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Spectrochimica Acta Part A 58 (2002) 83–89

SPECTROCHIMICA ACTA PART A

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Time resolved fluorescence spectroscopy of quercetin and morin complexes with Al^{3+}

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Received 9 March 2001; accepted 26 April 2001

Abstract

The association process of Al^{3+} with quercetin and morin in methanol was studied by electronic absorption and emission spectroscopies. The number of species in solution with different absorption spectra were determined by the method of Rank analysis of the absorbance matrix, and the stoichiometries of the complexes were evaluated using the Job method. The number of fluorescent species in solution were calculated from the Rank analysis method of the time resolved emission spectra (TRES), and compared with a global analysis of the decay surface using a proper multi-exponential decay model. The association of Al^{3+} with morin gives rise to two complexes with 1:1 and 2:1 (morin: Al^{3+}) stoichiometries, but in both species the association of the cation involves the carbonyl and 3-hydroxyl groups of the pyrone ring. The fluorescence decay surface of this system is biexponential and the lifetimes of the 1:1 and 2:1 complexes are 4.3 and 2.0 ns, respectively. The association of Al^{3+} with quercetin forms preferentially two complexes with 1:1 and 1:2 (quercetin: Al^{3+}) stoichiometries where the first cation binds to the site of the pyrone ring but the second one is bound to the cathecol group of the molecule. However, the multichelation character of the quercetin ligand allows larger aggregates to be formed, thereby the species Al_2Q_3 is also detected in methanol. The lifetime of the 1:1 complex is about 2.7 ns, while for 1:2 and 3:2 complexes the lifetimes measured are 3.5 and 1.8 ns, respectively.

Keywords: Flavonoids; Aluminum (III); Chelation; Fluorescence lifetimes; TRES and Rank analysis

1. Introduction

Quercetin and morin are phenolic compounds derived from hydroxyl substitutions on the flavone chromophore. They complexate with metal cations to form stable products which in several cases are highly fluorescent, a property which has been explored in analytical methods of metal and ligand identification [1–10]. For instance, quercetin and morin are well known reagents for determination of aluminum(III) traces in water and in biological samples. Besides these analytical applications, the chelation of mono and polyhydroxy flavones with cations is an important factor in their bioactivity as carriers and regulators of metal concentration. Free or

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1386-1425/02/$ - see front matter
PII: S1386-1425(02)00515-7

complexated, these compounds are also anti-oxidants and free radical scavengers in biological systems [5,11–13].

The enhancement of the fluorescence signal upon chelation of flavones with a nonparamagnetic metal is related to the inhibition of the excited state intramolecular proton transfer (ESPT) process [14–21] between hydroxyl and 4-keto groups of the cromone ring. The ESPT mechanism, which occurs in several hydroxyl substituted flavones, give rises to a fast excited state equilibrium between the normal and tautomeric forms, and therefore to dual fluorescence usually with low emission quantum yields at room temperature.

Most of the studies of quercetin and morin association with metals have focused attention on the investigation of the stoichiometry of the complexes and determination of possible sites of binding [4–10]. In the present work, these points are also addressed, but are discussed together with the electronic excited state properties of quercetin and morin complexes with aluminum (III) in methanol solution.

The number of species in solution with different absorption spectra is determined by the Rank analysis method of the absorbance matrix, and the stoichiometry of the complexes is evaluated using the Job method. The number of fluorescent species in solution is defined by the Rank analysis method of the time resolved emission spectra (TRES) matrix. The lifetime of the complexes in solution is then determined from a global analysis of the decay surface using a proper multi-exponential decay model.

2. Experimental

The flavonoids quercetin and morin (Aldrich) were recrystallized from ethanol, and $Al(NO_3)_3.9H_2O$ (synth. $>99.5\%$) was used as received. Stock solutions of the reagents were prepared in methanol at 1 mM concentration. In all samples for absorption and emission spectroscopy measurements, the flavonoid concentration was 10 μM obtained from dilution of the stock solutions. The concentration of Al(III) was varied by the addition of small amounts of its stock solution, via a microsyringe, followed by a stirring period to allow the equilibrium condition of the complexation process to be reached. The systems were also investigated in acidic methanol solution containing 0.01 M of nitric acid.

