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Comparative photodegradation studies on 3-hydroxyflavone: influence of different media, pH and light sources

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Abstract

3-Hydroxyflavone (3-OH-F) photochemistry in solution has been rationalized in terms of an excited state intramolecular proton transfer (ESIPT), which involves the free 3-hydroxy group interacting with the *ortho*-carbonyl. This photo-rearrangement occurs rapidly and is strongly influenced by the physico–chemical properties of the solvent, which plays an essential role in determining whether a photo-oxidation or a photo-induced molecular rearrangement takes place.

3-OH-F photoreactivity has been deeply investigated and the related mechanisms elucidated, as affected by various solvents, pH values and irradiation wavelengths, leading to different photodegradation rates and pathways.

Moreover, the influence of molecular encapsulation upon α- and β-cyclodextrins (α- and β-CyD) on the molecule photoreactivity has been examined, as a potential tool for increasing molecule photostability as well as minimizing photoinduced toxic effects on biosubstrates.

Keywords: Flavonoids; 3-Hydroxyflavone; Cyclodextrins; Photoreactivity; Photostability; Photo-rearrangement

1. Introduction

Flavonoids are a well-known class of natural pigments exhibiting a wide number of biological and medicinal properties (e.g. antioxidant, antiinflammatory, antiviral, hepatoprotective, etc.) as an outcome of their different in vivo action mechanisms [1–4].

They also play an essential role as screening pigments in protecting higher plants against short wavelength-induced damage [5–7]. Their importance is not confined to plants, however, since their strong UV-absorption could involve the protection of photosensitive biological targets such as DNA, lipids, proteins and coenzymes [8–10].

It is no wonder, therefore, that 3-OH-F, which we had formerly examined as a prototype of this class of compounds [11], exhibits not only luminescence, but also a singular photoreactivity. The reaction mechanism invoked to elucidate its sensitivity to light excitation is here briefly mentioned [12–16].

It can be assumed that a singlet excited state ^{1}ES, through a quick intramolecular electron transfer, turns into the corresponding phototautomer ^{1}PT which then

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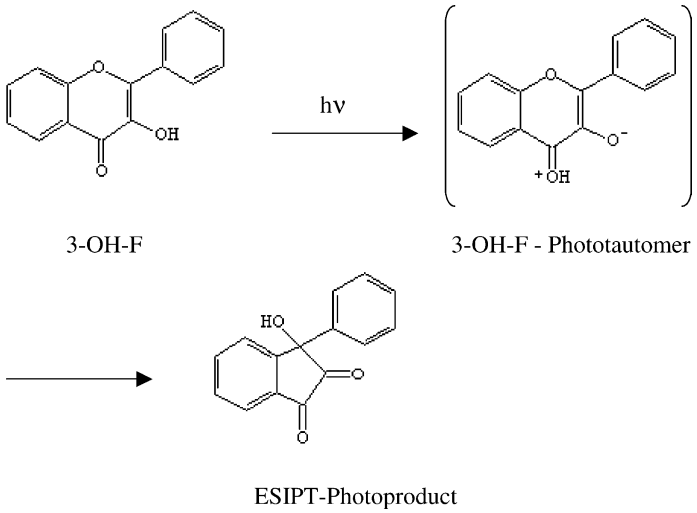

Fig. 1. Photo-rearrangement of 3-OH-F by excited state intramolecular proton transfer.

rearranges to the indandione penthatomic structure (see Fig. 1).

3-Hydroxyflavone, prototype of the flavonols, exhibited antioxidant activity in different biological assays: scavenging of damaging free radicals; binding of iron; DNA-protection against induced stress [17]. In view of a therapeutic use of the molecule and its derivatives, the present work was aimed to deeply investigate its behavior, as influenced both by the medium polarity and proticity: cyclohexane (Cy), acetonitrile (ACN) and methanol (MeOH). Since the molecule shows an equilibrium with its anionic and cationic forms, the influence of different pH values of the medium was afterwards evaluated: pH 7.4 (usually employed to assess the photoreactivity of pharmaceutical substances), pH 3.0 and 10.0 (mimicking the gastric and intestinal environments, respectively). Results show a remarkable influence of all the factors above cited on the molecule photodegradation rate and pathway upon UV-A and UV-C irradiation.

