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Kaempferol Complexation in Cyclodextrins at Basic pH

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The complexation of kaempferol with cyclodextrins (CDs) (β-, G_2-β-, and HP-β-CDs) in basic medium was studied, and the complexation constants (K_c) were calculated by using enzymatic, solubility, and fluorometric methods. This is the first time that a decrease in fluorescence has been observed as result of the analyte complexation by CDs. The highest K_c value for kaempferol complexation was obtained for HP-β-CDs. To establish the validity of the fluorometric method for determining the K_c between kaempferol and CDs, the same parameters were also determined by enzymatic and solubility methods. The enzymatic method was carried out by using horseradish peroxidase as oxidative enzyme, and the K_c values obtained were similar to those obtained by using the solubility method. However, the fluorometric method underestimated the K_c value by about 1.2-fold with respect to the other methods used. In all cases HP-β-CDs showed the highest K_c value, indicating that they are more efficient in the formation of inclusion complexes with kaempferol.

KEYWORDS: Kaempferol; cyclodextrins; complexation; K_c methods; stability; flavonols

INTRODUCTION

Flavonoids, polyphenolic phytochemicals found in fruits and vegetables, constitute a large group of secondary plant metabolites (*1*, *2*). They are particularly abundant in onions, apples, tea, and red wine (*3*). These natural products are of interest because of their proposed health-promoting effects as antioxidants (*4*) and as anticarcinogens (*5*). An inverse association between the intake of flavonols and flavones and the risk of coronary heart disease (*6−9*), stroke (*10*), and lung cancer (*11*) has been shown in epidemiological studies. They inhibit enzymes such as prostaglandin synthase, lipoxygenase, and cyclooxygenase, which are closely related with tumorigenesis (*12*, *13*), and induce detoxifying enzyme systems such as glutathione *S*-transferase (*14*).

Besides the interest shown by the food sector in developing functional foods, in recent years the cosmetic, pharmaceutical, and chemical industries have also become increasingly interested in antioxidants (*15*). Recent research efforts into antioxidants have focused on flavonoids with their strong free radical scavenging effects and metal ion chelating properties. Quercetin and kaempferol have also been described as efficient radical scavengers (*16*). However, their high hydrophobicity and sensitivity to external agents such as air, light, and oxidative enzymes constitute a serious problem for their bioavailability, formulation, and manipulation in the elaboration of functional foods. To avoid this limitation, the inclusion of kaempferol in cyclodextrins (CDs) has been suggested as a possible solution.

CDs are a group of naturally occurring cyclic oligosaccharides derived from starch with six, seven, or eight glucose residues linked by $\alpha(1\rightarrow4)$ glycosidic bonds in a cylinder-shaped structure and denominated α-, β- and γ-cyclodextrins, respectively. The central cavity of these molecules is hydrophobic, whereas the rims of the surrounding walls are hydrophilic. This hydrophobic cavity forms inclusion complexes with a wide range of organic and inorganic guest molecules (*17*), altering their physicochemical behavior and reducing their undesirable effects. In the pharmaceutical, cosmetics, and food industries, CDs have been used as complexing agents to increase the water solubility of various compounds, such as drugs, vitamins, and food colorants (*18−20*). It has been demonstrated that complexation can considerably increase the solubility, stability, and bioavailability of the guest molecule.

In recent years, numerous studies on flavonoid complexation in different types of CDs have been carried out (*16*, *21−25*). Many of them deal with flavonol complexation, in order to increase their aqueous solubility and stability at different working conditions (*21*, *25−28*). In those studies the effect of pH on the complexation constant between flavonols and CDs was stated.

Hydroxylated flavonols, such as myricetin, quercetin, and kaempferol, have been demonstrated to be particularly effective antioxidants in many studies (*29−31*). However, the enzymatic method has been used to determine the K_c value of only myricetin and quercetin (*25*).

In this paper, the complexation of kaempferol with three different types of CDs (β-, G_2-β-, and HP-β-CDs) at pH 9.0 was carried out. For that purpose, the effect of the complexation of kaempferol on its fluorescence intensity has been studied, and the results obtained were used to evaluate the complexation constant (K_c). Moreover, the K_c values obtained with this fluorometric method were compared with those calculated by both enzymatic and solubility methods at basic pH.

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MATERIALS AND METHODS

Highly stabilized peroxidase from horseradish (254 units/mg of solid) was obtained from Sigma (Madrid, Spain). Kaempferol and hydrogen peroxide were supplied by Aldrich (Madrid, Spain). HP-β-CDs were purchased from TCI Europe (Antwerp, Belgium). G_2-β and β-CDs were kindly supplied by Amaizo, American Maize-Products Co. (Hammond, IN). All other chemicals used were of analytical grade.

