

Study of flavonoids/ β -cyclodextrins inclusion complexes by NMR, FT-IR, DSC, X-ray investigation[☆]

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Abstract

Flavonoids are natural substances with a lot of biological activities, including the antioxidant one. Their use in pharmaceutical field is, however, limited by their aqueous insolubility. As the formation of the inclusion complexes can improve their solubility in water, the flavonoids hesperetin, hesperidin, naringenin and naringin have been complexed with β -cyclodextrin (β -CD) by the coprecipitation method and studied in solution and in solid state by NMR, FT-IR, differential scanning calorimetry and X-ray techniques. The effects of complexation on the chemical shifts of the internal and external protons of β -CD in the presence of each flavonoid were observed. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Hesperetin; Hesperidin; Naringenin; Naringin; β -Cyclodextrin; NMR; FT-IR; DSC; XRD

1. Introduction

Flavonoids are diphenylpropane derivatives that include flavanols, flavanones, anthocyanidins, flavons and flavonols [1]. More than 4000 flavonoids have been found in plants, fruits and vegetables. They are frequently components of the human diet and have gained recent interest be-

cause of their broad pharmacological activities, including the antioxidant [2–9].

Cyclodextrins (CDs) are cyclic α (1–4) linked glucose oligomers having six (α), seven (β) and eight (γ) glucose units. Their molecules have a torus shape and characteristic dimensions which increase from α -CD to γ -CD. Because of their geometry, and as the surface of the internal cavity is relatively hydrophobic, in contrast to the hydrophilic character of the external hydroxyl faces, CD molecules easily form inclusion complexes with a wide variety of molecules and molecular ions [10]. This property is the main base for their wide application in chemistry and in separation

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technology [11]. These complexes were employed to increase the aqueous solubility of drugs [12,13] and the stability of labile drugs [14,15] and to improve their bioavailability [16,17].

In this work were prepared inclusion complexes of hesperetin, hesperidin, naringenin and naringin in β -cyclodextrin (β -CD), that were analysed, both in solid state and in aqueous solution, by using NMR, FT-IR, differential scanning calorimetry (DSC) and X-ray techniques. Studies aimed to value characteristics of pharmaceutical

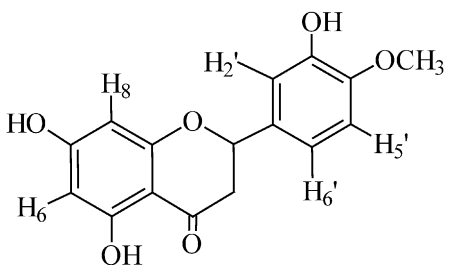
interest, such as solubility in aqueous media, dissolution rate, chemical stability and bioavailability are being carried out.

These flavonoids were chosen because of their well known pharmacological activities [18–20], but their use in pharmaceutical field is limited by their low aqueous solubility.

Proton NMR spectroscopy was used to determine the stoichiometry of the complex and to obtain useful information about the complex geometry in solution. In fact, insertion of a guest

Table 1

Chemical shifts (ppm) for the protons of hesperetin and of β -CD in the free state and in the pure complex (complex 1:1)

			
H hesperetin	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₆ e H ₈	6,311	6,189	0,122
H _{2'} , H _{5'} e H _{6'}	6,931	6,746	0,185

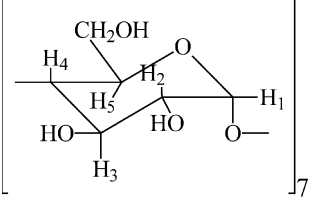
			
H β -CD	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₁	5.060	5.053	0.007
H ₂	3.638	3.632	0.006
H ₃	3.956	3.946	0.010
H ₄	3.575	3.570	0.005
H ₅	3.838	3.816	0.022
H ₆	3.870	3.851	0.019

Table 2

Chemical shifts (ppm) for the protons of hesperidin and of β -CD in the free state and in the pure complex (complex 1:1)

H hesperidin	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₆ e H ₈	6,253	6,185	0,068
H _{2'} , H _{5'} e H _{6'}	7,089	6,832	0,257

H β -CD	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₁	5.060	5.036	0.024
H ₂	3.638	3.615	0.023
H ₃	3.956	3.912	0.044
H ₄	3.575	3.559	0.016
H ₅	3.838	3.759	0.079
H ₆	3.870	3.839	0.031

molecule into the hydrophobic cavity of a CD results in the modification of the NMR signals of both the drug and the host molecule [21]. In this case they form 1:1 complexes.