Absorption measurements were performed on a Hitachi U-2000 spectrophotometer. Corrected steady-state fluorescence spectra were recorded on a CD-900 Edinburgh spectrofluorimeter. For measurements, air equilibrated samples in 1 cm quartz cuvettes were thermostated at 298 K. Fluorescence quantum yields were determined by using as standard the dye acridine orange, assuming a value of 0.4 in methanol. Fluorescence decay surfaces were measured by time correlated single-photon counting technique using a CD-900 Edinburgh spectrometer operating with a hydrogen-filled nanosecond flash lamp at 40 kHz pulse frequency. The data were analyzed using global multiexponential and time resolved emission spectra (TRES) routines of the Edinburgh Instruments Level 2 software.

In the Rank analysis of the absorbance matrices, a modified version of the program Triang by Hartley, Burgess and Alcock was used [22]. In the case of the TRES data, the number of counts of the traces were divided by the maximum value to form a scaled emission spectra matrix for Rank analysis. The Rank or the number of species in solution with distinct spectra corresponds to the number of diagonal elements of the trigonal matrix which are larger in module than three times the corresponding value of the reduced error matrix. The Rank is determined as a function of the error assumed in the measurements.

3. Results and discussion

3.1. Absorption spectroscopy

The addition of Al^{3+} to a quercetin solution in methanol results in significant change of the absorbance spectrum of the flavonoid solution, with the appearance of a new band centered on 430 nm with a bathochromic shift of about 58 nm from the original band in absence of the metal. A

typical example of the spectral changes observed upon addition of Al^{3+} to quercetin solution is illustrated in Fig. 1.

In the case of morin solution, the addition of Al^{3+} gives first a hipsochromic shift with a change of the maximum from 380 nm to 355 nm, and the development of a shoulder at around 420 nm. Further addition of the metal leads to the growth of a new band centered on 420 nm, with an isosbestic point at 380 nm. Fig. 2 shows the two stage change of the absorption spectrum of the Morin/Al system. When the spectra of the flavonoids are taken in acidic methanol solution, the absorption bands observed are practically the same as before, but the growth of the new band in the red region of the spectrum is reduced in both cases indicating that the amount of the new species formed by metal complexation has changed. For the morin/Al system however, the initial behavior observed in methanol disappeared in acid medium. This result suggests that the hipsochromic shift of the absorption spectrum of morin upon addition of a small amount of $Al(NO_3)_3$ may be related to a change in the dissociation equilibrium of the 4′–hydroxyl group of morin ($pK_a = 9.0$) when adding the acid salt of Al.

All the spectral data obtained can be further analysed by looking at the number of species in solution with a distinct absorbance spectrum,

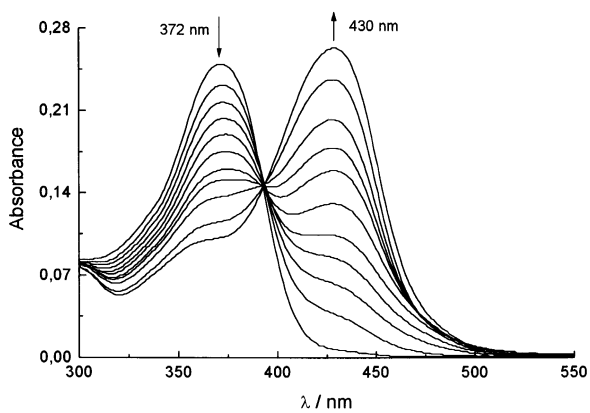


Fig. 1. Electronic absorption spectra of quercetin (10 μM) in methanol during titration with Al(III), concentration from 0–10 μM, $T = 298$ K.

