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Analytical Methods

# Rapid, simple and sensitive determination of the apparent formation constants of *trans*-resveratrol complexes with natural cyclodextrins in aqueous medium using HPLC

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**Abstract**

The study of the complexation of *trans*-resveratrol with natural cyclodextrins (CDs) in aqueous medium under different physicochemical conditions of pH or temperature is essential if this antioxidant compound is to be used successfully in the food industry as ingredient of functional foods, due its poor stability, bioavailability and solubility. In this paper, a rapid, simple and sensitive determination of the apparent formation constant of *trans*-resveratrol/CD complexes by HPLC in aqueous medium has been investigated for first time. It can be observed that *trans*-resveratrol forms a 1:1 complex with α-, β- and γ-CD. The highest value of the apparent formation constant ($K_F = 1922 \pm 89\ M^{-1}$) was found for β-CD and a strong dependence of $K_F$ on pH can be seen in the region where the *trans*-resveratrol begins the deprotonation of their hydroxyl groups. Moreover, an increase in the system's temperature produced a decrease in the values of $K_F$. Finally, to gain information on the mechanism of the *trans*-resveratrol affinity for CD, the thermodynamic parameters of the complexation were obtained.


*Keywords:* Resveratrol; Cyclodextrin; HPLC; Apparent formation constants; Aqueous medium

## 1. Introduction

*Trans*-resveratrol (3,5,4′-trihydroxy-stilbene) is a phytoalexin found in at least 72 species of plants distributed among 31 genera and 12 families (Jang et al., 1997). However, foods known to contain this natural antioxidant are limited to grapes, grape products, including wine, peanuts and derivatives and cranberries (Wang, Catana, Yang, Roderick, & van Breemen, 2002). Recently, *trans*-resveratrol has been detected in chocolate (Counet, Callemien, & Collin, 2006), cocoa (Counet et al., 2006), and tomato (Ragab, Van Fleet, Jankowski, Park, & Bobzin, 2006). In recent years, research into resveratrol has discovered several beneficial biological effects of this compound on human health (Latruffe et al., 2002). For these reasons, the increased intake of resveratrol from different dietary sources has been strongly recommended. However, the limited number of foods with high levels of resveratrol has led for the search for new strategies to attain this end. Recently, transgenic fruits like lettuce (Liu, Hu, Wang, Zhong, & Lin, 2006) or apple (Ruhmann, Treutter, Fritsche, Briviba, & Szankowski, 2006), with high levels of free resveratrol or its derivates have been developed.

*Abbreviations*: Resv, *trans*-resveratrol; CD, cyclodextrin; Resv-CD, *trans*-resveratrol-CD complex; Resv-$(CD)_2$, *trans*-resveratrol-$(CD)_2$ complex; m, mobile phase; s, stationary phases; $K_F$, apparent formation constant for the *trans*-resveratrol-CD complex in a 1:1 model; $K_{F1}$, apparent formation constant for the *trans*-resveratrol-CD complex in a 1:2 model; $K_{F2}$, apparent formation constant for the *trans*-resveratrol-$(CD)_2$ complex in a 1:2 model; $K_0$, equilibrium constant of *trans*-resveratrol between mobile and stationary phase; $K_1$, equilibrium constant of *trans*-resveratrol-CD complex between mobile and stationary phase; $K_2$, equilibrium constant of *trans*-resveratrol-$(CD)_2$ complex between mobile and stationary phase.

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Another alternative is being the use of resveratrol as an ingredient in the functional food industry as a fortifier and nutraceutical compound. The desire of the food industry of elaborate functional foods with high added value means that antioxidant compounds of a lipophilic nature, like *trans*-resveratrol, are needed in hydrophobic solvents. Although the benefits of this stilbene have been demonstrated, its use has several strong disadvantages as a functional ingredient is aqueous medium. Although *trans*-resveratrol is well absorbed by humans when taken orally, its bioavailability is quite low as a result of its rapid metabolism and elimination (Walle, Hsieh, Delegge, Oatis, & Walle, 2004; Wenzel & Somoza, 2005). Furthermore, the use of this stilbene as fortifier and nutraceutical is limited by its poor solubility in water (less than 0.05 mg/ml). Finally, the presence of conjugated double bounds in the structure of these compounds means that they are easily oxidised by different prooxidant agents (Fan & Mattheis, 2001). In recent years, some novel foods supplemented with different antioxidant compounds have appeared in the food market. However, the problems described which presents *trans*-resveratrol to be used as fortifier of foods in an aqueous medium has produced that none novel food has been enriched in this important antioxidant compound.