2. Experimental

2.1. Materials

All the flavonoids (hesperetin, hesperidin,

naringenin and naringin), potassium bromide and deuterium oxide, (D₂O, deuterium content 99.9%) were supplied by Sigma-Aldrich Chemie (Germany). β -CD was purchased from Fluka Chemie (Switzerland). Aceton and methanol were purchased from Merck (Darmstadt, Germany). All solutions were prepared using distilled deionised water and filtered through a 0.22 μm Millipore Filters (Bedford, USA). All other materials were of analytical reagent grade.

2.2. Apparatus

2.2.1. NMR

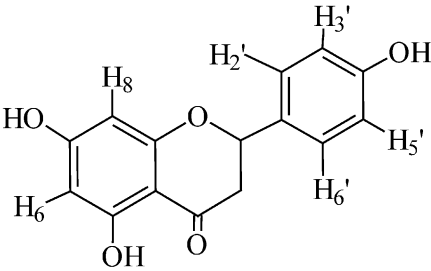
Proton NMR spectra were recorded at 25 °C on a Varian Gemini 300 MHz spectrophotometer, in D₂O. The chemical shifts are referred to D₂O signal at 4.8 ppm at 296 K.

2.2.2. Fourier transform infrared spectrophotometry

An IR spectrophotometer (FT-IR Perkin Elmer 1600) interfaced to a Pentium II 350 MHz processor was used for the analysis IR. The spectra were performed in KBr disks in a frequency range between 4000 and 450 cm⁻¹.

Table 3

Chemical shifts (ppm) for the protons of naringenin and of β-CD in the free state and in the pure complex (complex 1:1)

			
H naringenin	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₆ e H ₈	6,777	6,731	0,046
H _{2'} e H _{6'}	7,353	7,205	0,148
H _{3'} e H _{5'}	7,330	7,184	0,146

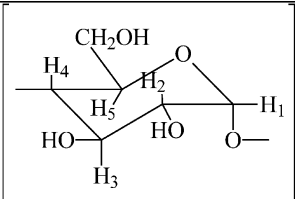
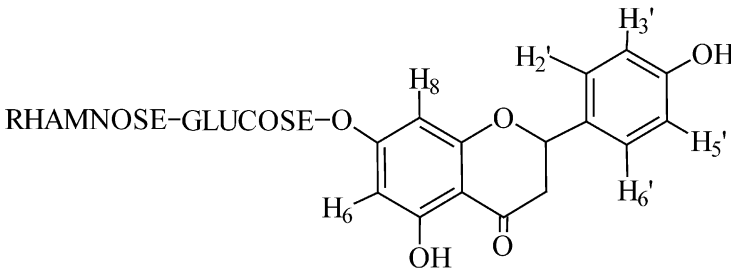
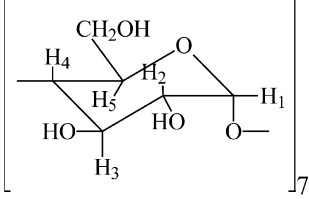
			
H β-CD	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₁	5.060	5.006	0.054
H ₂	3.638	3.588	0.050
H ₃	3.956	3.857	0.099
H ₄	3.575	3.528	0.047
H ₅	3.838	3.669	0.169
H ₆	3.870	3.794	0.076

Table 4

Chemical shifts (ppm) for the protons of naringin and of β -CD in the free state and in the pure complex (complex 1:1)

			
H naringin	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₆ e H ₈	6,241	6,125	0,116
H _{2'} e H _{6'}	7,430	7,153	0,277
H _{3'} e H _{5'}	6,967	6,696	0,271

