STUDY ON THE HESPERIDIN – CYCLODEXTRINS INTERACTIONS BY THIN LAYER CHROMATOGRAPHY

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In this study, the interaction of hesperidin with β-CD, HP-β-CD, β-CD sulfate was investigated by thin layer chromatography. The chromatographic conditions included: Kieselgel G_{25} stationary phase, mobile phase of butanol: acetic acid: water (4:1:5) and increasing concentrations of the three cyclodextrins. Visualization was performed by examination in UV light and by exposure to iodine vapors. Also, the thermodynamic parameters were calculated: the Gibbs free energy change (ΔG^0_{tr}), free energy change (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0). The results show that the best inclusion capacity is obtained for HP-β-CD, the inclusion process is favored by increasing the concentration of cyclodextrin, and by increasing the temperature.

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Introduction

Hesperidin (Figure 1) is a flavanone glycoside (hesperetin-7-rutinoside) and the most abundant flavonoid in citrus fruits. It is found in highest quantities in the peel and membranous parts of sweet orange and lemon. Hesperidin reduces the permeability and fragility of capillary walls and can be used in chronic venous insufficiency, haemorrhoids, varicose veins, various ulcers and bruising. Hesperidin also manifests other effects on the vascular system, such as: antihypercholesterolaemic, antihyperlypidemic, calcium channel blocker, antihypertensive and diuretic effects.[1]

Numerous studies confirm a significant antiinflammatory activity of hesperidin, both *in vivo* and *in vitro*, possibly through the inhibition of eicosanoid synthesis and/or antioxidant free radical scavenger activity. One study showed analgesic activity in mice on subcutaneous administration, exerted through a peripheral mechanism.[2]
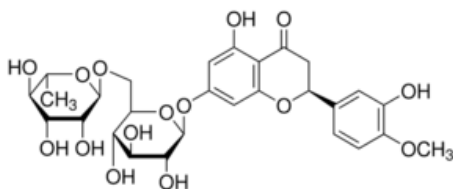
Figure 1. Hesperidine structure

Like most other flavonoids, hesperidin manifests antioxidant activity and radical scavenging properties. Lately, a considerable amount of research has been carried out on the anticarcinogenic activity of hesperidin against different human cancer cell lines, with remarkable results.[3] The sedative effect of hesperidine was also investigated, as the property to decrease bone density loss.[4,5]

Hesperidin has found to possess antibacterial, antifungal, antiviral properties, antiallergic and antianaphylactic activities. Further, it has been shown to inhibit platelet and cell aggregation and the activity of some enzymes (hyaluronidase, aldol reductase, aromatase). The aglycone, hesperetin, protects liposomes from UV-irradiation induced peroxidation and might be successfully employed as a topical photo-protective agent.[6]

Since hesperidin is poorly soluble in water it can be a challenge when using it in different pharmaceutical formulations. In order to increase the solubility of the substance, one of the most used methods is complexation with cyclodextrins, because the cyclodextrin - substance complex has the ability to change the physicochemical properties of the inclusion compound. Complexation can be defined as the formation of the reversible interaction between the active substance and cyclodextrin in order to form a new compound by means of intermolecular forces such as: covalent bonds, Van der Waals bonds, ion – dipole forces, dipole – dipole forces, hydrogen bonds. Some of the chemical and physical properties that can be modified during complexation include: increased stability and solubility of substances, protection against degradation, reduced gastric toxicity and irritancy, masking the unpleasant smell and taste, enhanced bioavailability of substances.[7]

The inclusion constants (K) and dissociation constants are a measure of changes in the physicochemical properties of a compound as a result of the inclusion process. The thermodynamic parameters, i.e. Gibbs free energy change (ΔG^0_{tr}), free energy change (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0) can be calculated according to the effect of temperature on the stability constants of the complex.[8,9]

The interaction between the active compounds and cyclodextrins can be investigated by various analytical methods, such as: in solid state-thermal methods, IR spectroscopy, X-ray diffractometry, Scanning Electron Microscopy, Thin Layer Chromatography, in solution - electrochemical methods, spectroscopic methods (UV-Vis, NMR, RES, fluorescence).[7]

This study investigates the interaction between hesperidin and three types of cyclodextrins by thin layer chromatography, calculating the inclusion constants and the thermodynamic parameters of the inclusion process.[8-13]

Materials and method

Materials

Hesperidin, beta cyclodextrin (1135 g mol^{-1}), HP-β-CD (1460 g mol^{-1}) and β-CD-sulfated sodium salt (3277 g mol^{-1}) were obtained from Sigma-Aldrich (USA). Chromatographic plates were coated with silica gel G$_{25}$ 0.25 mm (Sigma-Aldrich, Switzerland), 10 x 20 cm, 0.2 mm thickness. All the reagents and substances used were of analytical grade.