For these reasons, the complexation of *trans*-resveratrol in aqueous medium with compounds which increase its stability, bioavailability and solubility is strongly desirable. The use of cyclodextrins (CDs) is promising in this respect. These cyclic oligosaccharides consist of α (1–4) linked α-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface (Saenger, 1980). The most important functional property of CDs is their ability to form inclusion complexes with a wide range of organic guest molecules. Because CDs are able to increase the bioavailability of different compounds, their use in the food industry is increasing (Szente & Szejtli, 2004). Although the effect of CDs on *trans*-resveratrol has been reported in very few publications (Bru, Sellés, Casado-Vela, Belchí-Navarro, & Pedreño, 2006; Morales, Bru, García-Carmona, Ros Barceló, & Pedreño, 1998; Marier et al., 2002), the complexation of *trans*-resveratrol by CDs was not characterized in these studies.

Many physico-chemical methods have been successfully used to characterize inclusion complexes (Connors, 1997; López-Nicolás, Bru, & García-Carmona, 1997; López-Nicolás, Bru, Sánchez-Ferrer, & García-Carmona, 1995), etc. However, no researchers have investigated, as far as we know, the formation of *trans*-resveratrol-CD complexes in aqueous medium under several physico-chemical conditions of pH or temperature and such information is essential if this stilbene is to be used successfully in the food industry. Recently, Bertacche, Lorenzi, Nava, Pini, and Sinico (2006) calculated the apparent stability constants of the interaction between resveratrol and CDs using phase solubility diagrams, although several days of experiments are required. However, the low water solubility of *trans*-resveratrol and the length of the experiments, which may lead to the degradation of *trans*-resveratrol because of its high instability, do not make this method the best system for studying the apparent formation constants of *trans*-resveratrol complexes with CDs by in aqueous medium. On the opposite, in our paper we calculate these constants in a very short time and to avoid solubility problems. Moreover, the above authors did not analyze possible variation of the apparent stability constants in essential conditions for the production of functional foods such as temperature, pH or the thermodynamic parameters. Recently, our group studied the *trans*-resveratrol-β-CD interaction in a medium with high levels of organic solvent (López-Nicolás, Núñez-Delicado, Pérez-López, Carbonell, & Cuadra-Crespo, 2006). However, potential applications of these results not include their use in the food industry because the apparent complexation constants were not determined in a 100% aqueous medium, indispensable fact to use the *trans*-resveratrol-CD complexes as ingredients of novel foods.

Although the use of CDs as HPLC modifier has been reported by several authors, the novelty and significance of this paper stem from the fact that is the first time where the complexation in aqueous medium of *trans*-resveratrol by CDs is reported. Moreover, and bearing in mind the little information on the complexation of this antioxidant by CDs, one aim of the present work was to examine the retention mechanism of *trans*-resveratrol in an aqueous medium. For this, three types of natural CDs with status GRAS were used (α-, β- and γ-CD). The effect of the concentration and structure of CD and the organic solvent in the mobile phase on the capacity factor was discussed and the apparent formation constants of CD inclusion complexes were obtained. Moreover, the effect of pH, temperature and organic solvent concentration on the apparent formation constants of *trans*-resveratrol-CD inclusion complexes was discussed. Finally, in order to gain information about the mechanism aspect of the *trans*-resveratrol affinity for CDs, the thermodynamic parameters were obtained using a van't Hoff plot.

## 2. Material and methods

### 2.1. Materials

Biochemicals were purchased from Fluka (Madrid, Spain). *Trans*-resveratrol was from Sigma-Aldrich (Steinhelm, Deutschland) and was used without further purification. α-, β- and γ-CD were purchased from Sigma-Aldrich (Madrid, Spain) and used as received. The water used in this study was of HPLC grade purchased from Scharlau Chemie S.A. (Barcelona, Spain). Anhydrous D-glucose was supplied by Prolabo (Fontenoy-Sous-Bois, France).