			
H β -CD	δ_{free}	δ_{c}	$\Delta\delta$ (ppm)
H ₁	5.060	5.053	0.007
H ₂	3.638	3.633	0.005
H ₃	3.956	3.923	0.033
H ₄	3.575	3.575	0
H ₅	3.838	3.756	0.082
H ₆	3.870	3.847	0.023

2.2.3. Differential scanning calorimetry

The DSC curves of the different samples were recorded on a Perkin-Elmer DSC-4 differential scanning calorimeter calibrated with indium (3.21 mg, 99.99% pure, melting point 156.6 °C) at heating rates of 10 °C min⁻¹. The thermal behaviour was studied by heating 1–5 mg of samples in aluminium crimped pans under nitrogen gas flow over the temperature range 30–250 °C. Measurements were made in duplicate.

2.2.4. X-ray powder diffractometry (XRD)

Powder X-ray diffraction patterns were obtained with a Philips PW 1050/25 diffractometer system with CuK α radiation ($\lambda = 1.5418$ Å) over the interval 2–45°/2 θ . The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 20 mA; time constant, 4 s; angular speed 1° (2 θ) min; 1°, –0.1° and –1° slit; angular range 2° < 2 θ < 45°.

2.3. Preparation of samples

The inclusion complexes of hesperidin, naringenin and naringin with the β -CD were prepared by the coprecipitation method.

The solvents utilized for the complex formation were water-miscible organic solvents, that allow the flavonoid dissolution and give clear solutions when added to CD.

The procedure consisted in mixing an aqueous solution of β -CD with an organic solution of hesperidin, naringin or naringenin, in order to obtain equimolecular amounts of the substances used.

Aceton was the employed organic solvent for naringenin and naringin, methanol for hesperidin.

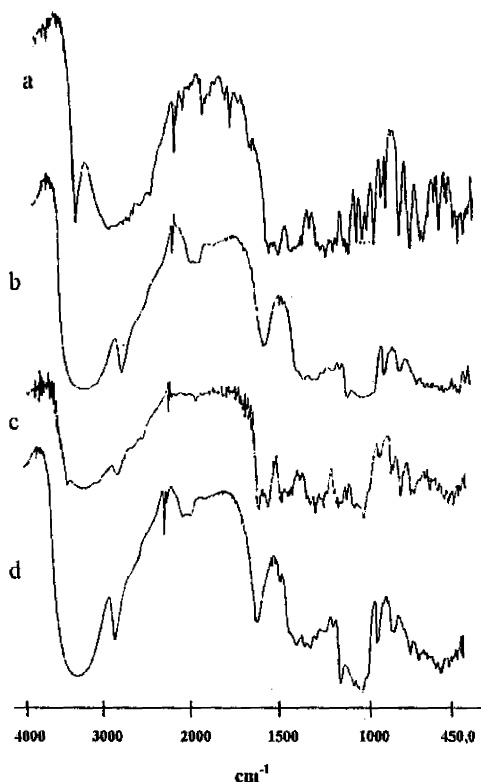


Fig. 1. FT-IR spectra: (a) hesperetin, (b) β -CD, (c) equimolecular physical mixture of hesperetin and β -CD, (d) inclusion complex.

