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# Complexation of resveratrol with cyclodextrins: Solubility and antioxidant activity

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ABSTRACT

The slightly water-soluble cancer chemopreventive polyphenol resveratrol (Res) and its inclusions with β-cyclodextrin (β-CD) and hydroxypropyl-β-cyclodextrin (HP-CD) have been investigated. The stoichiometric ratios and stability constants have been determined by phase-solubility measurements. In all cases 1:1 complexes are formed. The inclusion ability of HP-CD is larger than that of β-CD. The antioxidant activity of the Res complexes has been determined by the scavenging of the stable radical DPPH·. The scavenging capacity of the two complexes increases with increasing concentration of cyclodextrins. Res/HP-CD complex shows a higher scavenging capacity than Res/β-CD complex. The antioxidant activity of Res in free form has little difference with Res in complexed form at the same concentration.



## 1. Introduction

Resveratrol (3,4′,5-trihydroxystilbene, Res) is a naturally occurring phytoalexin, synthesised in response to injury or fungal attack (Fremont, 2000). It has been found in at least 72 plant species, a number of which are dietary components, such as mulberries, peanuts, and grapes (de la Lastra & Villegas, 2005; Fremont, 2000). Recently, Res has attracted great interest, due to the "French paradox"; despite fat-rich diets, mortality from coronary heart disease is lower in France than in other countries due to the moderate consumption of red wine (Nanji & French, 1986). Epidemiological studies have suggested that Res is one of the active ingredients of red wine responsible for decreased coronary heart disease mortality (Goldberg, Hahn, & Parkes, 1995). Some studies have shown that Res modulates lipid metabolism, protects low-density lipoproteins against oxidative and free radical damage (Brito, Almeida, & Dinis, 2002; Fremont, Belguendouz, & Delpal, 1999), and inhibits platelet activation and aggregation (Pace-Asciak, Hahn, Diamandis, Soleas, & Goldberg, 1995). It also has strong anticarcinogenesis effects and blocks the carcinogenesis stages of initiation, promotion and progression (Jang et al., 1997). Although the molecular basis for the biological activity of Res is not well understood, it is known that this molecule acts as a radical scavenger. However, Res is sparingly soluble in water, which may be responsible for its limited absorption upon oral administration. The limitation could be overcome by the formation of inclusion complexes with cyclodextrins (CDs).

In recent years, pharmaceutical applications of CDs as additives and drug-complexing agents have been growing rapidly. CDs are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity (Brewster & Loftsson, 2007). The hydrophobic cavity forms inclusion complexes with a wide range of guest molecules (Alvariza, Usero, & Mendicuti, 2007; Calabrò et al., 2004; Jullian, Moyano, Yañez, & Olea-Azar, 2007; Lucas-Abellán, Fortea, López-Nicolás, & Núñez-Delicado, 2007). Inclusion complex formation will modify physico-chemical properties such as solubility, stability and bioavailability of poorly water-soluble drugs (Calabrò et al., 2004; Karathanos, Mourtzinos, Yannakopoulou, & Andrikopoulos, 2007). Unmodified or unsubstituted β-CD has poor water solubility and is unsafe due to its nephrotoxicity. Therefore, several modified and relatively safe CDs have been made, such as hydroxypropyl-β-CD (HP-CD) and sulfobutyl ether-β-cyclodextrin (Brewster & Loftsson, 2007). The complexation of *trans*-Res with β-CD has been studied by reversed-phase liquid chromatography (López-Nicolás, Núñez-Delicado, Pérez-López, Barrachina, & Cuadra-Crespo, 2006).

The present work has involved the complexation of *trans*-Res with β-CD and HP-CD to improve its solubility. The stoichiometry and stability constants of the complexes were determined by evaluating drug–CD interaction in solution, using phase-solubility analysis. The antioxidant activity of two complexes was determined by scavenging of the stable radical DPPH·. The antioxidant efficacy (AE) value of the two complexes was obtained. The

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antioxidant capacity of Res in free form was compared with Res in complexed form, at the same Res concentration.