Methods

Chromatographic separation was achieved at room temperature by the ascending method. The mobile phase was butanol: acetic acid: water (4:1:5) containing different concentrations of cyclodextrin. The chromatographic plates were spotted with 20 μl solution containing 1 mg/ml hesperidin, at 2 cm distance from the bottom edge, and the separation chamber was saturated for 1 hour. The migration distance was 12 cm. Visualization was performed by examination in UV light and by exposure to iodine vapors. R_f values were calculated by the ratio between the distance covered by flavone and the distance covered by the mobile phase containing increasing concentrations of cyclodextrin.
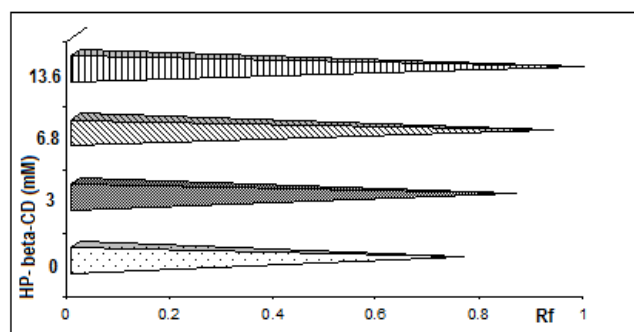
Results and discussions

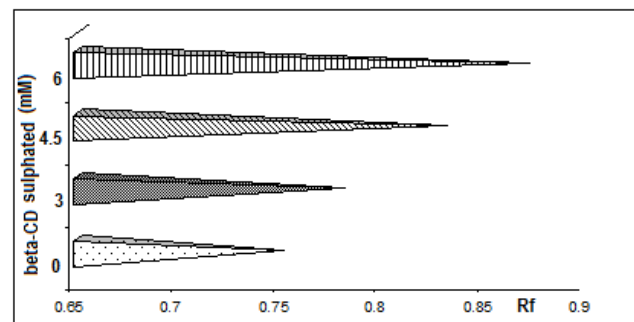Figures 2-4 show the effect of different concentrations of cyclodextrin on the R_f values of hesperidin.
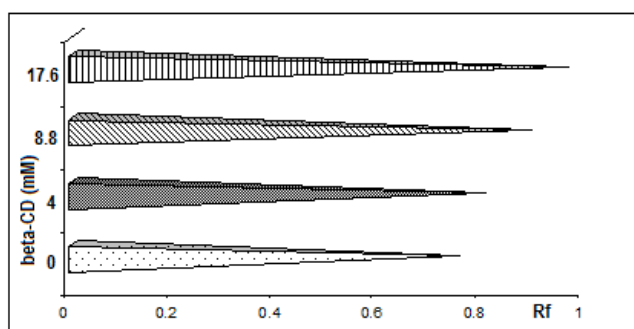
Figure 2. The effect of β-CD concentration on the R_f values of hesperidin

It is apparent that by raising the concentration of cyclodextrin in the mobile phase, the migration distance decreases and thus the value of R_f increases. The most obvious change in R_f values was observed for HP-β-CD, followed by β-CD and β-CD sulfate.

Figure 3. The effect of HP-β-CD concentration on the R_f values of hesperidin


Figure 4. The effect of β-CD sulfate concentration on the R_f values of hesperidin

From the diagrams obtained, where the R_f values are expressed based on the concentrations of cyclodextrins, the inclusion constants (K) were calculated according to the formula:

$$K = (slope/intercept)*1000$$

Figure 5 shows a comparison between the values of inclusion constants depending on cyclodextrins, and Figure 6 presents the values of dissociation constants ($1/k$) according to cyclodextrins.
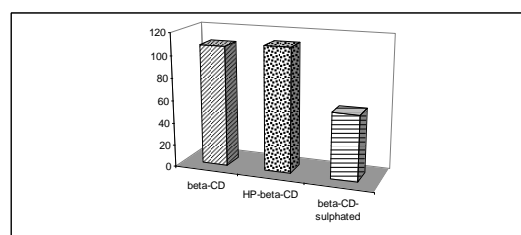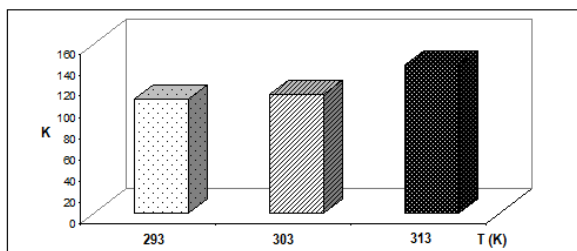

Figure 5. Inclusion constants of hesperidin depending on the cyclodextrin used
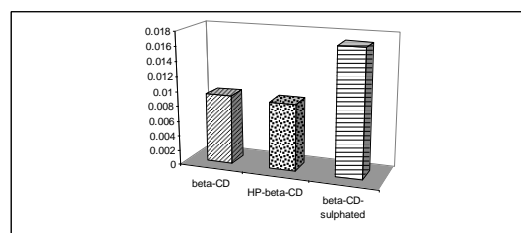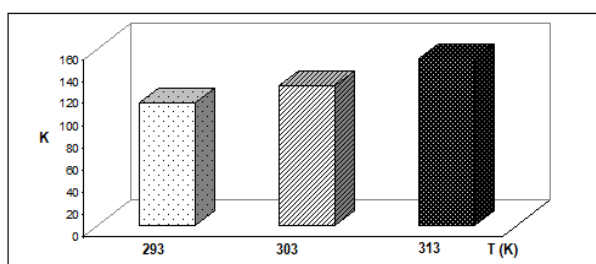

Figure 6. Dissociation constants of hesperidin depending on the cyclodextrin used

From the above figures it appears that the inclusion capacity decreases in order of HP-β-CD> β-CD> β-CD sulfate and HP-β-CD and β-CD compounds are more stable than those with β-CD sulfate. In order to determine the effect of temperature on the inclusion process, the values of inclusion constants were calculated at increasing temperatures: 293 K, 303 K and 313 K (Figures 7-9).

