

Determination of Complexation Parameters for β -Cyclodextrin and Randomly Methylated β -Cyclodextrin Inclusion Complexes of *p*-Cumalic Acid Using Reversed-Phase Liquid Chromatography

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Abstract

p-cumalic acid (PCA) i.e. hydroxycinnamic acid is a one of the major component of lignin. PCA can be found in a wide variety of edible plants including peanuts, beans, tomatoes, carrots, garlic etc. It is also found in wine and vinegar. PCA has strong antioxidant properties and is known to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines. According to that the main goal of present paper is to establish the basic properties of PCA complexes with some cyclodextrin. Host-guest complexation with cyclodextrins may be interesting to increase the bioavailability of PCA in functional food product. During the research an on-line complexation of *p*-cumalic acid with β -cyclodextrin (CD) and Randomly Methylated β -cyclodextrin (RMCD) was performed using RP-HPLC system. Due to the complex formation of CD with a guest molecule - PCA a significant changes in retention times of PCA was observed. The investigation was performed as a function of temperature as well as cyclodextrin concentration in liquid phase. As a result apparent formation constant – KF of PCA/CD and PCA/RMCD were calculated. Based on KF some thermodynamic properties were obtained including standard enthalpy, standard entropy and free Gibbs energy of host-guest complexation. It was shown that the stoichiometry of the complex in both cases is 1:1 and the complexation is an exothermic process. From the other hand the positive value of Gibbs free energy clearly designate the process as forced one. The increase in temperature influences on Gibbs free energy by means of increase of this parameter. Additionally the differences between CD and RMCD as hosting agents were shown.

Keywords

cyclodextrin, complexation, HPLC, thermodynamics, apparent formation constant

Introduction

Derivatives cinnamic acid (including the *p*-cumalic acid - PCA) are the most widely phenolic acid in plants. They occur in several forms as glycosides, depsydes as well as a free form.

PCA presented in the cereal grains is associated with arabinoxylans molecules, while in fruits and vegetables as esters of quinic acid and glucose³.

PCA (3-[4-hydroxyphenyl]-2-propenoic acid) is a plant metabolite with antioxidant, antimutagenic, anti-inflammatory, antimicrobial and other biological and pharmacological properties⁸. Natural sources of this compound are coffee, beans, fruits, vegetables, sunflower, beverages and aromatic herbs¹. The acid is a crucial substrate for enzymes producing resveratrol. In grape juice, the PCA can reach a concentration of 60 mg/L⁶.

A small amount of *p*-coumaric acid is esterified to arabinoxylans early in primary wall development in much the same way as ferulic acid, but later, during wall development, PCA is found more extensively esterified to lignin. The role of such high quantities of *p*-coumaric esters in maize lignin remains unexplained, but is probably involved in defense mechanism⁷.

CDs (cyclodextrins) are cyclic oligosaccharides derived from starch containing six (α CD), seven (β CD), eight (γ CD), nine (δ CD), ten (ϵ CD) or more (α -1,4)-linked α -D-glucopyranose units. Due to the chair conformation of the glucopyranose units, the CDs take the shape of a truncated cone or torus rather than a perfect cylinder².

The CDs have the shape of a truncated cone with interior cavity hydrophobic that allows the formation of inclusion complexes with hydrophobic guest substance, whereas the outer surface is hydrophilic, which makes the CDs soluble in water⁴. In recent years, HPLC has been increasingly used for observing and characterizing CD-guest inclusion complexes⁵.

On the other hand no information may be found in literature on PCA cyclodextrin complexation process. Due to the importance of PCA in food industry as well as pharmacy the increase in water solubility of this compound in water looks as an important task for modern organic chemistry.

Experimental

β -cyclodextrin used in this experiments was purchased from Roquette (France), methylated β -cyclodextrin was purchased from Wacker (Germany). *p*-cumaric acid was supplied from Aldrich (France). Methanol was purchased from Chempur (Poland).