## 2. Materials and methods

### 2.1. Materials

*trans*-Res (*trans*-3,4′,5-trihydroxy-stilbene) was purchased from Sigma (St. Louis, MO); its purity was 99% according to the manufacturer. β-CD and HP-CD (1.0 M substitution dextrin) were purchased from Sigma–Aldrich. DPPH· (2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl) free radical was purchased from Sigma–Aldrich. Solvent employed in the spectrophotometric analyses was of spectroscopic reagent grade. All other reagents were of analytical reagent grade. Deionised water from a Milli-Q system apparatus (Millipore Corp., Billerica, MA) was used throughout the experiments.

### 2.2. Methods

#### 2.2.1. UV–vis measurements

The UV–vis measurements of Res in the presence or absence of CDs were made in the range of 240–400 nm at 298 K. Res concentration was fixed at 25 μM while the CDs concentration was varied from 0 to 0.75 mM. All solutions were mixed thoroughly and reached equilibrium before measurements.

#### 2.2.2. Phase-solubility measurements

Phase-solubility measurements were carried out according to the method of Higuchi and Connors (1965). Excess amount of Res was added to 5 ml water containing increasing amounts of β-CD or HP-CD (ranging from 0 to 10 mM). The solutions were shaken for 24 h in a thermostatted bath at 298 K. To minimise photochemical degradation the flasks were covered with aluminium foil. After equilibrium was reached, suspensions were filtered through 0.45 μm cellulose acetate membrane filters to remove undissolved solid. An aliquot from each vial was adequately analysed on a Cary 100 UV–vis spectrophotometer (Varian Inc., Palo Alto, CA). The experiments were carried out in triplicate.

The stability constants, $K_C$, are calculated from the phase-solubility diagrams according to the Higuchi–Connors equation

$$K_C = \frac{\text{slope}}{S_0 \cdot (1 - \text{slope})} \quad (1)$$

where, $S_0$ is the solubility of Res at 298 K in the absence of CDs and slope means the corresponding slope of the phase-solubility diagrams.

#### 2.2.3. Determination of antioxidant activity by the scavenging of the stable radical DPPH·

The antioxidant activity was measured by DPPH· assay. In its radical form, DPPH· absorbs at 517 nm, but upon reduction by an antioxidant its absorption decreases.

An ethanolic solution of the radical DPPH· was prepared and protected from light. The linear relationship between radical concentration and absorbance was established. Res, Res/β-CD or Res/HP-CD samples were added to 60 μM DPPH· ethanolic solution. After the reaction reached equilibrium the absorbance at 517 nm was recorded. The blank reference cuvette contained ethanol. All measurements were performed in triplicate.

The stock solution of Res was prepared in 50% DMSO and kept in the dark at 277 K; the molar concentration was calculated from its molecular weight of 228. In order to compare the antioxidant capacity of free Res and complexed Res at the same concentration, the antioxidant capacity of different concentrations of free Res was measured.

#### 2.2.4. Data analysis

Reaction kinetics of complexes with DPPH· were tested. Times at steady state were determined. The percentage of DPPH· remaining at the steady state (DPPH· rem) was calculated as

$$\%DPPH^{\cdot} = \frac{A_f}{A_0} \times 100 \quad (2)$$

$A_0$ and $A_f$ correspond to the absorbances at 517 nm of the radical at the beginning and at steady state, respectively.

Antioxidant efficacy (AE) is calculated from following equation:

$$AE = \frac{1}{EC_{50}} \times TEC_{50} \quad (3)$$

where $EC_{50}$ is the amount of antioxidant needed to decrease the initial DPPH· concentration by 50%, $TEC_{50}$ is the time needed to reach the steady state at the concentration $EC_{50}$. This parameter is obtained by plotting the times at the steady state against the concentration for each antioxidant compound and is calculated graphically (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998).