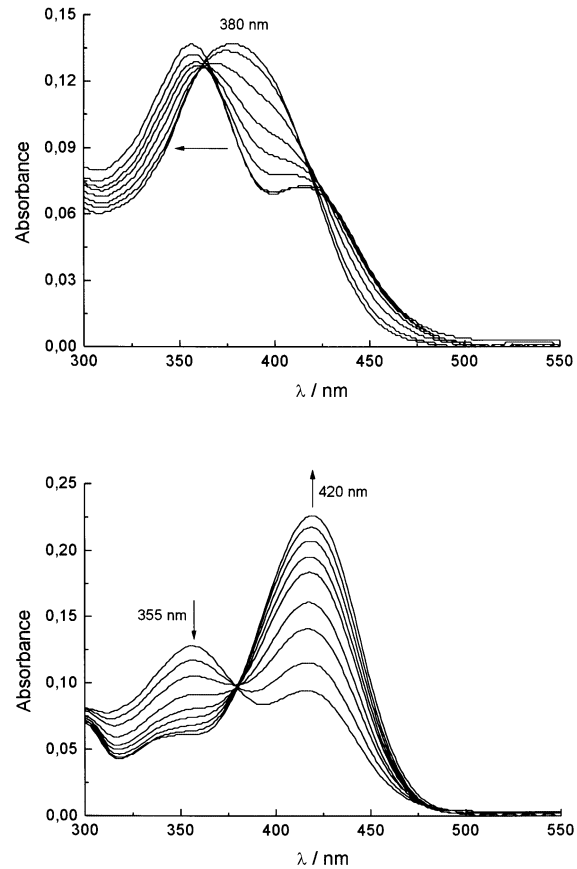


Fig. 2. Electronic absorption spectra of morin (10 μM) in methanol during titration with Al(III). Concentration range: 0–4 μM (top); 4–10 μM (bottom).

which corresponds to determination of the Rank of the absorbance matrix for a given error in the measurement. A typical plot of the number of species in solution as a function of the error in the measurement, taking the case of the quercetin/Al system, is illustrated in Fig. 3. For absorbance error in the range of 0.01 to 0.002, which corresponds to the longer flat line of the plot, the number of species for quercetin/Al is three and two in neutral and acidic methanol solution, respectively. For morin/Al system, the number of species depends on concentration of the added metal. From 0–10 μM of Al^{3+}, the number of species found in methanol is three. However, if the spectral analysis is performed excluding the data of the first additions of the metal, for in-

stance using only the spectral data for [Al] = 0.4 to 10 μM, the number of species is reduced to two. In acidic methanol solution, the number of species in solution with distinct absorption spectra is two, and it does not depend on the concentration range of added metal.

The stoichiometry of the complexation was investigated by using the Job method [22]. Considering a global equilibrium of Al (III) and n ligands (L) on the form,

$$\mathrm{Al} + n\mathrm{L} = \mathrm{AlL}_n, \quad (1)$$

n is determined from the plot of the absorbance as a function of the mole fraction x of the added ligand. In the absorbance maximum,

$$n = \frac{x_{\max}}{1 - x_{\max}} \quad (2)$$

A typical plot according to the Job method is presented in Fig. 4 for the Al/morin system in methanol and in acidic methanol. In methanol, the most probable stoichiometry is $n = 2$, while in acid medium, $n = 1$.

In the case of quercetin, the stoichiometry is well defined in acidic methanol where $n = 1$, but in methanol solution, more than one type of complex is formed, giving rise to species like Al_2Q, AlQ and Al_2Q_3. The Job plots for quercetin/Al system in methanol are presented in Fig. 5.

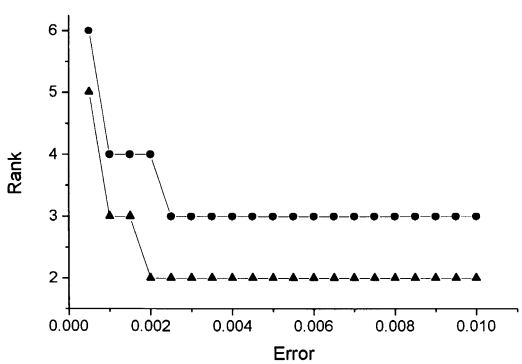


Fig. 3. The number of species in solution (Rank) of Quercetin/Al(III) as a function of the error in absorbance measurements. (●) methanol solution; (▲) methanol + 0.01 M HNO_3.