Fluorometric Method. Steady-state fluorescence measurements were performed with a Shimadzu RF 5301 PC spectrofluorometer. Emission fluorescence spectra were acquired in the 330−700 nm interval, at a fixed excitation wavelength of 351 nm. The reaction medium contained 20.96 μM kaempferol and increasing concentrations of β-, G_2-β-, and HP-β-CDs prepared in 100 mM sodium borate buffer (pH 9.0) to reach a final volume of 3 mL at 25 °C.

The fluorescence intensity at any wavelength (F) can be related to the CD concentration by the equation (*32*)

$$F = F_0 + \frac{(F_\infty - F_0)K_c[\mathrm{CDs}]_t}{1 + K_c[\mathrm{CDs}]_t} \quad (1)$$

where F_∞ is the fluorescence intensity when total kaempferol has been complexed in CDs and F_0 is the fluorescence of kaempferol in the absence of CDs. Experimental data of F as a function of [CDs] can be fitted to eq **1**, using as initial parameters (K_c and F_∞) those calculated from the analysis of the experimental data obtained using the Benesi−Hildebrand equation for 1:1 complexes (double-reciprocal plot) (*33*):

$$\frac{1}{F - F_0} = \frac{1}{F_\infty - F_0} + \frac{1}{(F_\infty - F_0)K_c[\mathrm{CDs}]_t} \quad (2)$$

The K_c value can also be calculated by Scatchard's equation for the 1:1 complex, which relates the enhanced fluorescence intensity with CD concentration (*34*):

$$\frac{F - F_0}{[\mathrm{CDs}]_t} = (F_\infty - F_0)K_c - (F - F_0)K_c \quad (3)$$

Enzymatic Method. The enzymatic oxidation of kaempferol was followed spectrophotometrically in a Shimadzu model UV-1063 at the absorption maximum of the kaempferol oxidation product ($\lambda_{315\mathrm{nm}}$, ε_{315} = 22144 M^{-1} cm^{-1}). Unless otherwise stated, the standard reaction medium at 25 °C contained 100 mM sodium borate buffer (pH 9.0), 400 μM H_2O_2, 21 μM kaempferol, 0.37 unit of horseradish peroxidase, and increasing concentrations of CDs, in a final volume of 1 mL. The steady state was calculated from the linear zone of the product accumulation curve. A reference cuvette containing all of the components of the reaction medium, except the enzyme, was used as control.

Assuming that the presence of increasing concentrations of CDs in the reaction medium gives rise to an increasing inhibitory effect on the kaempferol oxidation rate (due to the formation of a 1:1 inclusion complexes) and that free flavonol is the only form of substrate which peroxidase can use, the complexation constant (K_c) can be calculated by using the equations previously described by our group, in which a Michaelis−Menten modified equation for free substrate is used (*35*):

$$[\mathrm{flavonol}]_f + [\mathrm{CD}]_f \overset{K_c}{\leftrightarrow} [\mathrm{flavonol{-}CD}] \quad (4)$$

$$K_c = \frac{[\mathrm{flavonol{-}CD}]}{[\mathrm{flavonol}]_f[\mathrm{CD}]_f} \quad (5)$$

$$[\mathrm{flavonol}]_f = (-([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1) + \sqrt{([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1)^2 + 4K_c[\mathrm{flavonol}]_t})/2K_c \quad (6)$$

$$V = \frac{V_m[(-([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1) + \sqrt{([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1)^2 + 4K_c[\mathrm{flavonol}]_t})/2K_c]}{K_M + [(-([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1) + \sqrt{([\mathrm{CD}]_t K_c - [\mathrm{flavonol}]_t K_c + 1)^2 + 4K_c[\mathrm{flavonol}]_t})/2K_c]} \quad (7)$$

Solubility Method. Phase solubility diagrams were constructed according to the method of Higuchi and Connors (*36*). Excess amounts of kaempferol were added to aqueous solutions of increasing concentrations of β-, G_2-β-, and HP-β-CDs up to 100 mM (15 mM in the case of β-CDs, its solubility limit), in 10 mL of 100 mM sodium borate buffer (pH 9.0) at 25 °C. The samples were maintained in an ultrasonic bath for 90 min to reach equilibrium. The aqueous solutions were filtered through a 0.2 μm membrane filter and diluted in 80% ethanol−water. The kaempferol concentration was spectrophotometrically determined ($\varepsilon_{370\mathrm{nm}}^{\mathrm{EtOH}\ 80\%}$ = 22890 M^{-1} cm^{-1}).