Figure 7. The effect of temperature on inclusion constants of hesperidin in β-CD

Figure 8. The effect of temperature on inclusion constants of hesperidin in HP-β-CD
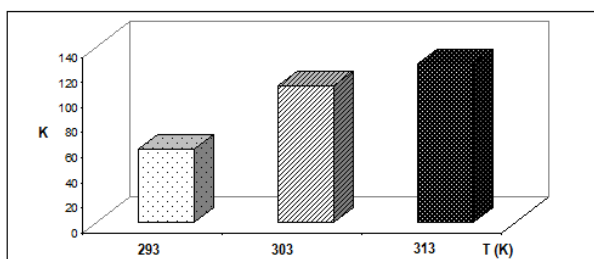
Figure 9. The effect of temperature on inclusion constants of hesperidin in β-CD sulfate

The values of inclusion constants rise with the increasing temperature, which indicates that the high temperature favors the inclusion process. Also, the thermodynamic parameters were calculated: Gibbs free energy change (ΔG^0_{tr}) (Table 1), free energy change (ΔG^0) (Table 2), enthalpy change (ΔH^0) and entropy change (ΔS^0) (Table 3), using the following equation:

$$\Delta G^0_{tr} = -RT \log \frac{R_f}{R_{f0}}$$

where

R = gas constant

T = absolute temperature of the reaction

R_f/R_{f0} = the ratio between the R_f values of hesperidin with CD and the R_f values of hesperidin without CD.

$$\Delta G^0 = -2.303RT \log K,$$

where

K = inclusion constant of the complex made

Table 1. ΔG^0_{tr} (kJ mol^{-1}) values depending on CD concentration

	CD concentra-tion, mM	ΔG^0_{tr}, kJ mol-1		
		293 K	**303 K**	**313 K**
β-CD	4	- 0.068	0.239	0.703
	8.8	- 0.178	0.155	0.523
	17.6	- 0.258	0.042	0.453
HP-β-CD	3	- 0.13	0.681	0.546
	6.8	- 0.212	0.506	0.43
	13.6	- 0.280	- 0.082	0.307
β-CD sulfate	3	- 0.041	0.314	0.569
	4.5	- 0.106	0.222	0.322
	6	- 0.154	0.120	0.345

Negative values of ΔG^0_{tr} indicates advantageous conditions for migration of hesperidin in the presence of cyclodextrin, especially for HP-β-CD. Values decline with increasing concentrations of CD, which demonstrates that the reaction becomes more favorable with larger CD concentrations. However, at greater temperature the ΔG^0_{tr} values become positive, which indicates that a higher temperature is unfavorable for the migration of hesperidin along the stationary phase.

Table 2. ΔG^0 (kJ mol^{-1}) values depending on temperature

Temperature	293 K	303 K	313 K
β-CD	- 11.28	- 11.76	- 12.73
HP-β–CD	- 11.35	- 12.08	- 12.93
β-CD sulfate	- 9.79	- 11.71	- 12.46

Negative values of ΔG^0 show that the inclusion process takes place spontaneously. Given the van't Hoff equation and plotting log K vs $1/T$, the slope will give us the value of enthalpy change (ΔH^0):

$$\text{Slope} = \frac{\Delta H_0}{2.303R}$$

The standard entropy change (ΔS^0) for the complexation reactions was calculated using the equation:

$$\Delta G^0 = \Delta H^0 - T \Delta S^0$$

Positive ΔH^0 values indicate an endothermic process and positive ΔS^0 values can be attributed to the transfer of hesperidin into the cyclodextrin cavity and to the formation of hydrophobic bonds.

Table 3. ΔS^0 (J mol^{-1} K^{-1}) and ΔH^0 (kJ mol^{-1}) values depending on the cyclodextrin used

	ΔS^0	ΔH^0
β-CD	0.042	1.10
HP-β–CD	0.043	1.28
β-CD sulfate	0.044	3.21

Conclusions

The present paper analyzed the interaction between hesperidin and β-CD, HP-β-CD and β-CD sulfate by thin layer chromatography. The results show that, the reaction between hesperidin and cyclodextrins with the formation of inclusion compounds is an endothermic and spontaneous process, and is more effective at increasing temperatures and CD concentrations. The best inclusion and more stable compounds were obtained with HP-β-CD.

Acknowledgements

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