Fig. 4. Job plot of the Morin/Al(III) system. Absorbance measurements at 420 nm. (●) methanol solution; (▲) methanol + 0.01 M HNO_3.

3.2. Fluorescence spectroscopy

The addition of Al to quercetin and morin solutions gives rise in both cases to an intense fluorescence signal which increases with metal concentration. Excitation and emission spectra for the quercetin/Al system are shown in Fig. 6 for illustration. However, no time-resolved data have been reported and analyzed with discussion of the excited state properties in correlation with the number of complexes formed and their stoichiometries. With this aim, the decay surface of each system is analyzed by the global method [23]. In this procedure, multiexponential decay function with linked lifetimes at different emission

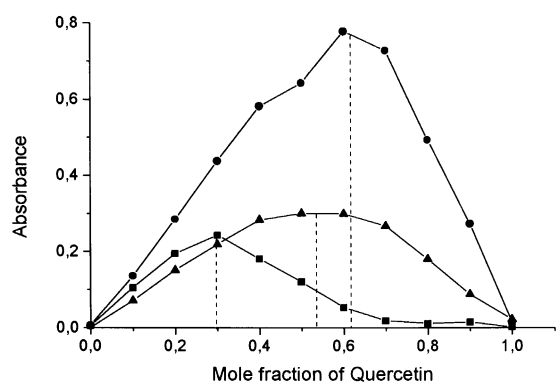


Fig. 5. Job plot of the Quercetin/Al(III) system. (●) methanol solution, and (▲) methanol + 0.01 M HNO_3 with absorbance measurements at 430 nm; (■) in methanol at 475 nm.

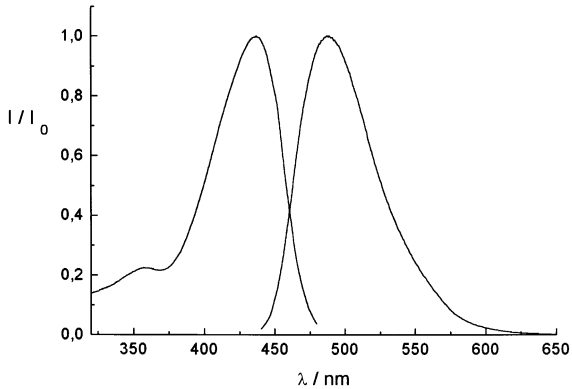


Fig. 6. Normalized excitation and emission spectra of Quercetin/Al(III) in methanol with both species at 10 μM.

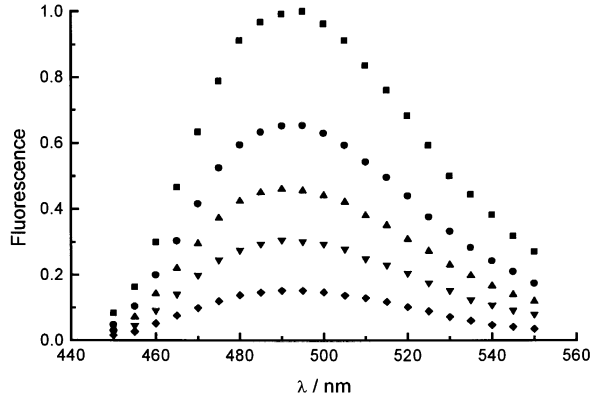


Fig. 7. Scaled TRES data obtained at 3.0, 4.5, 6.0, 7.5, and 9.0 ns after the excitation pulse. [Quercetin] = [Al(III)] = 10 μM, $\lambda_{exc} = 430$ nm, $T = 298$ K.

wavelengths is used as the fitting condition. The results found indicate that decay surface is biexponential in most of the systems and only monoexponential in the case of morin/Al in acidic methanol. The lifetimes measured are listed in Table 1.