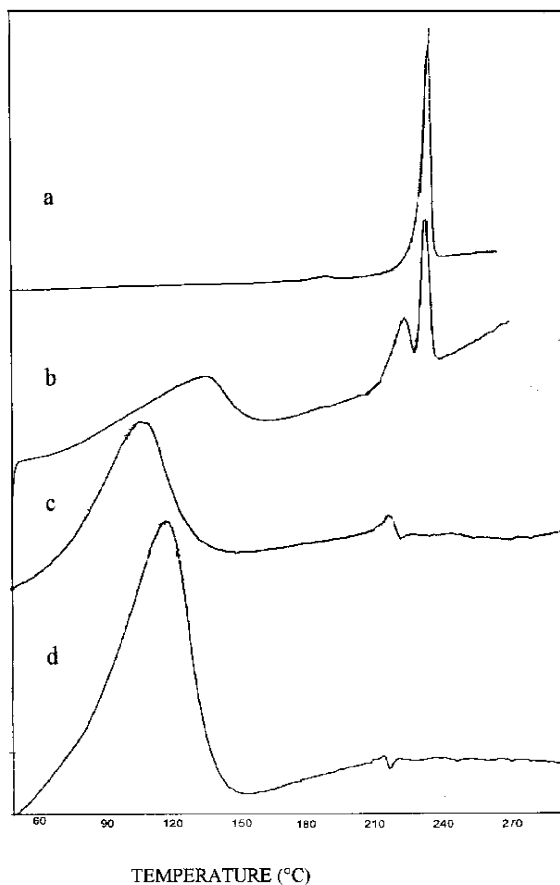


Fig. 2. DSC curves: (a) hesperetin, (b) equimolecular physical mixture of hesperetin and β -CD, (c) β -CD, (d) inclusion complex formed between β -CD and hesperetin.

Solutions were shaken at room temperature for 72 h and then dried under reduced pressure.

A 4×10^{-6} M solution of hesperetin in methanol was prepared and stored in the dark at 4 °C for about an hour. Evaporation of the solvent was done in a water bath. An appropriate amount of β -CD solution was added and the mixed solution was shaken for 72 h.

2.4. Preparation of the physical mixture

The calculated and exactly weighed (1:1 molar ratio) amounts of flavonoids and β -CD were pulverised in a ceramic mortar and carefully mixed.

3. Results and discussion

3.1. $^1\text{H-NMR}$ spectra

In the present work, the NMR technique was used to elucidate in detail the structures of the inclusion complexes of flavonoids with β -CD in aqueous solution.

The smooth variation of chemical shifts as a function of variation of the β -CD: flavonoid ratio and the absence of new peaks arising from the pure complex indicated that, on the NMR time scale, the inclusion process is a fast exchange regime.

This implies that any determination or comparison of spectral parameters dedicated to an estimation of the amount of complex present is not relevant unless the association constant is known; so the value of $\Delta\delta$ observed may be used as a physical parameter related with the complex concentration, because it represents the chemical shifts difference between the free and bound states [22–24].

The peak assignments of free flavonoids, β -CD and their complexes in the $^1\text{H-NMR}$ spectra are summarised in Tables 1–4 where $\Delta\delta = \delta_{\text{complex}} - \delta_{\text{free}}$. Assignment of all signals was previously performed.

Table 5
X-ray diffraction images for hesperetin, hesperidin, β -CD and their complexes

Hesperetin			Complex			β -CD			Hesperidin			Complex		
<i>d</i> (Å)	$^{\circ}2\theta$	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	$^{\circ}2\theta$	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	$^{\circ}2\theta$	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	$^{\circ}2\theta$	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	$^{\circ}2\theta$	<i>I</i> / <i>I</i> ₀
11.94	7.4	67.33	19.62	4.5	43.86	19.62	4.5	54	10.27	8.6	12.87	19.62	4.5	18.35
8.04	11.0	8.33	8.34	10.6	61.40	14.24	6.2	7	7.22	12.2	40.12	9.82	9.0	19.27
6.23	14.2	21.33	7.13	12.4	100	9.82	9.0	37	6.43	13.7	19.76	8.26	10.7	57.80
6.02	14.7	73.33	6.04	14.6	29.82	8.26	10.7	29	5.67	15.6	100	7.07	12.5	100
5.64	15.7	10.33	5.75	15.4	47.37	7.49	11.8	14	5.42	16.3	32.93	6.00	14.7	19.27
5.15	17.2	100	5.21	17.0	56.14	7.02	12.6	100	4.50	19.7	36.53	5.71	15.5	40.37
4.98	17.8	58.66	5.03	17.6	38.60	6.50	13.6	5	4.24	20.9	16.77	5.15	17.2	50.46
4.39	20.2	7.33	4.92	18.0	40.35	5.98	14.8	18	4.13	21.5	25.15	4.92	18.0	37.61
4.21	21.1	41	4.72	18.8	52.63	5.71	15.5	19	3.97	22.3	19.76	4.69	18.9	45.87
4.03	22.0	11.33	4.55	19.5	66.66	5.47	16.2	8	3.92	22.6	32.93	4.50	19.7	62.38
3.90	22.8	22	4.27	20.8	45.61	5.18	17.1	30	3.73	23.8	13.77	4.25	20.9	37.61
3.83	23.2	39.33	3.90	22.8	54.38	5.00	17.7	10	3.56	24.9	29.94	4.13	21.5	21.10
3.75	23.7	54	3.68	24.1	38.60	4.92	18.0	29	3.40	26.2	11.98	3.88	22.9	41.28
3.54	25.1	28.33	3.29	27.1	36.84	4.79	18.5	14	3.09	28.8	13.47	3.64	24.4	23.85
3.45	25.8	26				4.69	18.9	44	2.78	32.1	14.37	3.53	25.2	23.85
3.37	26.4	86.66				4.50	19.7	48				3.26	27.3	29.36
3.21	27.8	15				4.27	20.8	29				3.11	28.7	16.51
3.12	28.6	10.66				3.91	22.7	37				2.79	32.0	16.51
3.01	29.6	70.33				3.86	23.0	34						
2.51	35.8	7.66				3.77	23.6	14						
						3.66	24.3	23						
						3.53	25.2	14						
						3.46	25.7	20						
						3.29	27.1	24						
						3.12	28.6	7						
						3.01	29.6	6						
						2.96	30.2	5						
						2.79	32.0	11						
						2.58	34.8	26						
						2.55	35.1	19						
						2.49	35.9	11						

