperetin (M^{-1})	Naringenin (M^{-1})
15	609.80	700.57
25	234.48	355.06
35	228.69	182.13
45	129.99	96.07

Table 1 shows the values of the stability constants, K_c, of hesperetin and naringenin with β-CD, at different temperatures (15–45 °C). It can be observed that these values decrease as the temperature increase, revealing the temperature influence on stability of the complexes. The phase solubility data allow other information to be obtained, as the thermodynamical parameters involved in the complex formation. The integrated form of Van't Hoff equation (Eq. (2)) permits to calculate the values of enthalpy and entropy changes, depending on the variations of the stability constants with temperature [11–14,18]:

$$\ln K_c = -\frac{\Delta H^{\mp}}{RT} + \frac{\Delta S^{\mp}}{R}. \quad (2)$$

The Van't Hoff plots for the complexes hesperetin/β-CD and naringenin/β-CD show a linear behavior, as reported in Figs. 5 and 6. The relative thermodynamic parameters were calculated and are shown in Table 2. The negative values of enthalpy changes indicate that the interaction processes of both hesperetin and naringenin with β-CD are exothermic.

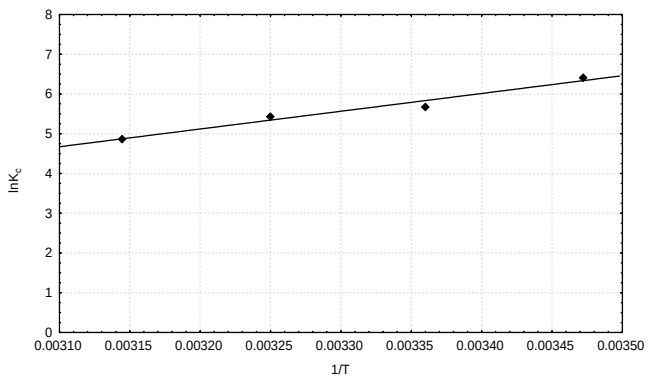

Fig. 5. Van't Hoff plot of the formation of the complex between hesperetin and β-cyclodextrin.

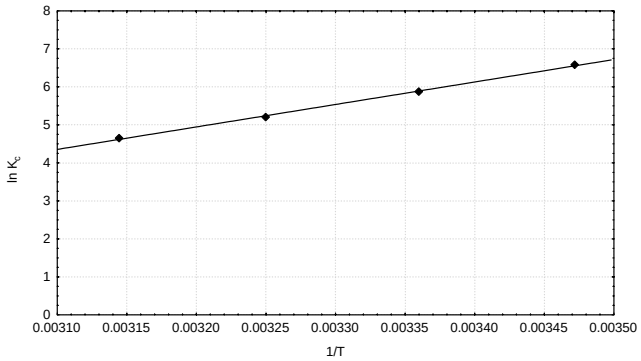

Fig. 6. Van't Hoff plot of the formation of the complex between naringenin and β-cyclodextrin.

They could derive from new interactions, as hydrophobic ones, due to the displacement of the water molecules from the cavity of the β-CD made by the more hydrophobic flavanones; increase of Van der Waals interactions between the molecules; formation of hydrogen bonds and other low energy interactions.

The changes of entropy are also negative in both these processes; this behavior can be explained considering that the complexation causes a decrease in translational and rotational degrees of freedom of the complexed molecule as compared with the free ones, giving a more ordered system.

These results indicate that the complexation of naringenin and hesperetin with β-CD has occurred.

The free energy changes ($\Delta G^{\mp}_{25}$) for the interactions involved in the complex formation were calculated using the Gibbs equation (Eq. (3)):

$$\Delta G^{\mp}_{25} = \Delta H - T\Delta S. \quad (3)$$

The negative values of $\Delta G^{\mp}_{25}$ for hesperetin and naringenin, in Table 2, show that both complexations are spontaneous processes.

Table 2
Thermodynamic values for complex formation of hesperetin and naringenin with β-CD

	Hesperetin	Naringenin
$\Delta H^{\mp}$ (kJ mol^{-1})	−35.49	−50.14
$\Delta S^{\mp}$ (J mol^{-1} K^{-1})	−71.29	−119.65
$\Delta G^{\mp}_{25}$ (kJ mol^{-1})	−14.25	−14.48

4. Conclusions

In this paper, β-CD was therefore used to improve undesirable properties of hesperetin and naringenin, such as solubility and, consequently, bioavailability.

The solubilizing effect of β-CD is related both to its ability to form inclusion complexes and to the intrinsic solubility of the host molecule in water.

The dissolution profiles showed that the solubility of the two flavonoids is influenced by changes of pH of the media: in basic ambient hesperetin and naringenin are in the dissociate form and are more soluble. The complexation improves the dissolution of these flavonoids in different degree in relation to their intrinsic solubility in the media and to the complexation effect itself.

Phase solubility study pointed out the formation of 1:1 stoichiometric complexes between flavanones and β-CD, also influenced by temperature variations. In particular, stability constants K_c were evaluated and evidenced a decreasing complex stabilities with increasing the temperature, thus proving an exothermic and spontaneous process of association.

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