Moreover, 1:1 inclusion complexes of the molecule were obtained in solution with α- and β-CyDs, which represent an extremely active area of research with regard to drug photostability and phototoxicity [18–22]. Beyond these concerns, CyDs also provide a useful mimicking model for shedding light on drug interactions with biological systems [23].

In the case of 3-OH-F, the CyD-microenvironment was found to be responsible for remarkable changes in the photoreactivity of the molecule, which exhibited a higher photochemical stability.

2. Experimental

2.1. Chemicals

3-Hydroxyflavone ($C_{15}H_{10}O_3$, FW = 238.2) was purchased from Sigma–Aldrich (Milan, Italy) and re-crystallized from a benzene–methanol mixture.

Its pureness was assayed by fluorescence spectroscopy and thin layer chromatography (TLC).

α- and β-cyclodextrins, both HPLC grade, were obtained from Fluka Chemie (Switzerland) and used without further purification.

All the solvents employed (Cy, ACN and MeOH) of spectroscopic grade were provided from Merck (Darmstadt, Germany).

Phosphate buffer solutions (pH 3.0, 7.4 and 10.0) were prepared according to Italian Pharmacopoeia (F.U.), X° ed.

Solutions were filtered through 0.45 μm PVDF Whatman® filters (Clifton, NJ). Water was distilled and deionised prior to use, then filtered through 0.22 μm Millipore® GSWP filters (Bedford, USA).

2.2. Apparatus

UV-Vis absorption spectra were recorded with a Lambda 45 Perkin-Elmer double-beam spectrophotometer (scanning speed = 7.5 nm/min; slit = 2); interfaced to a PC for data processing (software: UV-Win Lab, from Perkin-Elmer). Spectrofluorimeter quartz cells with 10 mm pathlength (Hellma) were employed.

Fluorescence emission spectra were obtained by a LS/5B Perkin-Elmer spectrofluorimetric equipped with a Hamamatsu photomultiplier.

Photolysis was performed using monochromatic light obtained from a high pressure Hanovia Hg lamp, with a Bausch-Lambs monochromator to isolate 254 nm wavelength.

On a semi-preparative scale, irradiation was carried out using a multi-lamp photoreactor (model MLU 18, Applied Photophysics, London, UK) provided with a six twin-lamp modules arrangement of UV-A "black-light". Solutions were placed in a round-bottom quartz vessel (34 mm × 360 mm, NS joint 114/23). pH values of the buffered solutions were measured with a 3310 Jenway pH meter (±0.1).

Mixed solvents systems were obtained by addition of 3-OH-F organic solutions (2.10^{-4} M) to the phosphate buffer solutions at various pH values, with a final ratio of 15:85 (by vol.). Thus, the pH value of the original aqueous component was quoted.

2.3. Irradiation

Solutions of 3-OH-F (3.5×10^{-5} M) were exposed to UV-A radiation in the photoreactor equipped with "black light" phosphor lamps, emitting in the 310–390 nm range (prominent spectral emission at 350 nm).

A cooling fan mounted on the photoreactor's base plate avoided overheating of the samples, which were continuously stirred to ensure homogenous exposure to artificial light. A vessel containing the same analyte was covered with aluminium before exposure, to serve as a blank.

Irradiation at 254 nm (UV-C) was performed with the Hg lamp. The incident photon flux on 3 ml solution in the quartz cell (optical path 10 mm) was ca. 10^{15} to 10^{18} quanta s^{-1} for the phosphor lamps and 8×10^{14} quanta s^{-1} for the 254 nm radiation.

Light intensity measurements were made by the ferrioxalate actinometer.