The K_c value was calculated using the equation described by Higuchi and Connors (*36*)

$$K_c = \frac{\mathrm{slope}}{S_0(1 - \mathrm{slope})} \quad (8)$$

where S_0 is the solubility of kaempferol in 100 mM sodium borate buffer (pH 9.0).

RESULTS AND DISCUSSION

Because of the ability of CDs to form complexes, the fluorescence signal of the complexed analyte is generally quite strongly enhanced; indeed, the fluorophore entrapped in the CD's internal cavity is isolated from the surrounding water molecules, and its excited state is shielded from any quenching processes. In the present paper, the effect of different types of CDs on the fluorescence spectral properties of kaempferol has been investigated. The fluorescence spectra were recorded in sodium borate buffer (100 mM, pH 9.0) at increasing CD concentrations (**Figure 1A**). The addition of increasing concentrations of β-, G_2-β-, or HP-β-CDs to the reaction medium did not lead to changes in the maximum excitation and emission wavelengths (data not shown). However, in all cases increasing CD concentrations resulted in a corresponding decrease in the fluorescence signal (**Figure 1**), the relative magnitude of this decrease being dependent on the type of CD employed (**Figure 1B**). This is the first time that a reduction in the fluorescence signal has been observed as result of analyte complexation by CDs, in contrast to the increase described for the complexation of other compounds by CDs (*37−41*). As can be seen from **Figure 1**, HP-β-CDs showed a more pronounced fluorescence decrease (**Figure 1B**), probably due to a greater propensity to form inclusion complexes with kaempferol. The reduction in fluorescence was 3.5-, 2.6-, and 2.4-fold for HP-β-, β-, and G_2-β-CDs, respectively.

The effect of increasing the CD concentration was to gradually reduce the fluorescence of kaempferol, in contrast to the behavior generally observed for other organic compounds (*25*,*41−45*). For HP-β-CDs, the emission intensity decreased with increasing CD concentration to reach a value of 2 mM, after which it remained constant (**Figure 1B**). In the case of β- and G_2-β-CDs, the plateau was reached at a CD concentration of around 5 mM (**Figure 1B**). In all CD types studied, the plateau indicated the maximum level of complexation of kaempferol by CDs. The lowest plateau value was observed in the case of HP-β-CDs, indicating the higher affinity of this type of CD to complex kaempferol.

The stoichiometry of the inclusion complexes formed between kaempferol and the types of CDs studied and the magnitude of the corresponding complexation constants (K_c) are derived from experimental data analysis.

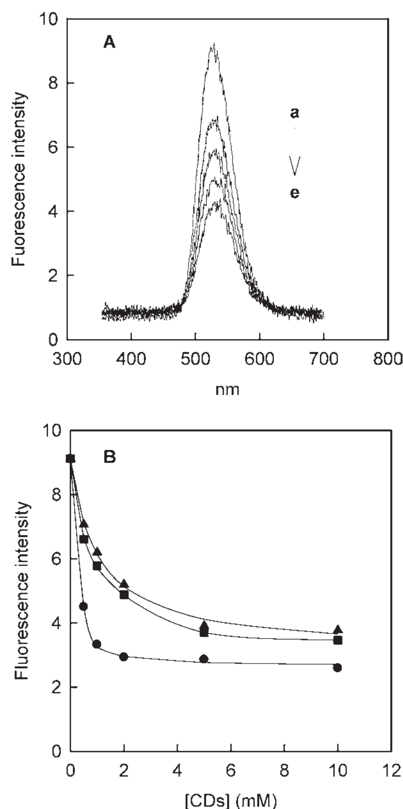

Figure 1. (**A**) Fluorescence spectra of kaempferol with various concentrations of β-CDs λ_{exi} 351 nm: (a) 0, (b) 0.5, (c) 1, (d) 2, and (e) 5 mM β-CDs. (**B**) Fluorescence intensities of kaempferol at 539 nm versus different concentrations of CDs: (●) HP-β-CDs, (■) β-CDs, and (▲) G_2-β-CDs. The lines show the best fits to eq 1.