## 3. Results and discussion

### 3.1. UV–visible measurements

The UV–visible absorption spectra of Res in the absence or presence of β-CD and HP-CD are shown in Fig. 1. The two kinds of CDs do not absorb in the range of measures. Res has strong absorbance with a peak at about 304 nm; its molar absorption coefficient is about 39,500 $M^{-1}$ $cm^{-1}$. The UV spectrum of Res is different in the presence of CDs. As shown in Fig. 1, β-CD causes small red shifts of the maximum peak position of Res (from 304 to

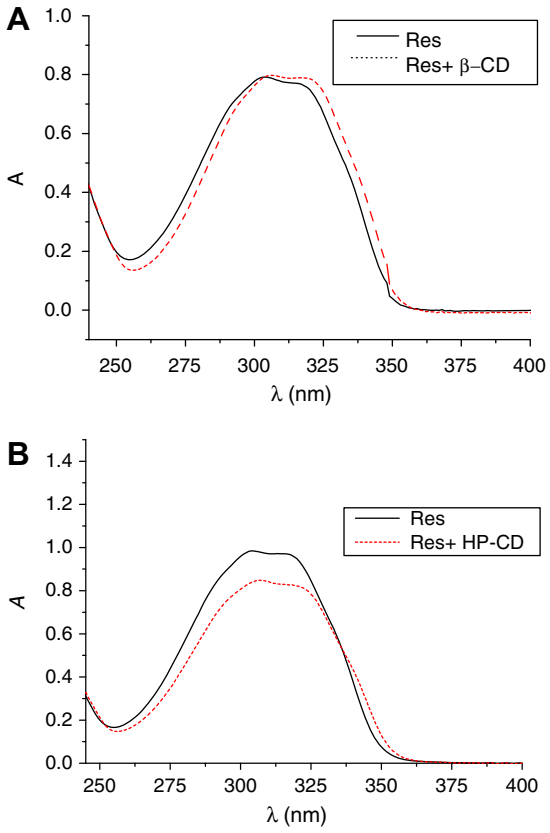


**Fig. 1.** UV–vis absorption spectra of Res in the presence of β-CD (A) or HP-CD (B) at 298 K. Concentration of Res is 25 μM, concentration of β-CD or HP-CD is from 0 to 0.75 mM.

306 nm), while the absorbance is slightly increased, giving rise to a molar absorption coefficient of 40,800 $M^{-1}$ $cm^{-1}$. When recording the absorption in the presence of HP-CD, a red shift of 3 nm is produced, while the absorbance is decreased, giving a molar absorption coefficient of 34,300 $M^{-1}$ $cm^{-1}$. The red shifts indicate that the chromophore of the drug is displaced to a more hydrophobic environment upon the drug-CDs binding (Calabrò et al., 2004). The molar absorption coefficient of 40,800 $M^{-1}$ $cm^{-1}$ at 306 nm or 34,300 $M^{-1}$ $cm^{-1}$ at 307 nm is used to determine the concentration of Res in a β-CD or in HP-CD complex.

### 3.2. Phase-solubility measurements

The two kinds of CDs enhance the aqueous solubility of Res. As shown in Fig. 2, the phase-solubility diagrams of Res with β-CD or HP-CD show a linear relationship between the amount of Res solubilised and the concentration of CDs in solution ($A_L$ type diagram). According to the theory of Higuchi and Connors (1965), this may be attributed to the formation of soluble 1:1 Res/CD inclusion complexes. The stability constants $K_C$ of the complexes are calculated from the slopes of the linear phase-solubility plots, according to the methodology described before. The $K_C$ value of Res/HP-CD system (6778 $M^{-1}$, 298 K) is larger than that of Res/β-CD system (1815 $M^{-1}$, 298 K), which suggests that HP-CD exhibits stronger inclusive ability than native β-CD. The fact implies that the cavity of modified CD provides a better protective microenvironment. This is probably because the hydroxypropyl substitutions enlarge the opening of native β-CD and destroy the strong intramolecular hydrogen bond network, which lets guest molecules access the HP-CD cavity easily and give a higher stability constant (Brewster & Loftsson, 2007).

### 3.3. Scavenging study of DPPH·

DPPH·, a stable and commercially available free radical, has been extensively applied on the study of antioxidant activity of natural compounds. It is easy to perform, highly reproducible and comparable with other methods (Villaño, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007). Progressive discoloration of DPPH· in the presence of Res indicated that Res is acting as an antioxidant. The rate of the DPPH·-scavenging reaction was measured by monitoring the decrease in absorbance at 517 nm due to DPPH·. No decay was observed when CDs were mixed with DPPH·. The equilibria of the reactions between DPPH· and Res, and Res complexes are reached in different times (Fig. 3). DPPH·-scavenging activity of complexes are shown in Fig. 4. The percentage of DPPH· remaining shows a linear decrease with increasing concentration of CDs. Furthermore, the scavenging activity of the Res/HP-CD complex is greater than that of the Res/β-CD complex. This is attributed to their being more Res complexed in HP-CD.