### 2.2. Equipment and experimental procedure

Twenty microliters of *trans*-resveratrol (prepared at a concentration of 0.05 mg/ml in methanol) were injected for HPLC analysis using a Merck-Hitachi L-6200 pump

(Merck-Hitachi, Darmstadt, Germany) and a diode array detector Shimadzu SPD-M6A UV (Shimadzu, Kyoto, Japan).

For the aqueous mobile phase studies a commercially available Agilent Zorbax Bioseries GF-450 (Santa Clara, USA) (250 × 9.4 mm I.D. 6 μm particle size) was used. For all experiments the mobile phase flow-rate was systematically controlled at 1.00 ± 0.01 mL/min and the UV detector was operated at 306 nm.

Mobile phases were prepared according to the following procedure. After fabrication of the desired aqueous mobile phase (0.2 M sodium acetate was used as buffer for pH 4.0, 0.2 M sodium phosphate for pH 7.0–8.5 and 0.2 M sodium borate for pH 9.0–10.0), an accurately weighed amount of α-, β- or γ-CD was added to 250 mL of this binary mixture in a 500 mL volumetric flask. When total dissolution at ambient temperature was observed, the remaining amount of solvent was added to reach a final mobile phase of 500 mL. The maximum quantity of CD that can be dissolved in such binary mixtures has been reported elsewhere (Chatjigakis, Donzé, & Coleman, 1992). The concentrations of CD used were 0, 0.5, 1, 1.5 and 2 mM. Whenever the mobile phase solution was changed, the column was previously conditioned for at least 1 h with the new solution mixture at a flow-rate of 1.0 mL/min. The column void volume, $t_0$, was determined using reagent grade copper sulfate solution (0.01 mg/mL) as described by Clarot, Clédat, Battu, and Cardot (2000). This volume was systematically controlled through out the experimental program.

### 2.3. Temperature studies

The retention factor was determined at the following temperatures: 15.0, 20.0, 25.0 and 37.0 ± 0.1 °C. The temperature was controlled using a Model Lachron-L 7350 (Merck-Hitachi (Japan)). The standard enthalpy and entropy of *trans*-resveratrol transfer from the mobile phase to the CD can be calculated using the following thermodynamic relationship (Al Omari, Zughul, Davies, & Badwan, 2006):

$$\ln K_F = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (1)$$

where $K_F$ is the apparent formation constant of the inclusion complex, $T$ is the temperature, $R$ is the gas constant and $\Delta H^\circ$ and $\Delta S^\circ$ are standard enthalpy and entropy changes of complexes formation in the mobile phase. For a linear plot of ln $K_F$ versus $1/T$, the slope and intercept are $-\Delta H^\circ/R$ and $\Delta S^\circ/R$, respectively. The Gibbs free energy change for the interactions that take place during the inclusion process may be found by the following equation

$$\Delta G = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

### 2.4. Statistical treatment

*Trans*-resveratrol was eluted in triplicate and the corresponding capacity factors were calculated and systemically tested under different experimental conditions. Tolerance curves and intercepts are described in Section 3.

## 3. Results and discussion

### 3.1. Effect of mobile phase additives on trans-resveratrol retention

To study the possibility of an inclusion complex being formed between D-glucose (molecules included in the CD structure) and *trans*-resveratrol and to examine the possible effect of CD on solvent strength of the mobile phase, various amounts of D-glucose (3.5 and 7 mM), corresponding to 0.5 and 1 mM of β-CD in the number of glucose units (each molecule of β-CD contains seven units of D-glucose in a ring), were added to the aqueous mobile phase at pH 7.0 and the retention of *trans*-resveratrol checked. The effects of different additives to the mobile phase on the retention of *trans*-resveratrol are depicted in Table 1 for an aqueous medium.

