FLUORESCENCE STUDY OF THE ENCAPSULATION OF STILBENOIDS BY CYCLODEXTRINS

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INTRODUCTION

Pterostilbene is a naturally occurring phytoalexin which has been identified in several plant species. Among its pharmacological properties are a wide range of biological activities such as antihyperglycemic, antioxidative, anticancer, etc. Because of pterostilbene shows very poor solubility in water and low bioavailability, the encapsulation of this potent antioxidant with types of molecules which can increases its bioavailability, solubility and stability is strongly desirable, as it is in the case of cyclodextrins (CDs).

Bearing the above in mind, the main objective of this work was to analyze the encapsulation mechanism of pterostilbene with different types of natural and modified CDs under various experimental conditions. The stoichiometry and complexation constants (*KF*) values for the pterostilbene-CD complexes are evaluated.

MATERIALS AND METHODS

• **Materials:** Natural (α-, ß- and ?-CD) and modified CDs (HP-ß-CD, methyl-ß-CD and ethyl-ß-CD), trans-resveratrol and trans-stilbene were purchased from Sigma-Aldrich (Madrid, Spain). Pterostilbene was from Sequoia Research Products Limited (Pangbourne, U.K.).

• **Methods**: Fluorescence intensity was measured in a Kontron SFM-25 spectrofluorimeter equipped (Zurich, Switzerland) with thermostatically controlled cells. Excitation and emission bandwidths were both set at 2 nm. The excitation and emission wavelengths for stilbenoids were 330 and 374nm respectively.

RESULTS AND DISCUSSION

EFFECT OF THE CYCLODEXTRIN STRUCTURE ON ENCAPSULATION CONSTANTS. *KF* values were determined, using changes in fluorescence spectroscopic, with different types of CD in an attempt to characterize the interaction between pterostilbene and the host CD at a molecular level.

Three types of natural CD with GRAS status and approved recently as additives in the European Union were used to this end. As regards the different species, α-, ß- and ?-CD, it can be observed that the highest *KF* value (*KF* =8120 M^{-1}) was found for ß-CD, followed by α-CD (*KF* =4920 M^{-1}) and, finally, ?-CD (*KF* = 361 M^{-1}).

As ß-CDs were the most effective for complexing pterostilbene, different types of modified ß-CDs were studied, adding different functional groups to the macrocyclic ring. As can be seen in Figure 1, HP-ß-CD showed the highest *KF* value followed by ethyl-ß-CD, methyl-ß-CD and, finally, the natural CDs.

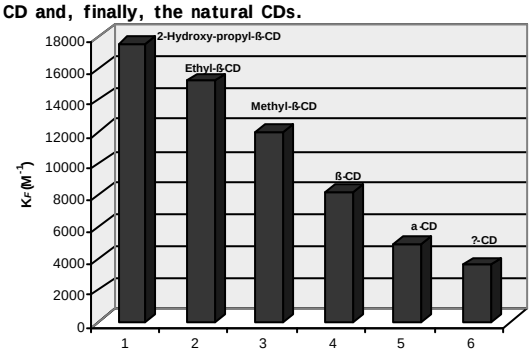

Figure 1. Effect of the structure of modified and natural CDs on the complexation constant (*KF*) of pterostilbene-CD complexes at 25 °C and pH 7.0.

EFFECT OF pH ON THE ENCAPSULATION OF PTEROSTILBENE BY HP-?-CD.

Figure 2 shows the significant dependence of *KF* on pH, passing from a stable value of around 17520 ± 981 M^{-1} (pH 5.5-7.5) to about 10050 ± 740 M-1 (pH 7.5-10.0). The strong decrease in the *KF* value coincides with the region where the stilbenoids begin the deprotonation of their hydroxyl groups. Indeed, a pH values higher than the pterostilbene pKa, the encapsulation is disadvantaged.

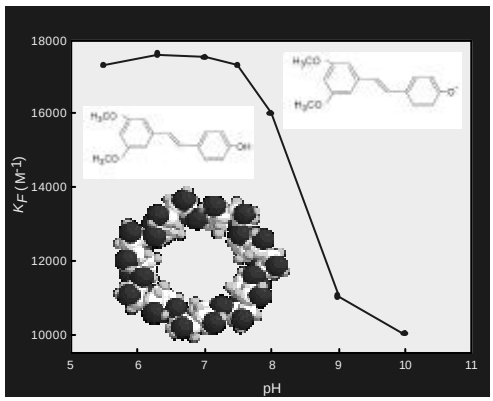

Figure 2. Effect of pH on the complexation constant (*KF*) of pterostilbene-HP-ß-CD complexes at 25 °C.

EFFECT OF THE STRUCTURE OF DIFFERENT STILBENES ON THE ENCAPSULATION CONSTANTS OF THE PTEROSTILBENE/ HP-?-CD COMPLEXES.

The *KF* values and the stoichiometry for the encapsulation of three stilbenoids are presented in Table 1. The complexes formed between both pterostilbene and *trans*-resveratrol with HP-ß-CD presented a 1:1 stoichiometry. Moreover, the interaction was more effective for the *trans*-resveratrol complexes than for the pterostilbene complexes. However, the *trans*-stilbene complexes showed a 1:2 stoichiometry. This means that one molecule of *trans*-stilbene may be encapsulated by two molecules of HP-ß-CD, each one of which encapsulated to *trans*-stilbene through one of the sides of its structure.

Complex	K_F (M^{-1})	K_{F12} (M^{-2})	Correlation coefficient	
			1:1	*1:2*
Resv/CD	24880	----------	0.99	0.91
Ptero/CD	17520	----------	0.99	0.86
Stilb/CD	--------	$1.01*10^9$	0.88	0.99

Table 1. *KF* values and correlation coefficients for 1:1 and 1:2 *stilbenoids*-HP-ß-CD complexes at 25 °C and pH 7.0.

CONCLUSIONS

In this work we propose the pterostilbene encapsulation with CDs which facilitates the "solubilization" of this antioxidant and protects it against prooxidants agents. Potential applications of the resulting of pterostilbene-CD complexes can be found in the pharmaceutical and food ingredient industries due their high solubility and stability. Moreover, the use of pterostilbene-CD complexes could slow down the rapid metabolism and elimination of pterostilbene, improving its bioavailability.