All experiments were performed using HPLC chromatograph (Knauer, Germany) equipped with UVVIS spectrophotometric detector operating at 320nm. For experiments the mobile-phase flow-rate was set at 1 mL/min. Mobile phases were prepared according to the following procedure. Weighed amount of CD was added to the 500 mL of binary mixture [H₂O/MeOH/CH₃COOH 88:10:2 (v/v)] in a 1000 mL volumetric flask. When all amount of CD was dissolved, the remaining amount of solvent was added to reach a final volume of 1000 mL.

The final concentration of cyclodextrin was 0.0, 1.0, 2.5, 3.5, 5.0, 7.0, 10.0 mM. The effect of the temperature on the complexation process of PCA with β -CD and methylated β -CD was studied from 30 to 60 °C. 20 microliters *p*-cumaric acid (prepared by dissolved 0,01 mg in methanol) were injected at the HPLC column. Reversed-phase column C18 (Knauer, Germany) was used.

To determine the K_F values for the formed complex the following relationship was used:

$$\frac{1}{k} = \frac{1}{k_0} + \frac{K_F}{k_0} [CD]$$

where: k is the capacity factor of the solute, k_0 capacity factor in the absence CD, K_F is the formation constant of the inclusion complex.

To study thermodynamics parameters was used thermodynamic relationships:

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

where:

R- universal gas constant;

T- temperature;

K_F - formation constant of the inclusion complex;

ΔH° - standard enthalpy changes of formed complex;

ΔS° - standard entropy changes of formed complex.

To determine the free Gibbs energy changes was used:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

where:

ΔG° - Gibbs free energy change which takes place during the complex was formed⁵.

Results and discussion:

The formation of complex between *p*-cumaric acid and β -cyclodextrins has been confirmed using RP-HPLC (Reversed-Phase High Performance Liquid Chromatography). To determine the thermodynamic parameters retention time of the formed complex and retention time the blind peak were needed.

Complexation constant strongly depends of the organic modifier. For this reason experiments started on selection of the best organic modifier in mobile phase. The study focused on two solvents: methanol and acetonitrile. Selection was based on reports in the literature. The reason for choosing the methanol as a solvent was weak associations with cyclodextrin cavity, represented by the low value of K_m which was presented by Matsui and Mochida⁵.

It is also important to establish the appropriate concentration of the modifier in the eluent. It allows determining its effect on the formation of the complex and the retention time. Several authors reported that methanol concentrations higher than 30% in eluent reduce the inclusion process. Furthermore, the higher water concentration in eluent produced long retention time (Fig. 1), with the associated experimental error. According to the above mentioned information, it was decided to choose water: methanol eluent in ratio of 90:10 (v/v).

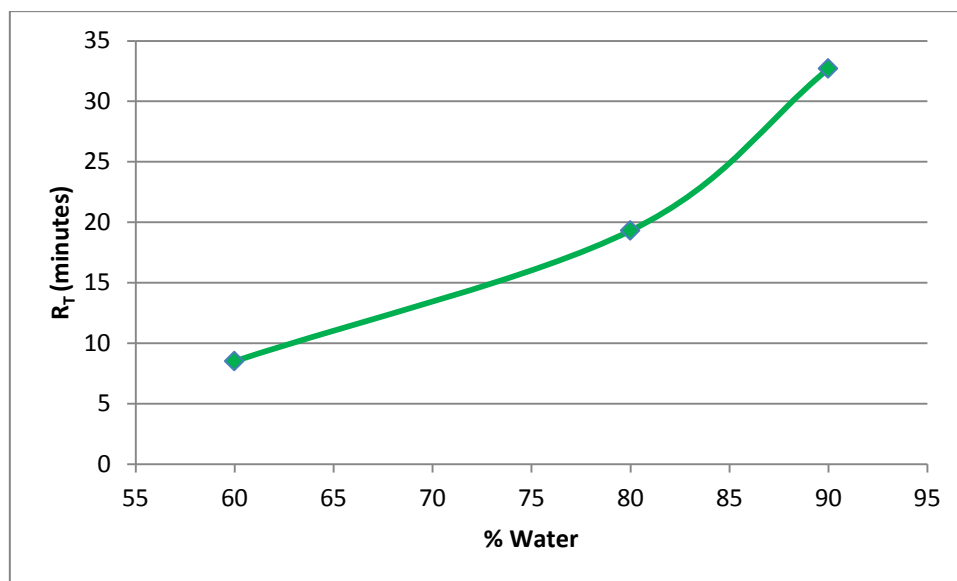


Fig. 1. Effect of water percentage on the retention time of PCA (0,001 mg/mL) in different methanol:water mobile phase with 10 mM β -CD in the mobile phase.