Time resolved emission spectra (TRES) may be obtained by slicing the decay surface at different times, and the whole set of spectra may be further investigated by Rank analysis of the emission data. Typical scaled TRES data are shown in Fig. 7. The corresponding number of fluorescent species as a function of the error in the relative intensity is plotted in Fig. 8. The longer flat region indicates the presence of two fluorescent species in the Al/quercetin system, in accordance with the global biexponential fitting and best chi-square value. The Rank of the scaled TRES data for the other systems are reported in Table 1. The result indicates a good correlation between the Rank of the TRES matrix and the number of exponential terms of the fluorescence decay process.

Concerning the results obtained, some useful generalizations may be written. In the morin/Al system in acidic methanol solution, the Rank of the absorbance matrix is 2, the main stoichiometry is 1:1, and the decay is monoexponential. It means that there are two molecular species in solution, the morin (M) and its fluorescence complex AlM with a lifetime of about 4.3 ns. In methanol, however, AlM_2 is formed preferentially but a small fraction of the complex AlM should be present because the Rank of the TRES matrix is 2 and the decay is biexponential. The recovered lifetimes have the values of 2.0 and 4.3 ns indicating that the short lived component may be ascribed to the AlM_2 species.

The association of quercetin with Al depends more critically on concentration of metal and solvent medium. In methanol/0.01 M HNO_3, the species AlQ is formed preferentially. The presence

Table 1
Stoichiometry of the complexes of Al(III) with Quercetin and Morin and their respective lifetimes from global analysis

Solvent	Stoichiometry	Lifetimes[a] (ns)	χ^2_{global}	Rank of TRES
Methanol	Al_2Q; Al_2Q_3	3.5; 1.8	1.090	2
	AlM; AlM_2	4.3; 2.0	1.024	2
Methanol + 0.01 M HNO_3	AlQ[b]	2.7; 4.9	1.102	2
	AlM	4.3	1.126	1

[a] The standard deviations of the lifetimes are about 0.3 ns.
[b] The major species formed. The biexpoential behavior is ascribed to a minor presence of the Al_2Q complex.

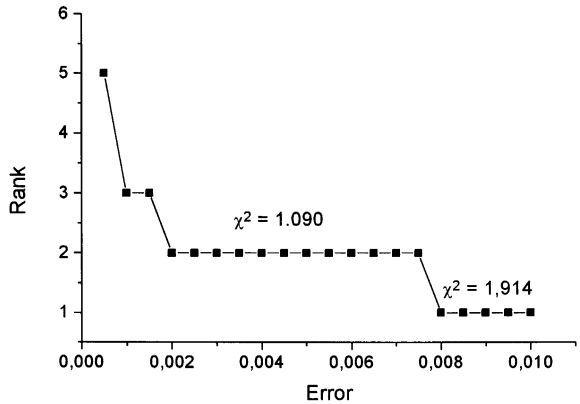


Fig. 8. Rank analysis of the TRES matrix of Fig. 7. χ^2 is the value obtained from global biexponential and monoexponential analysis.

of an intense fluorescence indicates that the binding site in AlQ is the 3-hydroxy and 4-keto groups of the pyrone ring. The decay is biexponential, but the main component with about 90% weight and lifetime of 2.7 ns should correspond to the AlQ complex. In methanol, the Rank of absorbance matrix is 3 indicating that two types of complex plus the free quercetin are in solution. The complexes Al_2Q and Al_2Q_3 are formed in larger amounts. The decay surface is biexponential with components of 1.8 and 3.5 ns. With addition of more Al, the decay becomes slow (see Fig. 9). Global analysis of a decay surface, obtained by measuring the fluorescence signal at different concentrations of Al(III) but at a fixed emission wavelength, indicates that the long lived component with a decay time of 3.5 ns governs the fluorescence deactivation at a high concentration of metal (see inset in Fig. 9). Thus the lifetime of 3.5 ns should be ascribed to Al_2Q formed preferentially when more metal is added. This result is in agreement with the increase in the fluorescence quantum yield (ϕ_F) from 0.08 up to 0.12 with concentration of Al(III).