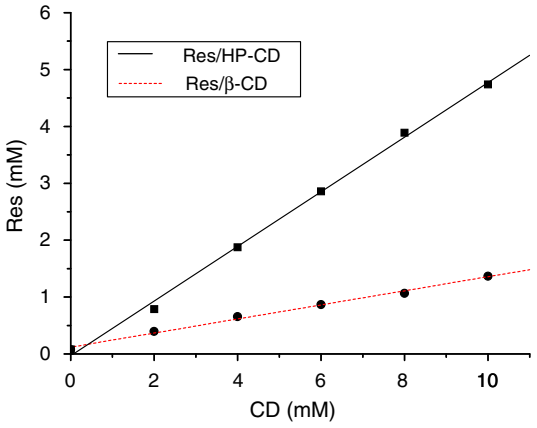


**Fig. 2.** Phase-solubility diagrams of Res/β-CD and Res/HP-CD system in water at 298 K.

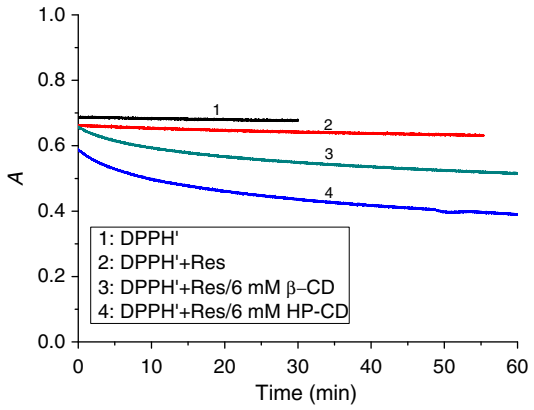


**Fig. 3.** Reaction curves between DPPH· and Res complexes at 298 K. Concentration of DPPH· is 60 μM; 0.1 ml of each sample were added to 3.9 ml of DPPH· solution.

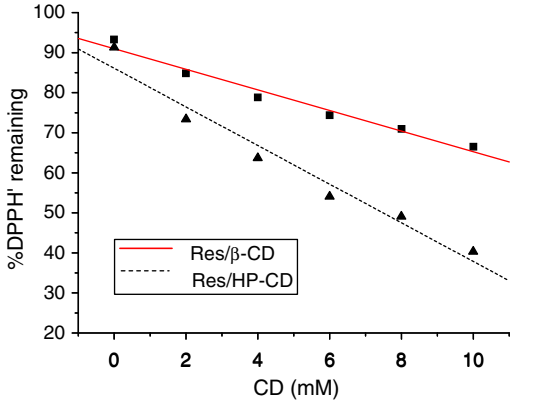


**Fig. 4.** DPPH·-scavenging activity of Res complexes at 298 K. Concentration of DPPH· is 60 μM.

### 3.4. Antioxidant efficacy

Antioxidant efficacy (*AE*) combines the antioxidant capacity of a compound ($EC_{50}$) and the rate of reaction towards the free radical ($TEC_{50}$). It seems to be a more appropriate parameter to better define a compound as an antioxidant (Sánchez-Moreno et al., 1998). The higher the *AE*, the higher the antioxidant activity of a compound is. The $EC_{50}$ values are calculated from Fig. 5 and are 79.4 μl for the Res/β-CD complex and 41.7 μl for Res/HP-CD complex. The $TEC_{50}$ values of Res complexes are calculated graphically and are 7.6 min for the Res/β-CD complex and 3.6 min for the Res/HP-CD complex, respectively. The $EC_{50}$ value of the Res/HP-CD complex is lower than that of Res/β-CD complex, which indicates that the Res/HP-CD complex shows a better antioxidant capacity. The $TEC_{50}$ value of the Res/HP-CD complex is also lower than that of the Res/β-CD complex and suggests that the Res/HP-CD complex reacts in a faster manner with DPPH·. This characteristic is of importance in biological systems, as free radicals have very short half-lives (Diplock et al., 1998). The *AE* value of the Res/HP-CD complex ($6.66 \times 10^3$ $l^{-1}$ $min^{-1}$) is about fourfold higher than that of the Res/β-CD complex ($1.66 \times 10^3$ $l^{-1}$ $min^{-1}$), which shows that the former acts as a better antioxidant than the latter.