The capacity factor, $k$, of *trans*-resveratrol decreased in the presence of β-CD at 0.5 mM and 1 mM, whereas the addition of D-glucose did not alter the $k$ values even though the concentration of D-glucose was the same as that of β-CD as regards the number of glucose units. These results agree with those obtained by Clarot et al. (2000) and López-Nicolás et al. (2006) and indicate that: (1) HPLC appears to be a satisfactory method for observing and characterizing *trans*-resveratrol-β-CD inclusion complexes; (2) the decrease in $k$ caused by the addition of β-CD to the mobile phase is due to the formation of an inclusion complex because no glucose/*trans*-resveratrol complexes exist, and (3) the possible elution modifications observed in the presence of β-CD cannot therefore be attributed to modifications in solvent strength.

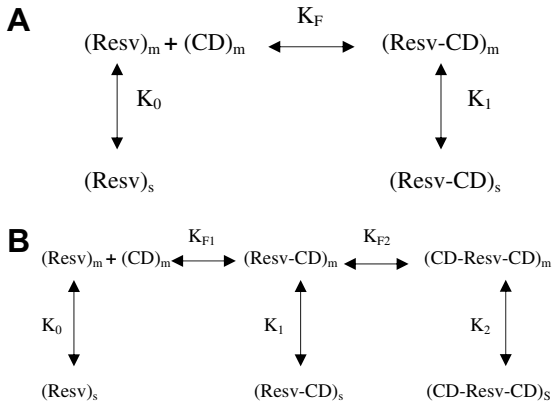
### 3.2. Mechanism of complexation of trans-resveratrol with CDs

In order to stablish the mechanism of complexation of *trans*-resveratrol with CDs in aqueous medium, Scheme 1A

Table 1
Effect of additives in mobile phase on capacity factors of *trans*-resveratrol ($k$)

| $k$ | | | | | |
|---|---|---|---|---|---|
| Mobile phase (% $H_2O$) | No addition | 0.5 mM β-CD | 1 mM β-CD | 3.5 mM D-glucose | 7 mM D-glucose |
| 100% | 2.54 ± 0.13 | 1.30 ± 0.09 | 0.88 ± 0.03 | 2.55 ± 0.12 | 2.59 ± 0.12 |

Flow-rate, 1.00 ± 0.01 mL/min; temperature, 25.0 ± 0.1 °C.




Scheme 1. (A) Equilibria proposed for a 1:1 *trans*-resveratrol-CD inclusion complex. (B) Equilibria proposed for a 1:2 *trans*-resveratrol-CD inclusion complex.

and B corresponding at 1:1 or 1:2 *trans*-resveratrol-CDs complexes were proposed. In the equilibria presented in both Scheme 1A and B, subscript m and s denote the mobile and stationary phases, respectively.

Assuming that the interaction of the *trans*-resveratrol-CD complex with the stationary phase is negligible, the relationship between the capacity factor, $k$, and the total analytical ($[CD]_T$) can be established. For this, an equation taking into account equilibria involving formation of 1:1 *trans*-resveratrol-CD complexes is proposed (Clarot et al., 2000; López-Nicolás et al., 2006; Matsui & Mochida, 1979; Moeder, O'Brien, Thompson, & Bicker, 1996). This equation permits the apparent formation constant to be determined from only a measurement of the *trans*-resveratrol retention time:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K_F}{k_0}[CD]_T \quad (3)$$

where $k$ is the capacity factor of the solute, $k_0$ the solute capacity factor in the absence of CD, $K_F$ is the apparent formation constant of the inclusion complex and ($[CD]_T$) is the total analytical CD mobile phase concentration.

In a previous paper published by Bertacche et al. (2006), the apparent formation constants of *trans*-resveratrol-CD complexes were calculated using long duration phase solubility diagrams. However, in the present work Eq. (4) permits us to determine the apparent formation constant with only one measurement of the *trans*-resveratrol retention time:

$$K_F = \frac{k_0}{[CD]_T} * \left(\frac{1}{k} - \frac{1}{k_0}\right) \quad (4)$$

On the other hand, we expanded the equilibria proposed to allow for 1:2 *trans*-resveratrol-CD complexation (López-Nicolás et al., 2006). The equilibria presented in Scheme 1B show the formation of a 1:2 *trans*-resveratrol-CD complex via a precursor 1:1 complex.