For the chosen eluent with the volume ratio 90:10 (water: methanol) fronting peaks (Fig. 2) were obtained chromatogram with. The addition of acetic acid (2% of the total volume of eluent) allowed for stabilization of the system and to obtain the correct chromatographic separation. Better symmetry of the peaks is due to decrease the acidity of surface $-OH$ methanol groups caused by the addition the acetic acid. This allows reducing the interaction with the polar groups complexed compound (Fig. 3). In addition, the acetic acid caused an increase of retention time of the complex PCA/ β -CD. For the following reasons the three component system water: methanol: acetic acid 88:10:2 (v/v) was used during the study because of the smallest possible effect the modifier on the resulting complexation process.

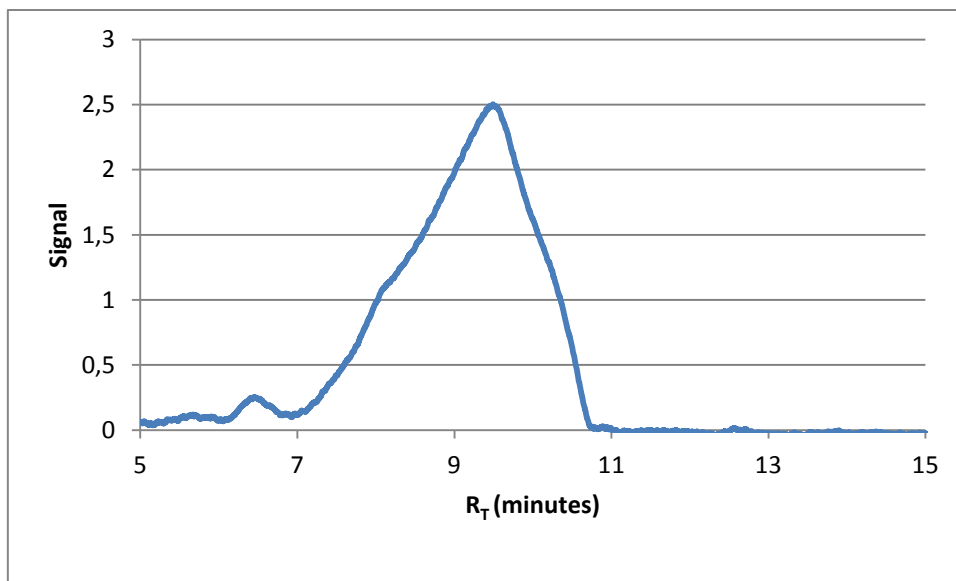


Fig. 2. Retention time of PCA (10 mg/mL) with 10 mM β -CD in the mobile phase without the acetic acid in mobile phase, 50 °C.