3. Results and discussion

3.1. Photolysis of 3-OH-F

UV-absorption spectrum of 3-OH-F is characterized by two broad bands, centered between 220–270 and 270–370 nm (Fig. 2). The so called "Band I", with a maximum around 339 nm, is relevant to the cynnamoilic portion of the molecule; the one around 237 nm ("Band II") can be attributed to the benzylic moiety. The absorption band around 304 nm arises from the pyronic ring. In Cy two weak "shoulders" are also evident, at 243 and 354 nm.

These spectral features are essentially unchanged in ACN, which is aprotic like Cy, but remarkably more polar. On the other hand, significant changes can be appreciated in methanol, a polar solvent with high dielectric constant and capable of forming H-bonding. Particularly, a significant shift towards longer wavelengths is noticed, with maxima at 343 nm for band I and 238 nm for band II.

The red-shift observed is in agreement with the fact that these main bands receive contributions from a π, π^* state, which is more polar than the ground state. Moreover, the fine vibrational structure of the spectrum is entirely lost due to the strong solvent–solute interactions.

In all cases, a drastic change in the shape of the spectra was observed as a function of the irradiation time, with a gradual decreasing of all the absorption bands.

The benzenoid band, which appears at shorter wavelengths, showed an ipsocrhomic shift in cyclohexane and acetonitrile, while in methanol it was red-shifted. The absorption band around 304 nm was blue-shifted in all the solvents.

In the two aprotic solvents (Cy and ACN), some isosbestic points were evident all throughout irradiation (e.g. in Cy at 263 and 280 nm), revealing the existence of an equilibrium between more species (presumably 3-OH-F and its photoproduct). Fig. 3 shows a set of UV spectra obtained during photolysis of 3-OH-F in Cy.

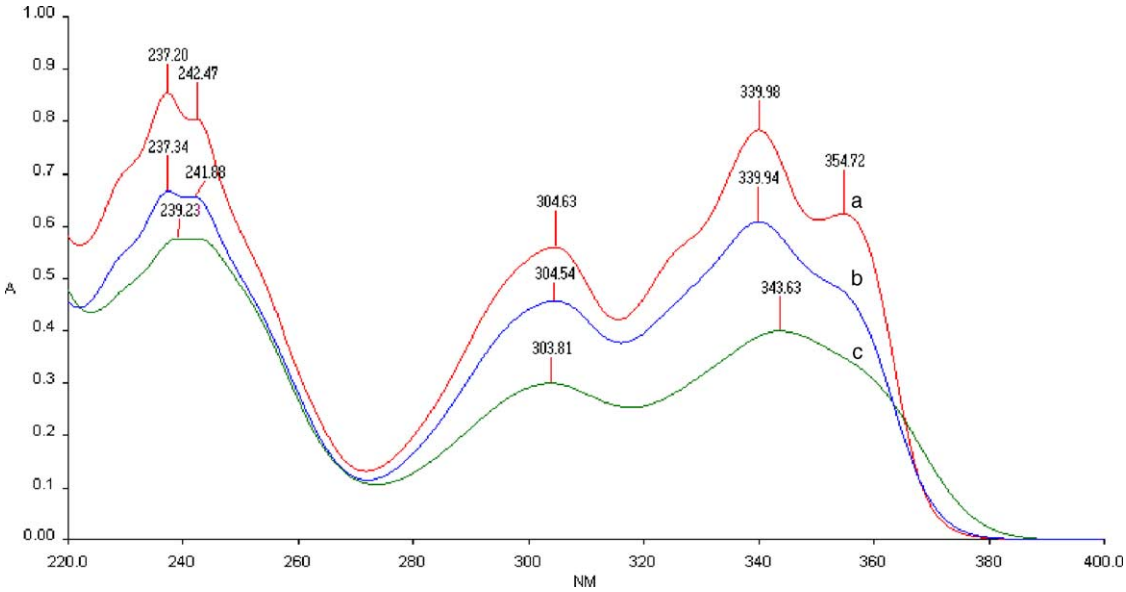

Fig. 2. UV-absorption spectra of 3-OH-F obtained in: cyclohexane (a); acetonitrile (b); methanol (c).