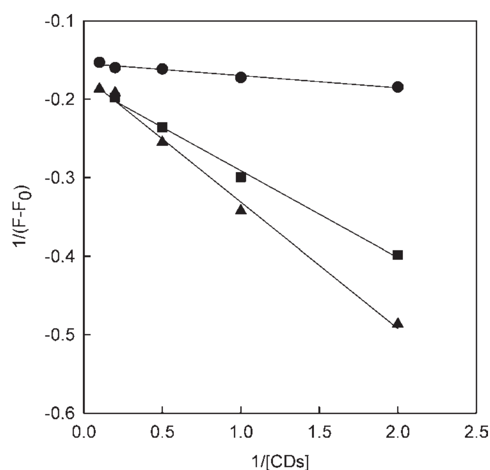

Figure 2. Benesi−Hildebrand plot for kaempferol-CDs: (●) HP-β-CDs, (■) β-CDs, and (▲) G_2-β-CDs.

The representation of $1/(F - F_0)$ versus 1/[CDs] (double-reciprocal plot), known as the Benesi−Hildebrand plot (**Figure 2**), provides a straight line for the three types of CDs used, indicating a 1:1 stoichiometry of the complexes in all cases. The negative values obtained for the slope and intercepts in the case of kaempferol were due to the decrease in the fluorescence intensity as the CD concentration increased. This linear relationship agrees with that described by the Benesi−Hildebrand equation (eq **2**). The linear plots of **Figure 2** can be used to determine K_c values by simply dividing the intercepts by the slopes (**Table 1**).

Table 1. Complexation Constant (K_c) Calculated by the Enzymatic, Solubility, and Fluorometric Methods

method	K_c β-CDs (M^{-1})	K_c G_2-β-CDs (M^{-1})	K_c HP-β-CDs (M^{-1})
fluorometric			
Benisi−Hildebrand	1622 ± 259	1056 ± 120	9872 ± 332
Scatchard	1555 ± 268	998 ± 109	9367 ± 325
enzymatic	2670 ± 245	2325 ± 312	11848 ± 258
solubility	2747 ± 210	2508 ± 150	11161 ± 302

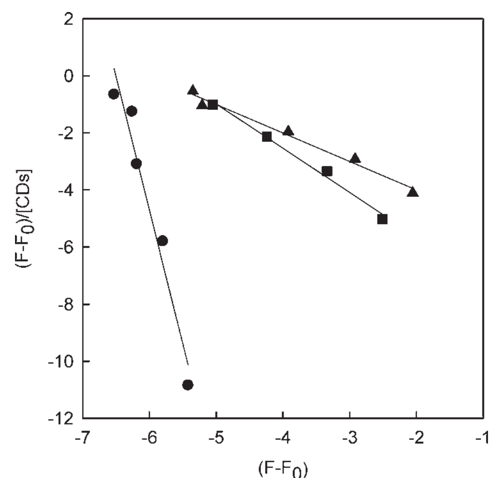

Figure 3. Scatchard plot for kaempferol−CDs: (●) HP-β-CDs, (■) β-CDs, and (▲) G_2-β-CDs.

Because Benesi−Hildebrand plots tend to place more emphasis on low CD concentrations than on higher values, the slope of the line is more sensitive to the ordinate values of the points for the lowest concentrations. The K_c values obtained for each type of CD are presented in **Table 1**. As can be deduced from the fluorescence data, the highest K_c value was obtained for HP-β-CDs (9872 ± 332 M^{-1}), whereas the native β- and modified G_2-β-CDs showed similar K_c values (1622 ± 259 and 1056 ± 120 M^{-1}, respectively), but much lower than that obtained for HP-β-CDs. These results contrast with the data obtained for other phenolic compounds, in which all modified β-CDs studied presented higher K_c values than native β-CDs (*25*, *41*).

To better estimate the binding constants, the nonlinear regression analysis (NLR) method was used, fitting the observed fluorescence intensity versus CD concentration (**Figure 1B**) to eq **1** and using the estimated K_c and F_∞ values obtained from the Benesi−Hildebrand plots as the initial parameters. **Figure 1B** shows the excellent convergence between the calculated and observed F values.

The fluorescence data (**Figure 1B**) were also used to determine the K_c values between kaempferol and CDs using Scatchard's method (*34*). In this case, when 1:1 complexes are formed, the relationship between the enhancement in fluorescence intensity $(F - F_0)$ and CD concentration is given by eq **3**. Replotting the data of **Figure 1B** as $(F - F_0)$/[CDs] versus $(F - F_0)$ (**Figure 3**) gave straight lines, confirming the 1:1 complex formation between kaempferol and the three studied types of CDs. The K_c values given by the slope of the plots (**Table 1**) were similar to those obtained using the Benesi−Hildebrand plot, corroborating that HP-β-CDs are the best type of CD for kaempferol complexation.