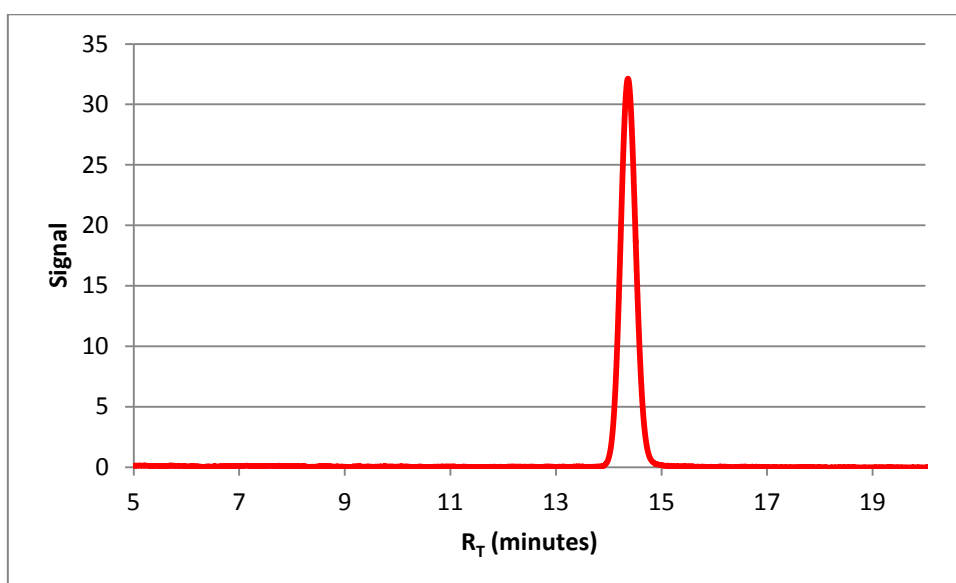


Fig. 3. Retention time of PCA (10 mg/mL) with 10 mM β -CD in the mobile phase with the acetic acid in mobile phase, 50 °C.

Because this is the first study where the interaction of PCA with β -CD and RMCD is studied, it was necessary to investigate the stoichiometry of this complex. In our study, a plot of $1/k$ versus $[\text{CDs}]$ gave a straight line with a linear correlation higher than 0.99, indicating that the presumed stoichiometry of the PCA/CDs complexes formed was 1:1 (Fig. 4 and Fig. 5). On the other hand, when $1/k$ was plotted against $([\text{CD}])^2$, a linear relationship was obtained (linear correlation of 0.96) which indicates that the stoichiometry of the inclusion complex is 1:2 (Fig. 6

and Fig. 7). But in this case the nature of the chosen stoichiometry of the reaction ability of the logarithm designates the complexation constant necessary for calculating the thermodynamic parameters. In addition the negative value for K_{I_2} stoichiometry 1:2 has no chemical sense.

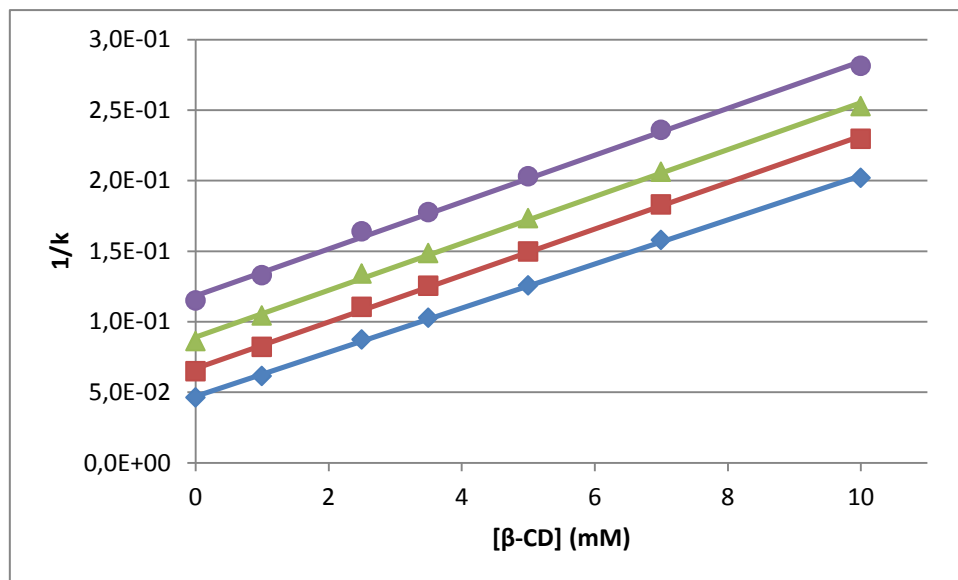


Fig. 4. $1/k$ versus $[\beta\text{-CD}]$ in tested temperatures. (\blacklozenge) 30°C; (\blacksquare) 40°C; (\blacktriangle) 50°C; (\bullet) 60°C.