Table 6
X-ray diffraction images for naringenin, naringin, β -CD and their complexes

Naringenin			Complex			β -CD			Naringin			Complex		
<i>d</i> (Å)	2θ	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	2θ	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	2θ	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	2θ	<i>I</i> / <i>I</i> ₀	<i>d</i> (Å)	2θ	<i>I</i> / <i>I</i> ₀
8.18	10.8	44.59	8.26	10.7	53.33	19.62	4.5	54	19.62	4.5	37.11	19.61	4.5	23.15
7.68	11.5	35.13	7.07	12.5	100	14.24	6.2	7	8.92	9.9	64.95	11.04	8.0	18.94
5.60	15.8	100	6.02	14.7	25	9.82	9.0	37	7.55	11.7	31.44	10.09	8.7	36.84
5.13	17.2	23.42	5.71	15.5	38.33	8.26	10.7	29	6.67	13.2	13.40	9.40	9.4	22.10
4.90	18.1	56.75	5.15	17.2	48.33	7.49	11.8	14	6.00	14.7	100	8.22	10.7	54.73
4.59	19.3	10.36	4.98	17.8	48.33	7.02	12.6	100	5.74	15.4	11.85	7.01	12.6	100
5.03	19.9	79.28	4.92	18.0	45	6.50	13.6	5	5.33	16.6	91.24	5.98	14.8	55.79
4.35	20.4	69.82	4.69	18.9	50	5.98	14.8	18	4.99	17.7	20.62	5.73	15.4	37.89
4.16	21.3	28.83	4.52	19.6	51.66	5.71	15.5	19	4.88	18.1	11.85	5.15	17.2	32.63
4.07	21.8	17.56	4.24	20.9	46.66	5.47	16.2	8	4.71	18.8	70.10	4.97	17.8	42.10
3.98	22.3	72.07	4.13	21.5	31.66	5.18	17.1	30	4.18	21.2	10.30	4.79	18.5	23.15
3.73	23.8	57.20	3.88	22.9	43.33	5.00	17.7	10	4.08	21.7	38.66	4.69	18.9	46.31
3.63	24.5	37.38	3.64	24.4	30	4.92	18.0	29	4.01	22.1	26.80	4.52	19.6	83.15
3.55	25.0	30.63	3.54	25.1	31.66	4.79	18.5	14	3.85	23.0	26.80	4.23	20.9	36.84
3.51	25.3	75.67	3.47	25.6	28.33	4.69	18.9	44	3.75	23.7	35.05	4.12	21.5	27.36
3.45	25.8	45.94				4.50	19.7	48	3.67	24.2	37.11	3.88	22.9	31.57
3.30	27.0	12.61				4.27	20.8	29	3.54	25.1	24.22	3.65	24.3	37.89
3.20	27.8	32.43				3.91	22.7	37	3.40	26.2	54.12	3.46	25.7	26.31
3.04	29.3	10.36				3.86	23.0	34	3.36	26.5	38.14	3.32	26.8	26.31
2.96	30.1	19.82				3.77	23.6	14	3.18	28.0	11.85	3.27	27.2	30.52
2.80	31.8	9.91				3.66	24.3	23	3.09	28.8	35.05	2.86	31.2	21.05
2.59	34.6	11.71				3.53	25.2	14	2.97	30.0	12.88			
2.47	36.3	10.81				3.46	25.7	20	2.73	32.8	12.37			
						3.29	27.1	24	2.29	39.3	12.37			
						3.12	28.6	7						
						3.01	29.6	6						
						2.96	30.2	5						
						2.79	32.0	11						
						2.58	34.8	26						
						2.55	35.1	19						
						2.49	35.9	11						