To establish the validity of the fluorometric method for the determination of K_c and to confirm the decrease in fluorescence observed after the complexation step, an enzymatic method was employed to calculate the complexation constant between

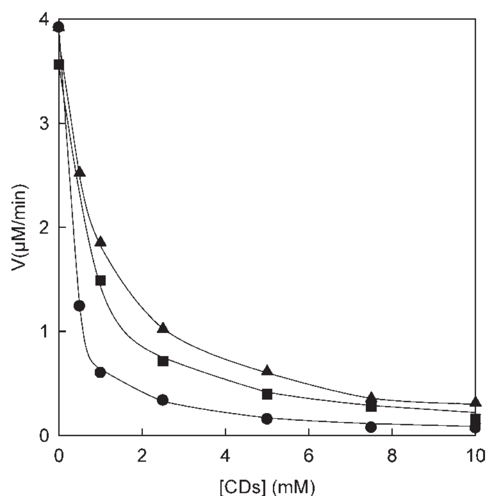

Figure 4. Effect of CDs on the oxidation of kaempferol by horseradish peroxidase in the presence of H_2O_2: (●) HP-β-CDs, (■) β-CDs, and (▲) G_2-β-CDs. The reaction medium at 25 °C contained 100 mM sodium borate buffer (pH 9.0), 20.96 μM kaempferol, 400 μM H_2O_2, 0.37 unit of horseradish peroxidase, and increasing concentrations of CDs.

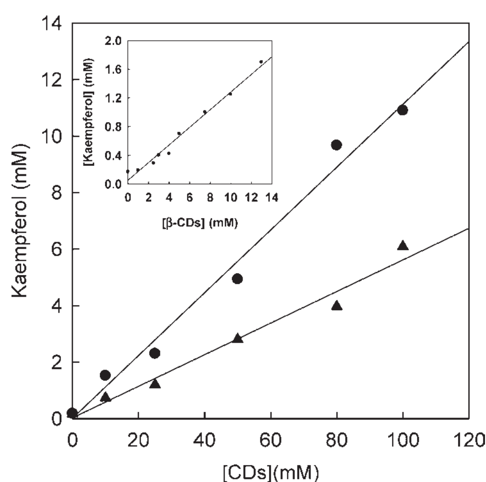

Figure 5. Phase solubility diagram of kaempferol with (●) HP-β-CDs and (▲) G_2-β-CDs in sodium borate buffer (100 mM, pH 9.0) at 25 °C. (Inset) Phase solubility diagram of kaempferol with (■) β-CDs in sodium borate buffer (100 mM, pH 9.0) at 25 °C.

kaempferol and CDs, using horseradish peroxidase and the H_2O_2 system.

The oxidation of kaempferol by horseradish peroxidase in the presence of H_2O_2 at pH 9.0 led to the formation of an oxidation product with a maximum at 315 nm (data not shown). In the presence of increasing concentrations of CDs, the oxidation rate of kaempferol by peroxidase decreased, indicating that kaempferol was complexed in the hydrophobic cavity of CDs, free kaempferol being the only form which peroxidase could use as substrate (**Figure 4**). This result was observed whether β-, G_2-β-, or HP-β-CDs were used, although the inhibition was higher in the case of HP-β-CDs (**Figure 4**, solid circles). Fitting points in **Figure 4** to eq **7**, K_c values for β-, G_2-β-, and HP-β-CDs were calculated, giving the following values: 11848 ± 258 M^{-1} for HP-β-CDs, 2325 ± 312 M^{-1} for G_2-β-CDs, and 2672 ± 245 M^{-1} for β-CDs (**Table 1**). It is important to note that HP-β-CDs showed the highest K_c values, indicating that they are the most efficient in the formation of inclusion complexes with kaempferol, as occurs in the case of the other flavonoids, myricetin or quercetin (*25*), and in the case of the stilbene resveratrol (*41*).