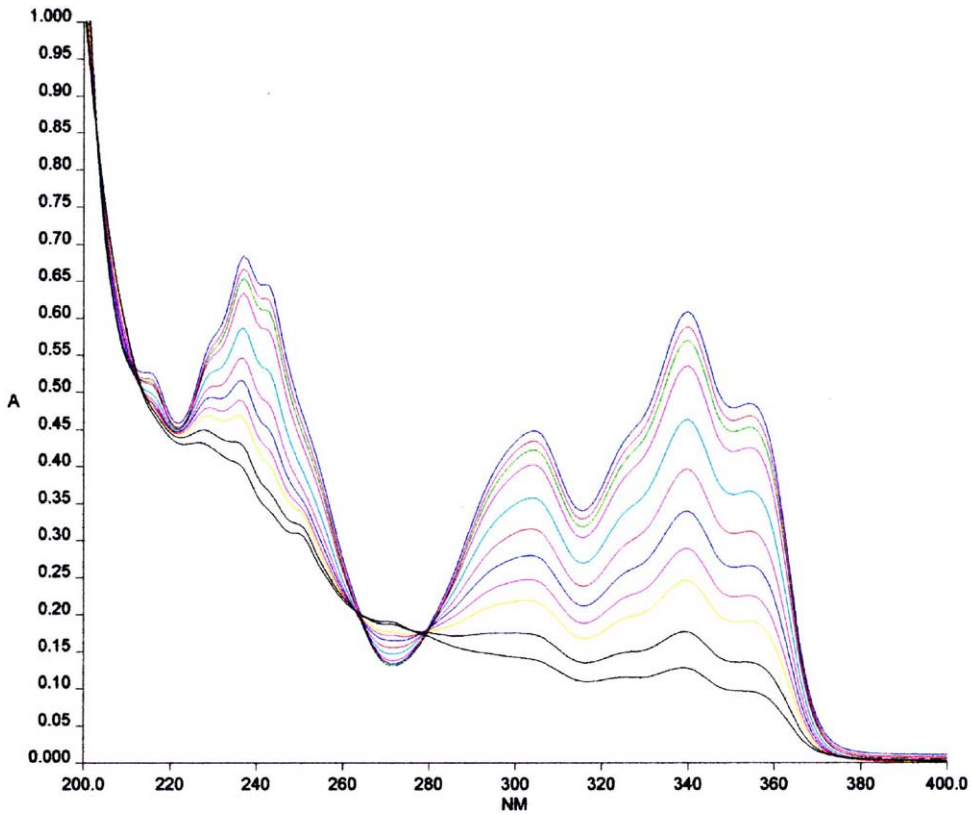

Fig. 3. Spectral changes observed for 3-OH-F after UV-A exposure (max 354 nm). Cyclohexane unbuffered solution.

The quantum yield for the disappearance of the flavonoid was measured at 313 nm up to 10% conversion, when the absorbance contribution arising from the degraded species was negligible, according to the following Eq. (1):

$$-\frac{\mathrm{d}[\text{3-OH-F}]}{\mathrm{d}t} = \Phi\frac{F}{V}I \quad (1)$$

where F is the light portion absorbed by the molecule ($1–10^{-A}$), I is light intensity at the irradiation wavelength and V is the volume of the irradiated solution. Since Φ value was found to be nearly one and according to Eq. (2):

$$-\frac{\mathrm{d}[\text{3-OH-F}]}{\mathrm{d}t} = \frac{\mathrm{d}[\text{photoproduct}]}{\mathrm{d}t} \quad (2)$$

it can be argued that no stable intermediates are formed, as confirmed by the invariability of the isosbestic points.

It is noteworthy that the photochemical reactions in ACN and Cy are not affected by molecular oxygen, which on the contrary plays a decisive role when the solvent is methanol. It can be assumed that in the latter case the flavonoid molecule undergoes a gradual decomposition (photo-oxidation), while a photo-rearrangement occurs in the former cases (ACN and Cy), mediated by intramolecular proton transfer in the excited state (ESIPT), as elsewhere mentioned.