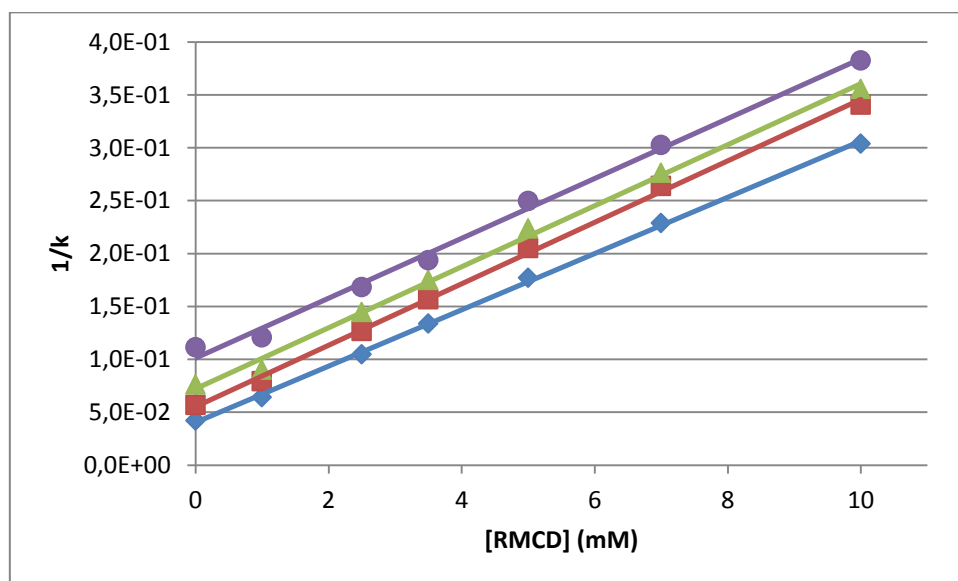


Fig. 5. $1/k$ versus $[\text{RMCD}]$ in tested temperatures. (\blacklozenge) 30°C; (\blacksquare) 40°C; (\blacktriangle) 50°C; (\bullet) 60°C.

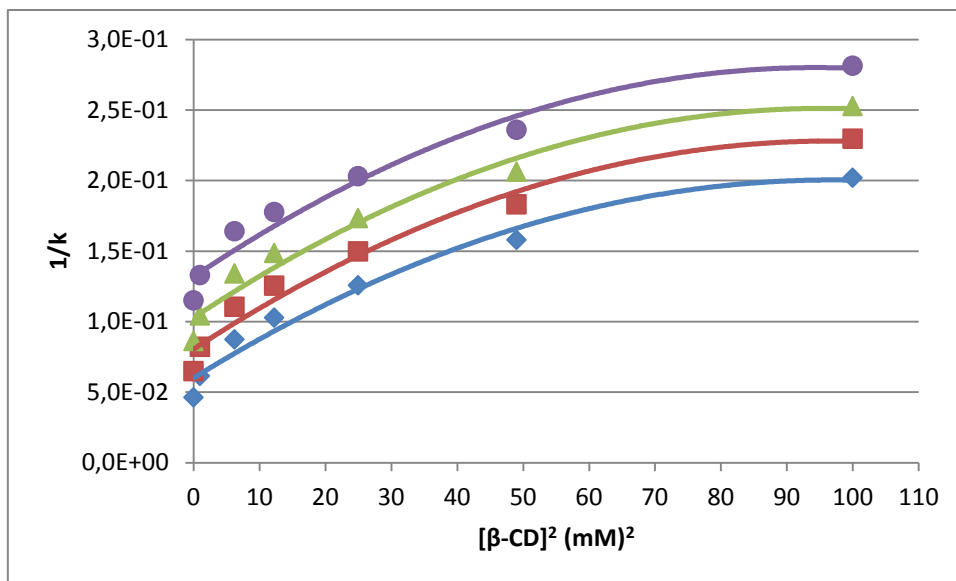


Fig. 6. $1/k$ versus $[\beta\text{-CD}]^2$ in tested temperatures. (♦) 30°C; (■) 40°C; (▲) 50°C; (●) 60°C.