As is shown in both Scheme 1A and B, when CD is added to the mobile phase, *trans*-resveratrol retention is governed by its partition between the mobile and stationary phases and the *trans*-resveratrol complexation with CD (López-Nicolás et al., 2006). Assuming that the interaction of the *trans*-resveratrol-CD complex with the stationary phase is negligible ($K_1 \approx 0$ and $K_2 \approx 0$) (López-Nicolás et al., 2006) and including terms that account for the possibility of a 1:2 *trans*-resveratrol-CD complex, a similar mathematical expression can be derived that describes the dependence of the capacity factor, $k$, of *trans*-resveratrol on the total analytical CD concentration in the mobile phase, $[CD]_T$:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K_{F_1}}{k_0}[CD]_T + \frac{K_{F1}K_{F2}}{k_0}[CD]_T^2 \quad (5)$$

Eq. (5) is an extension of Eq. (3) and includes a second-order term that accounts for the possibility of a 1:2 *trans*-resveratrol-CD complex formation. $K_{F1}$ is the apparent formation constant for the 1:1 *trans*-resveratrol-CD complex. $K_{F2}$ is the apparent formation constant for the 1:2 *trans*-resveratrol-CD complex and $k_0$ is the capacity factor of *trans*-resveratrol in the absence of CD modifier. Eq. (5) simplifies to Eq. (3) when a 1:1 *trans*-resveratrol-CD complex is the only complex formed; the apparent formation constant of the 1:2 *trans*-resveratrol-CD complex, $K_{F2,}$ is zero. In this case, a plot of the reciprocal of $k$ versus $[CD]_T$ should give a straight line. In the case of a 1:2 *trans*-resveratrol-CD complex formation, a plot of reciprocal of $k$ versus $[CD]_T$ should give a parabolic curve that fits Eq. (5). The values of $K_{F1}$ and $K_{F2}$ can be obtained by performing a second-order polynomial fit to the data.

### 3.3. Determination of the stoichiometry of trans-resveratrol-CD complexes

As far as we know, very few researchers have studied the effect of CD on *trans*-resveratrol properties (Bru et al., 2006; Marier et al., 2002; Morales et al., 1998). Moreover, the stoichiometry for CD complexes of *trans*-resveratrol in aqueous medium in different physico-chemical conditions (e.g. several temperatures or pHs) has not previously been studied. To determine the stoichiometric ratios for the *trans*-resveratrol-CD complexes formed, both Eqs. (3) and (5) were used. For this, the reciprocal of $k$ for *trans*-resveratrol was plotted as a function of [CD]. In Table 2, we can see the correlation coefficients arising from this plot determined in the different physico-chemical conditions studied in this paper. Whatever the mobile phase composition, the type of CD used or the physico-chemical conditions employed, the correlation coefficients corresponding to 1:1 complexes were always higher than those calculated for 1:2 complexes.

### 3.4. Effect of the CD structure on apparent formation constants

The complexation equilibrium constants between *trans*-resveratrol and CDs were determined with different types

Table 2
Correlation coefficients arising from Eqs. (3) and (5), (for 1:1 and 1:2 *trans*-resveratrol-CD complexes, respectively), with different mobile phases

| Mobile phase conditions | | | | Correlation coefficient | |
|---|---|---|---|---|---|
| Type of CD | $T$ (°C) | Buffer | pH | 1:1 using Eq. (3) | 1:2 using Eq. (5) |
| α-CD | 25.0 | 0.2 M sodium phosphate | 7.0 | 0.99 | 0.91 |
| γ-CD | 25.0 | 0.2 M sodium phosphate | 7.0 | 0.99 | 0.88 |
| β-CD | 25.0 | 0.2 M sodium phosphate | 7.0 | 0.99 | 0.90 |
| β-CD | 37.0 | 0.2 M sodium phosphate | 7.0 | 0.98 | 0.74 |
| β-CD | 25.0 | 0.2 M sodium acetate | 4.0 | 0.96 | 0.85 |

of CDs in an attempt to characterize the interaction between *trans*-resveratrol and the host CD at a molecular level. Three types of natural CDs with status GRAS (α-, β- and γ-CD) were used to this end. Fig. 1 shows the effect of CD concentration on the capacity factors of *trans*-resveratrol determined using increasing concentrations of α-, β- and γ-CD (0–2.0 mM). As is shown, the retention time strongly decreased as the concentration of α-CD and β-CD in the mobile phase increased, due to the formation of the *trans*-resveratrol-α-CD or *trans*-resveratrol-β-CD complexes, which enhanced the guest solubility in the mobile phase and reduced its residency time in the column. On the other hand, the presence of increasing concentrations of γ-CD produced a slight decrease on the retention time.