This process is hindered in MeOH, since the strong solvent–solute interactions (H-bonding) become competitive with the intramolecular bonding, hence affecting the excited state decay.

Irradiation at 254 nm resulted in a higher photodegradation rate in all the solvents, as it was predictable because of the greater energy of UV-C radiation with respect to UV-A (350 nm). Above all, the benzenoid absorption band was the most affected one, due both to the wavelength of the incident light (254 nm), and to the higher absorbitivity in this spectral range. The degradation profile of the molecule showed a negative exponential plot of the residual absorbance against irradiation time, consistently with an apparent first-order kinetic (see Fig. 4 for an example).

For quantitative applications, a linear relationship was found between absorbance at a fixed wavelength (237 nm) and the drug concentration. Calibration curves for 3-OH-F were obtained (in triplicate)

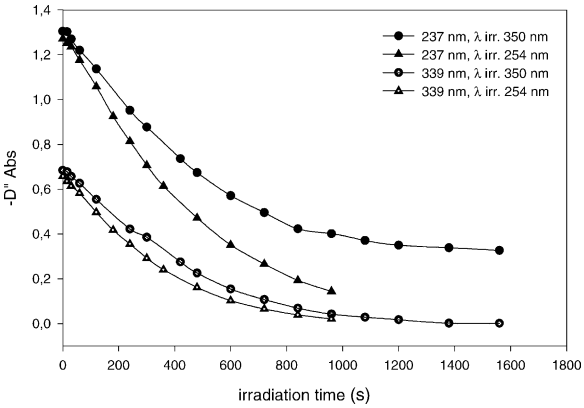

Fig. 4. Photodegradation profile of 3-OH-F upon UV-A and UV-C exposure. Cyclohexane unbuffered solutions.

in the concentration interval ranging from 2.37 to 23.7 $\mu g\,ml^{-1}$ and average absorbance values were subjected to linear regression analysis. Intercept, slope and correlation coefficient (r^2) of the calibration curves are summarized in Table 1.

3-OH-F in solution shows an equilibrium with its anionic and cationic forms, as a function of the acidic strength of the medium. Unbuffered solutions (pH 5.4) are characterized by the presence of the neutral form of the molecule, as revealed by the strong absorption band in the near UV, which is typical of π, π^* electronic transitions. These are generally the most pronounced ones and furthermore receive a positive contribution from polar solvents. When varying the pH of the medium, a significant batocrhomic shift could be observed at higher pH values (see Fig. 5), arising from salification of the free 3-OH group.

For the photolysis study, 0.375 ml of 3-OH-F/ACN solution (2×10^{-4} M) where diluted up to 2.5 ml with the phosphate buffer solutions. Irradiation of the buffered 3-OH-F solutions in ACN (pH 3.0, 7.4 and 10.0) showed how the different acidic strength of the medium influences not only the shape of the

Table 1
Linearity parameters for 3-OH-F UV-Vis determination

	ACN	MeOH	Cy
r^2	0.99993	0.99985	0.99986
Slope	69.58991	66.13308	82.4417
Intercept	0.002285	0.01725	0.02924