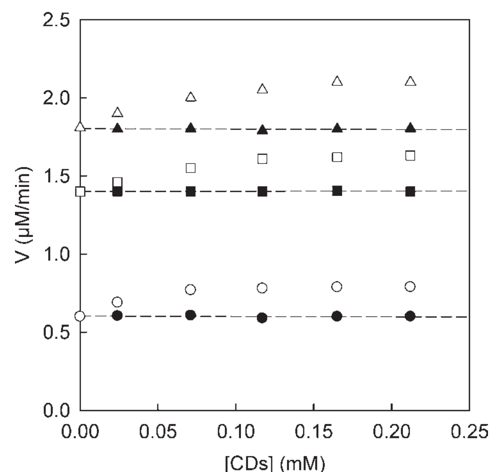

Figure 6. Cyclodextrin assay. The CD and kaempferol total concentrations used in each case were calculated to obtain the free kaempferol concentrations, by using the K_c values obtained with the enzymatic method and eq 6 ((●) 1.6 μM free kaempferol and increasing concentrations of HP-β-CDs; (■) 5.7 μM free kaempferol and increasing concentrations of β-CDs; (▲) 6.3 μM free kaempferol and increasing concentrations of G_2-β-CDs) and the fluorometric method ((○) 1.6 μM free kaempferol and increasing concentrations of HP-β-CDs; (□) 5.7 μM free kaempferol and increasing concentrations of β-CDs; (△) 6.3 μM free kaempferol and increasing concentrations of G_2-β-CDs). The oxidation of free kaempferol was followed in 100 mM sodium borate buffer (pH 9.0), using 0.37 unit of horseradish peroxidase.

These two methods were also compared with the solubility one described by Higuchi and Connors (*36*).

Phase solubility diagrams of kaempferol (pH 9.0) with β-, G_2-β-, or HP-β-CDs are shown in **Figure 5**. In all cases phase solubility diagrams showed a linear relationship between the amount of kaempferol solubilized and the concentration of cyclodextrin in solution (A_L type), indicating that the stoichiometry of complexes was 1:1 in all cases. The K_c values obtained by using this method are similar to those obtained in the enzymatic method (**Table 1**).

By comparison of the K_c values obtained for the complexation of kaempferol by β-, G_2-β-, and HP-β-CDs using the enzymatic, solubility, and fluorometric methods (**Table 1**), results showed that the enzymatic and solubility methods provided higher K_c values for all of the CDs studied than fluorometric one.

To determine which method is more accurate (enzymatic/solubility or fluorometric), the so-called CD assay (*35*, *41*, *46*), in which the total CDs and kaempferol concentrations are adjusted so that the free kaempferol concentration remains constant, was carried out for the all CDs studied. Using the K_c values determined enzymatically, by solubility studies or fluorometrically and eq **6**, the corresponding CD assay was performed. It was expected that when the concentration of effective free kaempferol remained constant, the rate of kaempferol oxidation by horseradish peroxidase (μM/min) could also be constant, regardless of the total kaempferol concentration. As shown in **Figure 6**, the results obtained using K_c values (solid symbols) determined enzymatically or by solubility studies were as expected (the enzymatic activity was independent of total CDs and kaempferol concentrations), but the result obtained using the fluorometrically determined K_c values (open symbols) deviated from expected results (the enzymatic activity did not remain constant), indicating that the free kaempferol concentration is not

the same in all cases; therefore, the K_c values used are not correct. Thus, the differences in the K_c values observed between the enzymatic/solubility and fluorometric methods (**Table 1**) are very important for knowing the real effective kaempferol concentration. We conclude that if fluorescence decay of kaempferol is used, the K_c will be undervalued and the free kaempferol concentration will be higher than expected, which could explain the increase in horseradish peroxidase activity observed in **Figure 6**. These results agree with those obtained for the complexation of resveratrol (*41*).

In conclusion, kaempferol fluorescence decreases when it is complexed in the hydrophobic cavity of CDs. This fluorescence decrease cannot be used to determine the K_c between kaempferol and CDs because it is underestimated. Therefore, in the case of kaempferol and other compounds that can be oxidized by different enzymes, the best method for calculating its K_c is the enzymatic one, because of the high specificity of the enzymes, which act only against free substrate. When the enzymatic method could not be used, the solubility study is an optional method to calculate K_c values. In addition, HP-β-CDs are the most effective CDs for complexing kaempferol, regardless of the K_c calculation method used.

LITERATURE CITED

(1) Herrmann, K. Flavonols and flavones in food plants: a review. *J. Food Technol.* **1976**, *11*, 433–448.

(2) Justesen, U.; Knuthsen, P.; Teth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *J. Chromatogr., A* **1998**, *799*, 101–110.

(3) Hertog, M. G. L.; Katan, M. B. Quercetin in food, cardiovascular disease, and cancer. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Dekker: New York, 1998; pp 447−467.

(4) Rice-Evans, C. A.; Miller, N. J. Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Dekker: New York, 1998; pp 199−219.

(5) Strube, M.; Dragsted, L. O.; Larsen, J. C. *Naturally Occurring Antitumourigens. I. Plant Phenols*; Nordiske Seminar-og Arbejdsrapporter 605; Nordic Council of Ministers: Copenhagen, Denmark, 1993.

(6) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **1993**, *342*, 1007–1011.

(7) Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **1995**, *155*, 381–386.

(8) Hertog, M. G. L.; Sweetnam, P. M.; Fehily, A. M.; Elwood, P. C.; Kromhout, D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphily study. *Am. J. Clin. Nutr.* **1997**, *65*, 489–494.