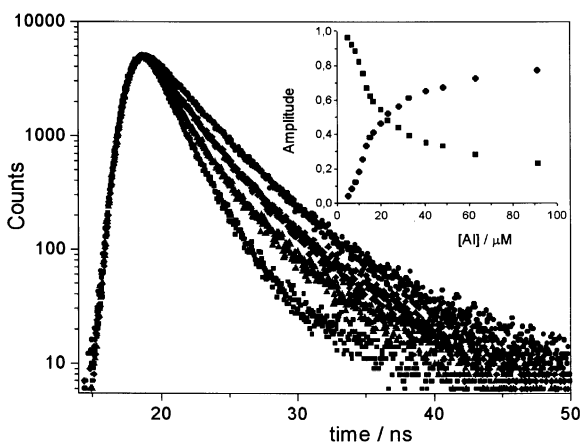


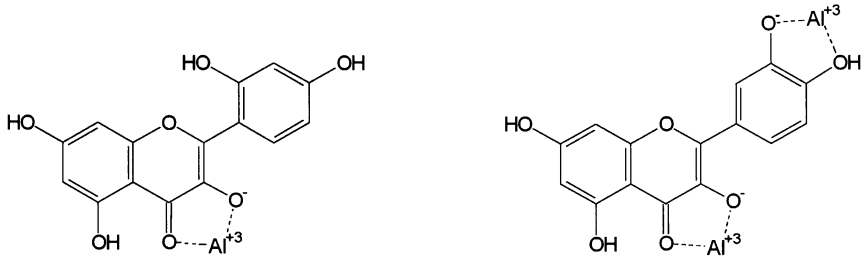
Fig. 9. Fluorescence decays of Quercetin/Al(III) system in methanol. [Quercetin] = 30 μM, [Al(III)] = 5, 10, 20, 80 μM from bottom to top. $\lambda_{exc} = 430$ nm, $\lambda_{em} = 495$ nm, $T = 298$ K. Inset: normalized amplitudes of the biexponential global fitting as a function of metal concentration. $\chi^2 = 1.072$; (■) $\tau_1 = 1.8$ ns; (●) $\tau_2 = 3.5$ ns.

The major species in acid medium are the complexes AlQ and AlM, and their ϕ_F values measured were 0.3 and 0.6, respectively. For the Al/morin system in methanol, the ϕ_F values of the AlM and AlM_2 complexes, measured in 5:1 and 1:5 metal:ligand concentration ratios, were 0.5 and 0.2, respectively. Note that the quantum yields measured are correlated with the lifetimes of the complexes (see Table 1). Since $\phi_F = k_F\tau$, the species with higher ϕ_F would have longer lifetimes if the radiative rate constant k_F remained constant.

The main sites of Al(III) binding to morin and quercetin are shown in the molecular models of Fig. 10. It should be emphasized that in all systems investigated, the $\pi^* \rightarrow \pi$ singlet emission appears because the binding of Al^{3+} to the carbonyl and 3-hydroxyl groups of the pyrone ring inhibits the intramolecular proton transfer in this site, which forms a nonemissive or low emission phototautomer at room temperature. In the samples investigated the fluorescence occurs always from the anion form of the 7-hydroxy group of the pyrone ring because the pK_a of the excited state is -2.2, the pK_a of the ground state is 7.4, and the photodissociation is a very rapid process according to results by Wolfbeis at al. [24].

4. Conclusions

In this contribution, several spectroscopy methods, including Rank analysis of absorbance and



Morin Quercetin

Fig. 10. Molecular models of Al(III) complexation with Morin and Quercetin.

TRES matrixes, stoichiometry by Job method, and global fitting with exponential decay functions, are used to determine the properties of the complexes formed by quercetin and morin with the cation Al(III) in methanol solution. The results obtained here are in agreement with previous studies [4–10] confirming that morin forms AlM and AlM_2 complexes, while quercetin is more likely to generate the AlQ and Al_2Q species, but larger aggregates, like the Al_2Q_3 complex, are not ruled out. The lifetimes of these species are determined. In general, the complexes with more than one ligand have a shorter lifetime than the monoligand complex, and therefore they contribute less to the fluorescence quantum yield in solution.

Acknowledgements

Financial support by FAPESP (Brazil) is gratefully acknowledged. ACG thanks CNPq for a graduate fellowship.

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