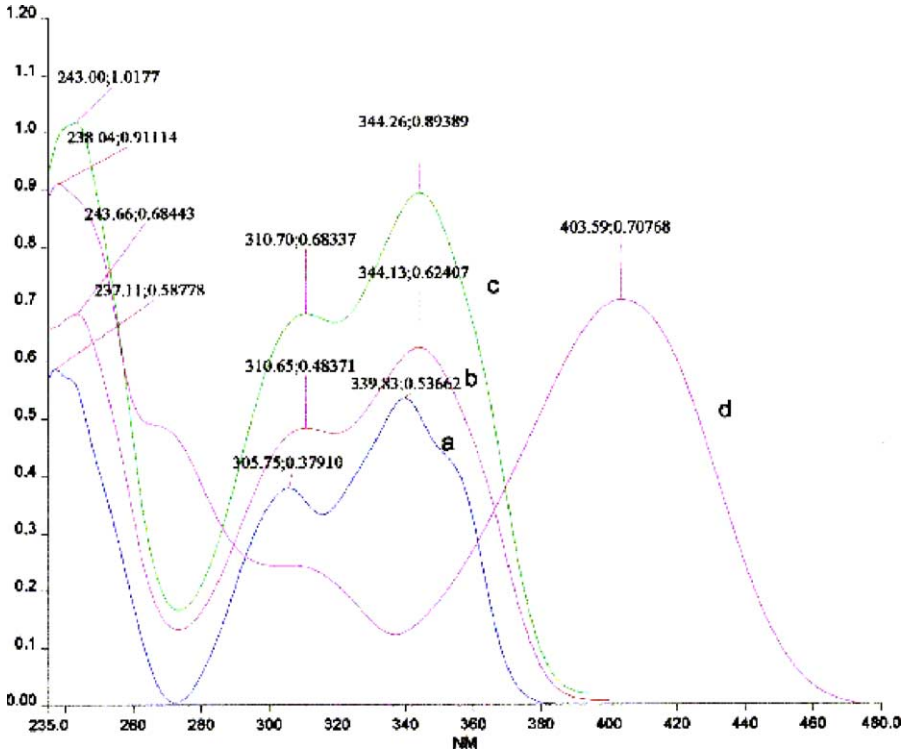

Fig. 5. UV-absorption spectra of 3-OH-F in acetonitrile: unbuffered solution (a); pH 3.0 phosphate buffer (b); pH 7.4 (c); pH 10.0 (d).

spectrum before irradiation, but also the degradation of the molecule upon light exposure (Fig. 6a–d). In more detail, the rate of photolysis was found to increase with the pH value of the medium; in all cases the photodegradation pathway was quite different from that in unbuffered solutions and new absorption contributes relevant to photoproducts were not longer detectable.

3.2. Photolysis of the inclusion complexes

Inclusion complexes of 3-OH-F with α- and β-CyD were obtained in solution by the co-precipitation method, from acetone:water (1:1, v/v) mixtures containing equimolecular amounts of the "host" and "guest" molecules. Inclusion compounds were characterized by means of DSC, UV-Vis, FT-IR, XRPD, ^{1}H-NMR analysis. Phase solubility studies gave a 1:1 stoichiometry of the complexes [17]. Spectrophotometric analysis and photolysis studies of the inclusion complexes (6×10^{-5} M 3-OH-F) were performed in water-acetonitrile (1:1, v/v) mixtures. In the UV-absorption spectra, a decrease in the molar extinction coefficients was observed, in both cases, in the presence of the CyD-microenvironment. Besides, a slight blue-shift of the maxima was noticeable, due to the "shielding" effect of the cavity on the main absorption bands, which arise from π, π* electronic transitions.

Unbuffered solutions of the host-guest systems (3-OH-F/α-CyD and 3-OH-F/β-CyD) exposed to UV-A radiation showed enhanced photostability with respect to the free molecule.

Measurements performed to 10% conversion of the starting compound revealed a ca. three-fold reduction of the photodegradation quantum yield, compared to that observed in the absence of CyDs.

A plot of the absorbance changes at 343 nm (maximum of band II) as a function of irradiation time, performed both in the presence and in the absence of α- and β-CyD, showed significant differences in the efficiency of the photochemical reaction. After 330 s irradiation, the absorbance decrease was 44.46% for 3-OH-F/α-CyD system and 38.06% for