The spectra for each flavonoid in the presence of β -CD are compared with the spectra for the individual components and in both cases there are clear differences between the spectra with and without β -CD. There is a significant shift of the signals referred to the phenilic moiety of hesperetin, hesperidin, naringenin and naringin, that indicates the interaction of this portion of the molecules with the CD.

Only the H₃ and H₅ protons, located inside the cavity, and the H₆ proton, located on the cavity rim at the narrow end of the molecule, are appreciably shifted.

¹H-NMR spectra show upfield shifts, due to

the diamagnetic anisotropy of the included guest, of the H₃ and H₅ proton signal joined to little shifts of H₆ signal, while H₁, H₂ and H₄ signals, located outside the cavity, are relatively unaffected (Tables 1–4); these results indicate the interaction of part of the molecule with the cavity of the β -CD and consequently the formation of an inclusion complex between each flavonoid and the CD.

The order of the upfield shift is found to be for hesperetin: H₅ ($\Delta\delta$ /ppm = 0.022) > H₆ (0.019) > H₃ (0.010) > H₁ (0.007) \cong H₂ (0.006) \cong H₄ (0.004); for hesperidin: H₅ ($\Delta\delta$ /ppm = 0.169) > H₃ (0.099) > H₆ (0.076) > H₁ (0.054) \cong H₂

(0.050) \cong H₄ (0.047); for naringenin: H₅ ($\Delta\delta$ /ppm = 0.079) > H₃ (0.044) > H₆ (0.031) > H₁ (0.024) \cong H₂ (0.023) > H₄ (0.016); for naringin: H₅ ($\Delta\delta$ /ppm = 0.082) > H₃ (0.033) > H₆ (0.023) > H₁ (0.007) \cong H₂ (0.005) > H₄ (0). The shifts in the protons located at the exterior of the torus (H₁, H₂ and H₄) are relatively small.

3.2. FT-IR spectroscopy

The FT-IR spectroscopic analysis confirmed the interaction and the complex formation between flavonoids and β -CD.

FT-IR spectra of the complex were compared to the physical mixture and pure substances ones. Changes in the characteristic bands of pure substances confirm the existence of the complex as a new compound with different spectroscopic bands.

– *Hesperetin* characteristic bands, i.e. the aromatic at 1581 and 1500 cm⁻¹, the –OH phenolic

at 1200 and 1350 cm⁻¹, and the methoxylic at 1250 cm⁻¹, disappear in the IR spectrum relative to the complex.

- *Hesperidin* characteristic bands, i.e. the aromatic at 1605 and 1510 cm⁻¹, the –OH phenolic at 3479 and 1205 cm⁻¹, the methoxylic at 1277 cm⁻¹ disappear in the IR spectrum of complex, while the intensity of the band relative to the carbonylic group decreases.
- *Naringenin* characteristic bands, i.e. the aromatic at 1510 and 1601 cm⁻¹, the –OH phenolic at 1200 cm⁻¹, change in the relative complex: the intensity of the aromatic bands decreases and the OH band disappears.
- *Naringin* characteristic bands, i.e. the aromatic at 1519 cm⁻¹, the –OH phenolic at 1205 and 1361 cm⁻¹ disappears in the relative complex, while the intensity of carbonylic band at 1649 cm⁻¹ decreases.