(9) Knekt, P.; Järvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478–481.

(10) Keli, S. O.; Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch. Intern. Med.* **1996**, *156*, 637–642.

(11) Knekt, P.; Järvinen, R.; Seppänen, R.; Hellövaara, M.; Teppo, L.; Pukkala, E.; Aromaa, A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. *Am. J. Epidemiol.* **1997**, *146*, 223–230.

(12) Baumann, J.; Bruchhausen, F. V.; Wurm, G. Flavonoids and related compounds as inhibitors of arachidonic acid peroxidation. *Prostaglandin* **1980**, *20*, 627–639.

(13) Laughton, M. J.; Evans, P. J.; Moroney, M. A.; Hoult, J. R. S. Inhibition of mammalian 5-lipoxygenase and cyclo-oxigenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem. Pharmacol.* **1991**, *42*, 1673–1681.

(14) Smith, T. J.; Yang, C. S. Effect of food phytochemical on metabolism and tumorigenesis. *Food Phytochemicals For Cancer Prevention I*; Huang, M. T., Ed.; American Chemical Society: Washington, DC, 1994; p 48.

(15) Maccarrone, M.; Catani, M. V.; Iraci, S.; Melino, G.; Agro, A. F. A survey of reactive oxigen species and their role in dermatology. *J. Eur. Acad. Dermatol. Venereol.* **1997**, *8*, 185–202.

(16) Mercader-Ros, M. T.; Lucas-Abellán, C.; Fortea, M. I.; Gabaldón, J. A.; Núñez-Delicado, E. Effect of HP-β-cyclodextrins complexation on the antioxidant activity of flavonols. *Food Chem.* **2009**, *118*, 769–773.

(17) Cai, Y.; Gaffney, S. H.; Lilley, T. H.; Magnolato, D.; Martin, R.; Spencer, C. M.; Haslam, E. Polyphenol interactions, part 4: model studies with caffeine and cyclodextrins. *J. Chem. Soc., Perkin Trans. 2* **1990**, 2197–2209.

(18) Loftsson, T.; Brewster, M. E. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. *J. Pharm. Sci.* **1996**, *85*, 1017–1025.

(19) Buschmann, H. J.; Schollmayer, E. Application of cyclodextrins in cosmetic products: a review. *J. Cosmet. Sci.* **2002**, *53*, 185–191.

(20) Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic: Dordrecht, The Netherlands, 1988.

(21) Bergonzi, M. C.; Bilia, A. R.; Di Bari, L.; Mazzi, G.; Vincieri, F. F. Studies on the interactions between some flavonols and cyclodextrins. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5744–5748.

(22) Calabrò, M. L.; Tommasini, S.; Donato, P.; Raneri, D.; Stancanelli, R.; Ficarra, P.; Ficarra, R.; Costa, C.; Catania, S.; Rustichelli, C.; Gamberini, G. Effects of α- and β-cyclodextrin complexation on the physico-chemical properties and antioxidant activity of some 3-hydroxyflavones. *J. Pharm. Biomed. Anal.* **2004**, *35*, 365–377.

(23) Jullian, C.; Moyano, L.; Yañez, C.; Olea-Azar, C. Complexation of quercetin with three kinds of cyclodextrins: an antioxidant study. *Spectrochim. Acta, Part A: Mol. Biomol. Spectrosc.* **2007**, *67*, 230–234.

(24) Tommasini, S.; Raneri, D.; Ficarra, R.; Calabró, M. L.; Stancanelli, R. J.; Ficarra, P. Improvement in solubility and dissolution rate of flavonoids by complexation with β-cyclodextrin. *Pharm. Biomed. Anal.* **2004**, *35*, 379–387.

(25) Lucas-Abellán, C.; Fortea, I.; Gabaldón, J. A.; Núñez-Delicado, E. Encapsulation of quercetin and myricetin in cyclodextrins at acidic pH. *J. Agric. Food Chem.* **2008**, *56*, 255–259.

(26) Wang, Y.; Oiao, X.; Li, W.; Zhou, Y.; Jiao, Y.; Yang, C.; Dong, C.; Inoue, Y.; Shuang, S. Study on the complexeation wiyh β-cyclodextrins and its derivatives by spectroscopy. *Anal. Chim. Act.* **2009**, *650*, 124–130.

(27) Pralhad, T.; Rajendrakumar, K. Study of freeze-dried quercetin-cyclodextrins binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis. *Pharm. Biomed. Anal.* **2004**, *34*, 333–339.