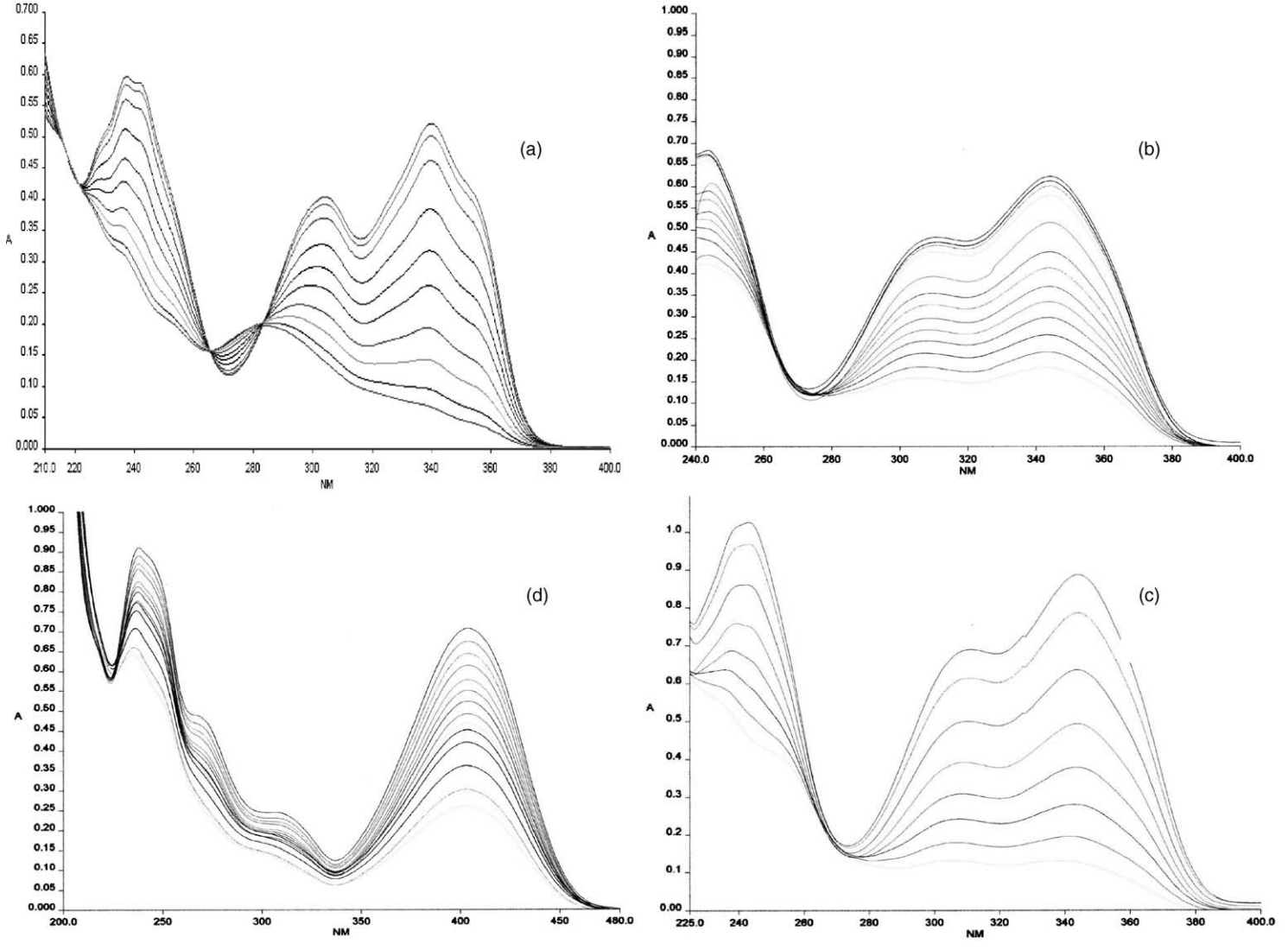

Fig. 6. Set of UV spectra obtained during photolysis of 3-OH-F/acetonitrile solutions: unbuffered solution (a); pH 3.0 phosphate buffer (b); pH 7.4 (c); pH 10.0 (d). Irradiation at 254 nm.