Fig. 1a–d shows, as an example, the IR spectra of hesperetin, β -CD, hesperetin/ β -CD physical mixture and inclusion complex.

3.3. DSC

The DSC curves of the raw materials, (flavonoids and β -CD) compared with those obtained by coprecipitation confirm not only an interaction between the flavonoids and β -CD, but also a real inclusion.

In fact, the formation of an inclusion complex was suggested by the absence of the melting endotherm of hesperetin at 226.73 °C, of hesperidin at 263.19 °C, of naringenin at 247.25 °C and of naringin at 249 °C in the DSC curves of the inclusion complexes. The physical mixtures show, instead, two endothermic peaks, one for the β -CD (at 125.18 °C) and one for the flavonoid (hesperetin at 226.73 °C, hesperidin at 263.19 °C, naringenin at 247.25 °C, and naringin at 249 °C).

The DSC curves of hesperetin, β -CD, hesperetin/ β -CD physical mixture and inclusion complex are shown in Fig. 2a–d.

3.4. X-ray diffractometry

The X-ray diffractometry patterns of the physical mixtures of flavonoids and β -CD are approxi-

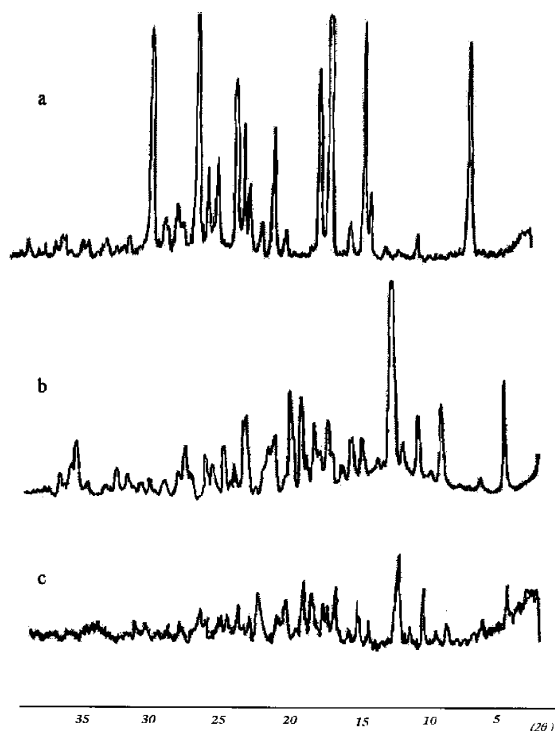


Fig. 3. X-ray diffraction profiles: (a) hesperetin, (b) β -CD, (c) inclusion complex formed between β -CD and hesperetin.

mately the superposition of the patterns of the raw materials. On the other hand, the coprecipitate products have completely different patterns in which it is no longer possible to distinguish the characteristic peaks of flavonoids (Tables 5 and 6), thus confirming the existence of new compounds.

Results show that flavonoids are completely included in the β -CD and, in particular, the differences in the interplanar spacing, relative diffraction peak intensities and diffraction angles confirm that the inclusion complexes have different crystalline structures.

The powder X-ray patterns of hesperetin, β -CD and inclusion complex, reported as an example, are shown in Fig. 3.

4. Conclusions

β -CD, for its ability to make complexes with flavonoids, may be useful in providing promising applications in pharmaceutical formulations.

All the data obtained from the NMR, FT-IR, DSC and X-ray studies showed that it is possible to obtain an inclusion complex (1:1), in solid state and in aqueous solution, between the cavity of β -CD and hesperetin, hesperidin, naringenin and naringin. This could improve, in a therapeutical formulation, the dissolution and subsequently the absorption of the drug.