Fitting the values of capacity factors to the equations previously described provides the corresponding $K_F$ (Fig. 1 inset). As regards the different species α-, β- and γ-CD it can be observed that the highest $K_F$ value ($K_F = 1922 \pm 89\ M^{-1}$) was found for β-CD, followed by α-*CD* ($K_F = 565 \pm 34\ M^{-1}$) and, finally, γ-CD showed the lowest $K_F$ value ($K_F = 55 \pm 4\ M^{-1}$). These results indicated that resveratrol interacts more strongly with β-CD, which was therefore considered the most suitable CD for the present investigation.

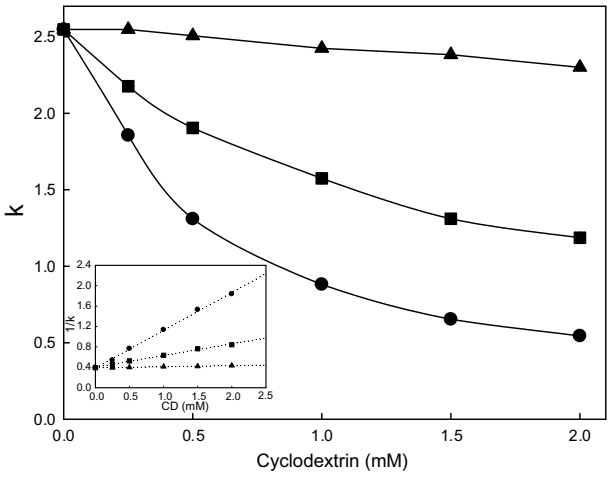



Fig. 1. Effect of CD concentration on the capacity factors of *trans*-resveratrol determined using increasing concentrations of α- (■), β- (●) and γ- (▲) CD (0–2.0 mM) in an aqueous mobile phase (0.2 M sodium phosphate pH 7.0) (flow-rate, 1.00 ± 0.01 mL/min, temperature, 25.0 ± 0.1 °C). Inset: Effect of CD concentration on the reciprocal values of capacity factors of *trans*-resveratrol. Each data point is the mean of three replicates.

If we compare the $K_F$ value found for β-CD in a 100% aqueous mobile phase ($K_F = 1922 \pm 89\ M^{-1}$) with the results obtained previously (López-Nicolás et al., 2006) for the complexation equilibrium constants between *trans*-resveratrol and β-CD in the presence of high percent of methanol in the mobile phase ($K_F = 588 \pm 29\ M^{-1}$ for a 50% v/v methanol/water mobile phase), we can observe as the $K_F$ value obtained in aqueous medium is higher than the determined in the presence of organic modifier. This behaviour can be explained in two ways: (1) the enhanced competition of methanol and solute for the β-CD cavity (Clarot et al., 2000; Moeder et al., 1996), since the association constant of methanol with β-CD is 0.32 $M^{-1}$ (Matsui & Mochida, 1979) and (2) the effect of methanol on the interaction of *trans*-resveratrol with both mobile and stationary phases. At high methanol percentage (50% v/v in binary mixtures methanol–water), a substantial amount of methanol can interact with β-CD, leading to competition with *trans*-resveratrol complexation. Nevertheless, the increase in $K_F$ values is interpreted by reference to hydrophobic interactions, which are known to play a key role in the inclusion process.

### 3.5. Effect of pH on apparent formation constants

The apparent formation constants for *trans*-resveratrol-β-CD complexation were determined in the pH range 4.0–10.0. Fig. 2 shows the effect of pH on the equilibrium constants. A strong dependence of $K_F$ on pH can be seen, passing from a stable value of about 1920 $M^{-1}$ to about 1314 $M^{-1}$ in just one pH unit, as happens during the titration of a weak ionizable group. As β-CD does not possess any ionizable groups it is presumably one of the hydroxyl groups of the *trans*-resveratrol that is being titrated. As shown in Fig. 2, the strong decrease in the $K_F$ value coincides with the region where the *trans*-resveratrol begins the deprotonation of their hydroxyl groups. In fact, the pH at which the drastic decrease of $K_F$ value is observed is very close to a *trans*-resveratrol $pK_a$ of 9.3 (dotted line) for the first of the hydroxyl group, as determined by Deak and Falk (2003).