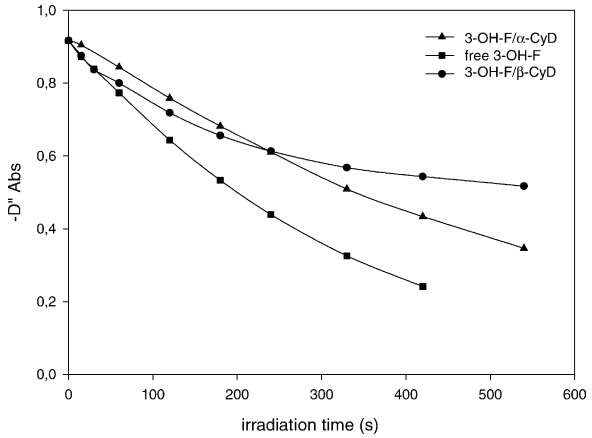

Fig. 7. Effect of CyD-complexation on 3-OH-F photolysis upon 354 nm irradiation (each point is the mean of three determination, average S.D. = 0.0055). Residual light absorption at 343 nm.

3-OH-F/β-CyD system, compared to 64.49% of the free molecule (Fig. 7).

Moreover, the formation of any photoproducts was quite suppressed, as only a gradual decrease of the absorption bands was evident, while maxima of absorbance remained unchanged.

4. Conclusions

The main evidence is that in all the buffered 3-hydroxyflavone solutions, no photoproducts were formed upon light irradiation, at any of the chosen pH values (pH 3.0, 7.4 and 10.0). On the other hand, the formation of a stable photoproduct was evident in the unbuffered 3-OH-F solution (pH 5.4), when the solvent was aprotic like acetonitrile and especially cyclohexane.

Table 2
Extent of 3-OH-F photodegradation as affected by pH

Solution	Time	Absorbance (∼339 nm)	Absorbance decrease (%)
Unbuffered[a]	0	0.344	
	4′	0.153	55.52
pH 3.0	0	0.182	
	4′	0.088	51.64
pH 7.4	0	0.257	
	4′	0.107	58.36
pH 10.0	0	0.098	
	4′	0.036	63.26

Acetonitrile solutions upon 254 nm irradiation. Photodegradation rate order: pH 3 < unbuffered < pH 7.4 < pH 10.0.

[a] pH=5.4.

This later evidence strengthens the hypothesis of an excited state intramolecular proton transfer. Most likely, the free 3-hydroxy group is kept from interacting with the adjacent carbonyl group, by the presence of the buffer.

Photo-rearrangement is likewise hampered in methanol, due to the strong solvent–solute interactions which become competitive with the intramolecular H-bonding.

As a further proof, the photolysis rate of 3-OH-F under UV irradiation was found to be pH-dependent, as summarized in Table 2 (for the decrease in absorbance at 339 nm).

Whatever the pH and the solvent, all experiments exhibited the expected first-order kinetics. As for the unbuffered 3-OH-F solutions, the change in pH was very rapid upon irradiation, yielding a gradual increase in $[H^+]$ as a function of light exposure time (measured pH value after ca. 50 min irradiation was 8.4).

Table 3
Extent of 3-OH-F photodegradation as affected by solvent

Solution	Time	Absorbance (∼237 nm)	Absorbance decrease (%)	Absorbance (∼343 nm)	Absorbance decrease (%)
ACN	0	0.705		0.344	
	6′	0.209	70.35	0.059	82.84
Cy	0	1.272		0.657	
	6′	0.613	51.80	0.240	63.47
MeOH	0	0.655		0.207	
	6′	0.171	73.89	0.063	69.56

Unbuffered solutions upon 254 nm irradiation.

The photostability of 3-OH-F in solution showed to be much affected also by polarity and proticity of the solvents employed, following the order: cyclohexane < acetonitrile < methanol (data are summarized in Table 3).

The inclusion of the molecule into α- and β-CyD cavity was effective in reducing the extent of photodegradation of the flavonoid. Moreover, the interaction with the host cage was responsible for remarkable changes in the drug photoreactivity, although a rationale role for the CyD-induced events cannot be proposed in this context.

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