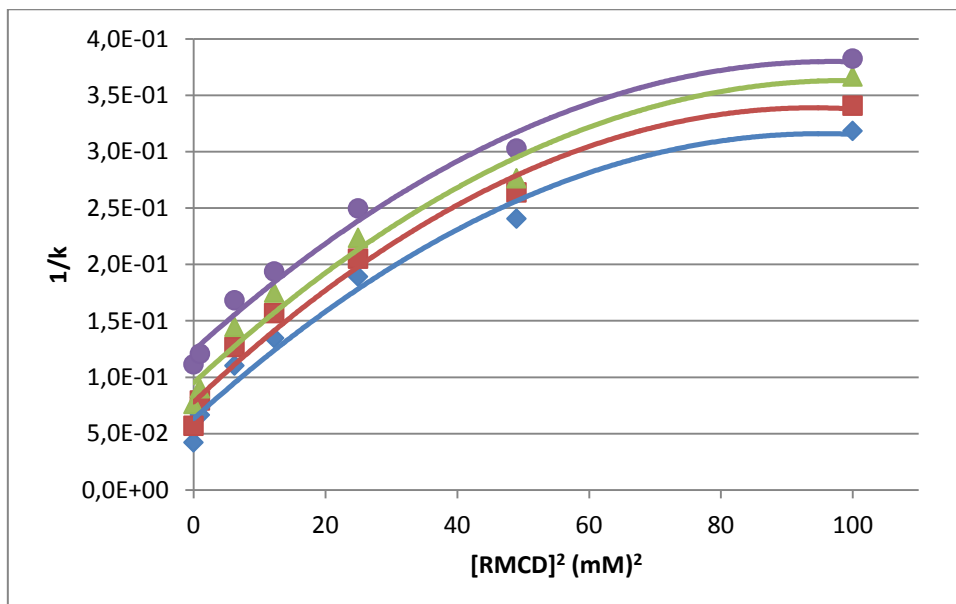


Fig. 7. $1/k$ versus $[\text{RMCD}]^2$ in tested temperatures. (♦) 30°C; (■) 40°C; (▲) 50°C; (●) 60°C.

The values of K_F and for complex PCA/cyclodextrin ($\beta\text{-CD}$, RMCD) are presented in Table 1.

Table 1. Values of K_f for complex PCA/cyclodextrin at different temperature.

| Degree [°C] | K_f [mM ⁻¹] | |
|-------------|---------------------------|--------|
| | β -CD | RMCD |
| 30 | 0,0464 | 0,0728 |
| 40 | 0,0491 | 0,0813 |
| 50 | 0,0481 | 0,0799 |
| 60 | 0,0470 | 0,0792 |

Next step was to study the thermodynamic parameters of the complexation process. According to that $\ln(K_f)$ versus $1/T$ was plotted. In case of both cyclodextrins a linear relationship was observed.

Values ΔS° (entropy change), ΔH° (enthalpy change) and ΔG (free Gibbs energy change) were calculated by the above mentioned equations, assuming that the slope is equal to $\Delta H^\circ/R$ and abscissa ΔS° . Enthalpy change allows establishing that the process is endo- or exothermic. Table 3 presents the calculated values of thermodynamic parameters.

Table 3. Values of thermodynamic parameters for the PCA/CD and PCA/RMCD complexes.

| CD | ΔS° [J·mol ⁻¹ ·K ⁻¹] | ΔH° [kJ·mol ⁻¹] | ΔG [kJ·mol ⁻¹] | | | |
|-------------|---|---|------------------------------------|-------|-------|-------|
| | | | 30 °C | 40 °C | 50 °C | 60 °C |
| β -CD | -31,05 | -18,797 | 75,30 | 78,40 | 81,51 | 84,61 |
| RMCD | -24,60 | -11,673 | 62,87 | 65,33 | 67,79 | 70,25 |

Conclusion:

The obtain K_f values and thermodynamic parameters allows to submit some conclusion:

1. in both cases the complexes of PCA and investigated cyclodextrin are formed with 1:1 stoichiometry,
2. the negative values obtained for enthalpy changes indicate the exothermic nature of the integration processes of PCA with β -CD,
3. increase in the temperature of the system leads to an increase in the Gibbs free energy changes,

4. the process isn't spontaneous, as seen for positive value obtain for the Gibbs free energy changes.
5. HPLC is a suitable tool for investigation of cyclodextrin complexation processes in broad range of temperature, however care should be taken in case in which organic eluents are in used.

All this conclusion come down to the single application that the complexation process depends for many factors e.g. temperature, pH, type of eluent, type of organic modifier, type of cyclodextrin.

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