A likely explanation for the dependence of $K_F$ on pH is that the protonated *trans*-resveratrol hydroxyl group forms a hydrogen bond with hydrophilic groups of CD at pH

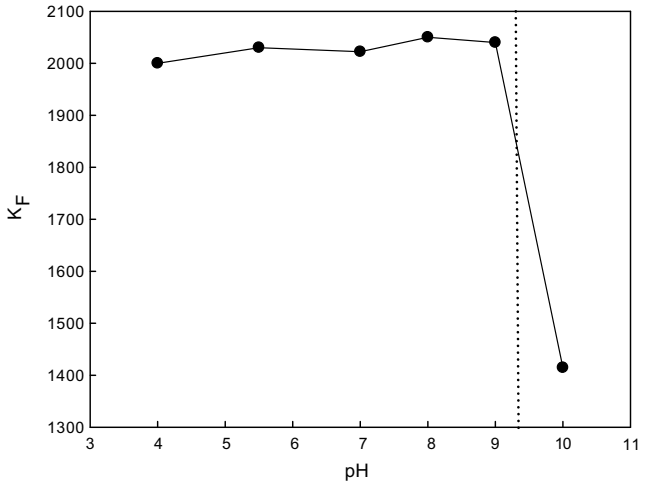



Fig. 2. Effect of pH on the apparent formation constant ($K_F$) of *trans*-resveratrol-β-CD complexes (flow-rate, 1.00 ± 0.01 mL/min, temperature, 25.0 ± 0.1 °C). Each data point is the mean of three replicates.

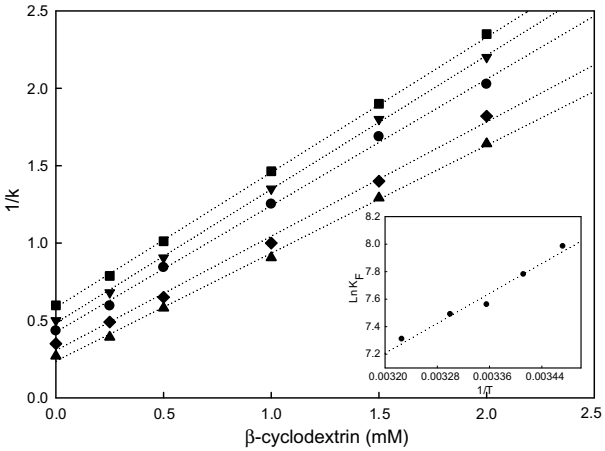



Fig. 3. Effect of β-CD concentration on the reciprocal of *k* for *trans*-resveratrol at four different temperatures: 15.0 °C (▲), 20.0 °C (♦), 25.0 °C (●), 30.0 °C (▼) and 37.0 °C (■). Inset: Vańt Hoff plot (ln $K_F$ vs. $1/T$, for *trans*-resveratrol-β-CD complexes with an aqueous mobile phase (0.2 M sodium phosphate pH 7.0) (flow-rate, 1.00 ± 0.01 mL/min).

values below the p$K_a$ value, as occurs with other ionized weak electrolytes. Indeed, hydrogen bonding is one of the most important types of interaction in the stabilization of inclusion complexes of guest molecules with CDs (Szejtli, 1988). This type of behaviour has been described by our group for the complexation of fatty acids by different types of CD (López-Nicolás et al., 1995).

The fact that the complexes between β-CD and the protonated form of *trans-resveratrol* were more stable than the interaction with the deprotonated forms of this lipophilic antioxidant is of great interest for the food industry, because the protonated form of *trans-resveratrol* presents several beneficial biological effects on human health. These include anticancer activity, cardioprotection, antioxidant activity, antiviral activity, cytogenetic activity, the inhibition of platelet aggregation, and antiinflamatory activity (Latruffe et al., 2002).