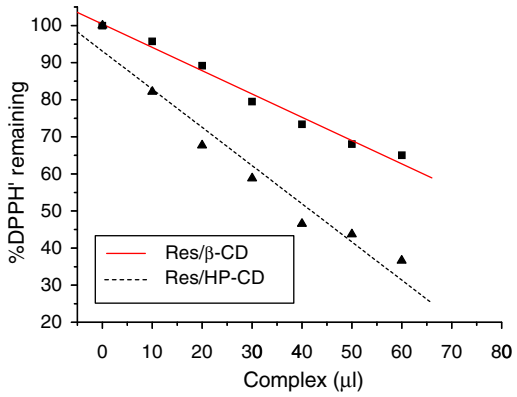


**Fig. 5.** The scavenging capacity of Res/6 mM complexes towards DPPH· free radical at 298 K. Concentration of DPPH· is 60 μM.

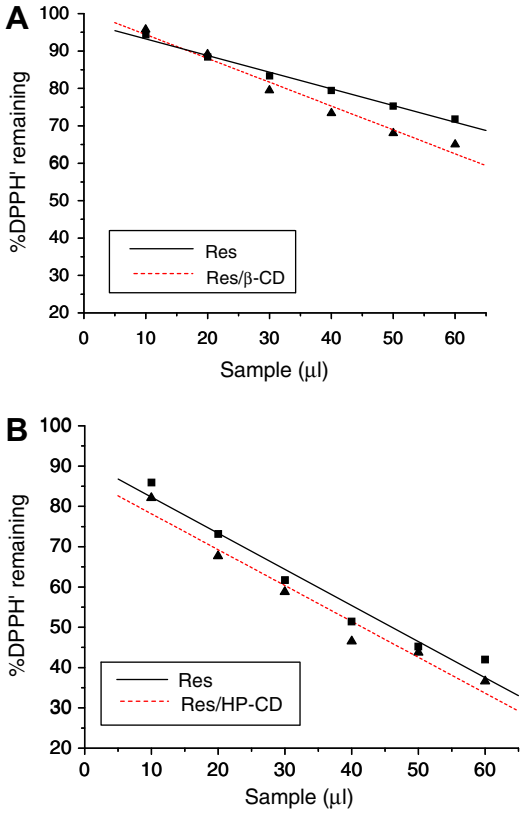


**Fig. 6.** The scavenging capacity of free Res and Res/CD complexes towards DPPH· at 298 K. (A) Res (0.68 mM), Res/β-CD (β-CD: 6 mM); (B) Res (2.72 mM), Res/HP-CD (HP-CD: 6 mM). Concentration of DPPH· is 60 μM.

### *3.5. Antioxidant capacity comparison of Res in free form with Res in complexed form*

In order to explore whether the inclusion has an influence on antioxidant capacity of Res, the scavenging capacity towards DPPH· of free Res and complexed Res at the same concentration were compared. The concentration of Res in Res/6 mM β-CD and Res/6 mM HP-CD complexes are 0.68 and 2.72 mM, respectively, determined by the molar absorption coefficients of 40,800 $M^{-1}$ $cm^{-1}$ at 306 nm and 34300 $M^{-1}$ $cm^{-1}$ at 307 nm. The scavenging capacities of Res in free form or in complexed form toward DPPH· free radical are shown in Fig. 6. The differences in scavenging capacity between free Res and complexed Res are little, which suggests that the inclusion process has little influence on the antioxidant activity of Res. This may offers an experimental basis for the usage of Res complexes as an antioxidant.

## 4. Conclusions

The limited water solubility of Res could be overcome by the formation of inclusion complexes with CDs. The solubility of Res increases with increasing CD concentration in the order of β-CD < HP-CD. Res/HP-CD complex shows a higher antioxidant efficacy both in terms of capacity and rate of scavenging DPPH· radical. The antioxidant activity of Res in free form has little difference with Res in complexed form at the same concentration, this result indicates that the complexes formed maintained the Res antioxidant activity. This work shows the potential usage of Res/CD complexes.

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