References

- [1] J.B. Harborne (Ed.), *The Flavonoids—Advances in Research Since 1986*, Chapman and Hall, New York, 1994.
- [2] P. Pietta, in: C.A. Rice-Evans, L. Packer (Eds.), *Flavonoids in Health and Disease*, Marcel Dekker Inc, New York, 1994, pp. 61–110.
- [3] V. Cody, E. Middleton, J.B. Harborne, A. Beretz (Eds.), *Plant Flavonoids in Biology and Medicine II. Biochemical, Cellular and Medicinal Properties*, AR Liss, Inc, New York, 1988.
- [4] P.C.H. Hollman, M.B. Katan, in: C.A. Rice-Evans, L. Packer (Eds.), *Flavonoids in Health and Disease*, Marcel Dekker Inc, New York, 1998, pp. 483–522.
- [5] J.A. Manthey, K. Grohmann, N. Guthrie, *Curr. Med. Chem.* 8 (2001) 135–153.
- [6] C. Yutzing, Z. Rongliang, J. Zhonghan, J. Yong, *Free Radic. Biol. Med.* 9 (1990) 19–21.
- [7] G. Cao, E. Sofic, R.L. Prior, *Free Radic. Biol. Med.* 22 (1997) 749–760.
- [8] P. Cos, L. Ying, M. Calomme, J.P. Hu, K. Cimanga, B. Van Poel, L. Pieters, A.J. Vlietinck, D. Vanden Berghe, *J. Nat. Prod.* 61 (1998) 71–76.
- [9] H. Ohshima, Y. Yoshie, S. Auriol, I. Gilibert, *Free Radic. Biol. Med.* 25 (1998) 1057–1065.
- [10] W. Saenger, *Angew. Chem. Int. Ed. Engl.* 19 (1980) 344–362.
- [11] W.A. Konig, *Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins*, Huethig Publishing Ltd., Heidelberg, Germany, 1992, pp 1–5.
- [12] J. Pitha, J. Milecki, H. Fales, L. Pannell, K. Uekama, *Int. J. Pharm.* 29 (1986) 73–82.
- [13] M. Brewster, M. Hora, J. Simpkins, N. Bodor, *Pharm. Res.* 8 (1991) 792–795.
- [14] N.D. Heindel, R.A. Egolf, J.S. Stefely, *J. Pharm. Sci.* 79 (1990) 862–865.
- [15] M. Brewster, T. Loftsson, K. Eastes, J.L. Lin, H. Fridriksdottir, N. Bodor, *Int. J. Pharm.* 79 (1992) 289–299.
- [16] T. Loftsson, M.E. Brewster, *J. Pharm. Sci.* 85 (1996) 1017–1025.
- [17] R.A. Rajewski, V.J. Stella, *J. Pharm. Sci.* 85 (1996) 1142–1169.
- [18] R. Ficarra, P. Ficarra, M.R. Di Bella, D. Raneri, S. Tommasini, M.L. Calabrò, M.C. Gamberini, C. Rustichelli, *J. Pharm. Biomed. Anal.* 23 (2000) 33–40.
- [19] C.A. Rice-Evans, N.J. Miller, G. Pagana, *Free Radic. Biol. Med.* 20 (1996) 933–956.
- [20] S.A.B.E. van Acker, D. van den Berg, M.N.J.L. Tromp, D.H. Griffioen, W.P. van Bennekom, W.J.F. van der Vijgh, A. Bast, *Free Radic. Biol. Med.* 20 (1996) 331–342.
- [21] H. Ohshima, Y. Yoshie, S. Auriol, I. Gilibert, *Free Radic. Biol. Med.* 25 (1998) 1057–1065.
- [22] P.L. Irwin, P.E. Pfeffer, L.W. Doner, G.M. Sapers, J.D. Brewster, G. Nagahashi, K.B. Hicks, *Carbohydr. Res.* 256 (1994) 13–27.
- [23] Y. Yamamoto, Y. Inoue, *J. Carbohydr. Chem.* 8 (1989) 29–46.
- [24] J.R. Moyano, M.J. Arias-Blanco, J.M. Gines, A.M. Rabasco, J.I. Perez-Martinez, M. Mor, F. Giordano, *J. Pharm. Sci.* 86 (1997) 72–75.