### 3.6. Effect of temperature on apparent formation constants

Different studies have evaluated the effect of the temperature of the medium on the equilibrium between CDs and some compounds. Normally, the inclusion complexes dissociate when temperature is increased. However, our group showed that an increase in the temperature of the medium was also able to increase the $K_F$ of some compounds such as fatty acids (López-Nicolás et al., 1995). In this paper the effect of temperature on the $K_F$ was studied for the *trans*-resveratrol-β-CD interaction at four different temperatures: 15.0, 20.0, 25.0 and 37.0 °C. To determine the stoichiometric ratios for *trans*-resveratrol-β-CD complexes formed at these temperatures, the reciprocal of *k* for *trans*-resveratrol was plotted as a function of β-CD concentration (Fig. 3). A correlation coefficient higher than 0.99 was found when using an aqueous solution at pH 7.0 as mobile phase at all the temperatures assayed, indicating the formation of 1:1 complexes. The values of $K_F$ for 15.0, 20.0, 25.0 and 37.0 °C were 2935 ± 93, 2400 ± 75, 1922 ± 89, 1497 ± 37 $M^{-1}$, respectively. Retention decreases with decreasing temperature because it is accompanied by a higher degree of complexation. Consequently, the concentration of free *trans*-resveratrol that can be adsorbed on the stationary phase diminishes.

### 3.7. Thermodynamic parameters for the trans-resveratrol-β-CD complexes

The integrated form of the van't Hoff equation (Eq. (1)) permits the changes in enthalpy and entropy values to be calculated, depending on the variations in the stability constants with temperature (Tommasini et al., 2004). To obtain information about the mechanism aspect of the *trans*-resveratrol affinity for β-CD in aqueous medium, the thermodynamic parameters were obtained from the van't Hoff plot. The ln $K_F$ versus $1/T$ was plotted for β-CD complexes. As shown in Fig. 3 (inset), the van't Hoff plot for the complex of *trans*-resveratrol-β-CD is a linear function between $K_F$ and the inverse of the absolute temperature ($1/T$), with a correlation coefficient higher than 0.99.

The values of $\Delta H°$ and $\Delta S°$ for the complex at pH 7.0 have been calculated with the corresponding Gibbs free energy at 25.0 °C. The enthalpy change is −22.56 kJ $mol^{-1}$, which is typical of the following types of interaction: hydrophobic interactions due to the displacement of water molecules from the cavity of β-CD; increase van der Waals interactions between the molecules; the formation of hydrogen bonds and other interactions. This negative value of enthalpy changes indicates that the interaction processes of *trans*-resveratrol with β-CD are exothermic. The changes in entropy are also negative ($\Delta S°$: −12.30 J/mol K) in these processes because the complexation causes a

decrease in translational and rotational degrees of freedom of the complexed molecule compared with the free ones, giving a more ordered system. Finally, in order to gain information about the possible spontaneity of the inclusion process, the Gibbs free energy change for the interactions that take place during the inclusion process at 25.0 °C was calculated. The value of $-18.89\ kJ\ mol^{-1}$ obtained indicates that the inclusion process is a spontaneous one.

## 4. Conclusions

The food industry's growing interest in functional foods of high added value emphasises the need for antioxidant compounds of a lipophilic nature in hydrophilic solvents. Because of *trans*-resveratrol is poor solubility and propensity to oxidation, its use as functional ingredient in novel foods (as a fortifier and nutraceutical compound) will depend on compounds which permit its "solubilization" and protection in hydrophilic foods. In this paper, the encapsulation of this bioactive substance in CDs is proposed to protect it from potential adverse reaction. To use such *trans*-resveratrol-CD complexes in functional foods, the first step is to determine the apparent formation constants in aqueous medium because this will permit us show the amount of *trans*-resveratrol complexed in equilibrium with free *trans*-resveratrol. The results presented in this paper show a rapid, easy to perform and accurate determination of the apparent formation constant of the *trans*-resveratrol-CD complexes in aqueous medium in several conditions of pH, temperature and type of CD. Moreover, the method presented reduces the analysis time necessary in liquid chromatographic methods.

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