(28) Dias, K.; Nikolaou, S.; De Giovani, W. F. Synthesis and spectral investigation of Al(III) catechin/β-cyclodextrins inclusion compounds. *Spectrochim. Acta, Part A* **2008**, *70*, 154–161.

(29) Cao, G.; Sofic, E.; Prior, R. Antioxidant and prooxidant behaviour of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **2007**, *22*, 749–760.

(30) Soobrattee, M. A.; Neergheen, V. S.; Luximon-Ramma, A.; Aruoma, O. I.; Bahorum, T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat. Res.* **2005**, *579*, 200–213.

(31) Wojdylo, A.; Oszmianski, J.; Czemerys, R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* **2007**, *105*, 940–949.

(32) Connors, K. A. *Binding Constants*; Wiley: New York, 1987; pp 103−108.

(33) Benesi, H. A.; Hildebrand, J. H. A spectrophotometric investigation on the interaction of iodine with aromatic hydrocarbons. *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707.

(34) Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **1949**.
(35) Lucas-Abellán, C.; Fortea, M. I.; López-Nicolas, J. M.; Núñez-Delicado, E. Cyclodextrins as resveratrol carrier system. *Food Chem.* **2007**, *104*, 39–44.
(36) Higuchi, T.; Connors, K. A. Phase solubility techniques. *Adv. Anal. Chem. Instrum.* **1965**, *4*, 56–63.
(37) Álvarez-Parrilla, E.; De la Rosa, L.; Torres-Rivas, F.; Rodrigo-García, J.; González-Aguilar, G. A. Complexation of apple antioxidants: chlorogenic acid, quercetin and rutin by β-cyclodextrins (β-CDs). *J. Incl. Phenom. Macromol. Chem.* **2005**, *53*, 11–129.
(38) Mahedero, M. C.; Muñoz de la Peña, A.; Bautista, A.; Aaron, J. J. An investigation of inclusion complexes of cyclodextrins with phenylurea herbicides by photochemically induced fluorescence. Analytical applications. *J. Incl. Phenom. Macromol. Chem.* **2002**, *42*, 61–70.
(39) Wagner, B. D.; Fitzpatrick, S. J. A comparison of the host-guest inclusion complexes of 1,8-ANS and 2,6-ANS in parent and modified cyclodextrins. *J. Incl. Phenom. Macromol. Chem.* **2000**, *38*, 467–478.
(40) Muñoz de la Peña, A.; Mora-Diez, N.; Mahedero-García, M. C.; Bohoyo-Gil, D.; Cañada-Cañada, F. A chemometric sensor for determining sulphaguanidin residues in honey simples. *Talanta* **2007**, *73*, 304–313.
(41) Lucas-Abellán, C.; Fortea, M. I.; Gabaldón, J. A.; Núñez-Delicado, E. Complexation of resveratrol by native and modified cyclodextrins: determination of complexation constant by enzymatic, solubility and fluorimetric assays. *Food Chem.* **2008**, *111*, 262–267.
(42) Marquez, J. C.; Hernadez, M.; Garcia-Sanchez, F. Enhanced spectrofluorimetric determination of the pesticide warfarin by means of the inclusion complex with β-cyclodextrin. *Analyst* **1990**, *115*, 1003–1005.
(43) Muñoz de la Peña, A.; Salanas, F.; Gomez, M. J.; Acedo, M. I.; Sanchez-Pena, M. Absorptiometric and spectrofluorimetric study of the inclusion complexes of 2-naphthyloxyacetic acid and 1-naphthylacetic acid with β-cyclodextrin in aqueous solution. *J. Inclus. Phenom. Mol. Recognit. Chem.* **1993**, *15*, 131–143.
(44) Frankewich, R. P.; Thimmaiah, K. N.; Hinze, W. L. Evaluation of the relative effectiveness of different water-soluble β-cyclodextrin media to function as fluorescence enhancement agents. *Anal. Chem.* **1991**, *63*, 2924–2933.
(45) Maafi, M.; Laassis, B.; Aaron, J. J.; Mahedero, M. C.; Muñoz de la Peña, A.; Salinas, F. Photochemically induced fluorescence investigation of α-cyclodextrin: Azure A inclusion complex and determination of analytical parameters. *J. Inclus. Phenom. Mol. Recognit. Chem.* **1995**, *22*, 235–247.
(46) López-Nicolás, J. M.; Bru, R.; García-Carmona, F. Enzymatic oxidation of linoleic acid by lipoxygenase forming inclusion complexes with cyclodextrins as starch model molecules. *J. Agric. Food Chem.* **1997**, *45*, 1144–1148.

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