JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2000; **13**: 141–150

Acidichromic effects in 1,2-di- and 1,2,4-trihydroxyanthraquinones. A spectrophotometric and fluorimetric study

C. Miliani, A. Romani and G. Favaro*

Dipartimento di Chimica, Università di Perugia, 06123 Perugia, Italy

Received 2 August 1999; revised 8 October 1999; accepted 11 October 1999

ABSTRACT: Absorption and fluorescence spectra of alizarin (1,2-dihydroxyanthraquinone) and purpurin (1,2,4-trihydroxyanthraquinone) were investigated as a function of pH in water–dioxane (2:1, v/v) in the pH range 2–14. Absorbance changes with pH are accompanied by color changes; by increasing the pH, the spectra shift to the red. These molecules exhibit fluorescence emissions over the whole pH range explored; fluorescence quantum yields increase with increasing pH and are greater for purpurin ($\Phi_F = 10^{-3}$–10^{-2}) than for alizarin ($\Phi_F = 10^{-4}$–10^{-3}). The fluorescence lifetimes were measured by the phase-shift technique for the three species derived from purpurin (neutral, τ = 150 ps; mono-anion, τ = 2600 ps and di-anion, τ = 380 ps), and only for the di-anion of alizarin (τ = 80 ps). Both spectrophotometric and fluorimetric titrations gave p*K*s of the first and second dissociation steps in the ground state. Excited-state p*K**s were calculated by the Förster cycle. In the ground state, purpurin (pK_1 = 4.7; pK_2 = 9.5) is a stronger acid than alizarin (pK_1 = 6.6; pK_2 = 12.4) in both the first and second dissociation steps; in the excited state, purpurin is a weaker acid in both dissociation steps. Copyright © 2000 John Wiley & Sons, Ltd.

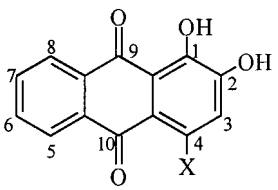
KEYWORDS: 1,2-dihydroxyanthraquinone; 1,2,4-trihydroxyanthraquinone; acidichromism; fluorescence yield; fluorescence lifetime; ground-state p*K*; excited-state p*K**

INTRODUCTION

Hydroxyanthraquinones are a class of molecules which have attracted wide interest from both applied and fundamental points of view. Their chromophore is contained in the anti-tumor anthracycline[1] and their activity in photo-induced intramolecular proton transfer makes them potential candidates for molecular storage devices.[2] Compared with anthraquinone, which has the lowest excited singlet state of n,π* character, the π,π* excitation energy of hydroxyanthraquinones is lower than the n,π*excitation energy.[3] Consequently, hydroxyanthraquinones absorb visible light and are colored, whereas anthraquinone is colorless. Most applications are based on their chromatic properties, which depend on the position and number of the hydroxy substituents. Owing to their relatively high triplet yields, they may work as photosensitizers of electron-transfer induced reactions.[4]

In this paper attention is focused on two hydroxyanthraquinones, alizarin and purpurin (Scheme 1). A mixture of alizarin and purpurin constitutes the red dye madder,[5,6] a colorant used in painting, which has been obtained from madder root (*Rubia tinctorum*) since 3000 BC. This colorant is also called Madder Lake because it is generally precipitated on to an inert inorganic substrate ($Al_2O_3 \cdot nH_2O$).

Since paintings are subject to deterioration because of aging and also due to atmospheric pollutants, it is important to know which chromatic changes they undergo when the environment changes. In recent decades, acidic rain has been one of the most important causes of pollution and may have attacked painted artifacts. One of the reasons why we undertook this study on the effect of pH on the absorption and fluorescence spectra of alizarin and purpurin is to contribute to the knowledge about the



X = H: Alizarin (1,2-HAQ)

X = OH: Purpurin (1,2,4-HAQ)

Scheme 1

**Correspondence to:* G. Favaro, Dipartimento di Chimica, Università di Perugia, 06123 Perugia, Italy.
Contract/grant sponsor: Consiglio Nazionale delle Ricerche.
Contract/grant sponsor: Ministero per l'Universitá e la Ricerca Scientifica e Technologica.

degradation processes in artistic paintings. Moreover, these molecules represent a special case where a modest structural change induces drastic modifications in the absorption and mainly fluorescence characteristics in organic solvents.[7] The different spectral behaviors of the two molecules have been explained considering the relative stability of intramolecular hydrogen bonding in the ground and in the excited states and on the basis of literature findings regarding similar molecules.[8–10] For 1,2,4-HAQ, the intramolecular hydrogen bond is maintained in the excited state with little variation in the molecular geometry. For 1,2-HAQ, the intramolecular hydrogen-bonded form is the most stable form in the ground state, whereas excited-state intramolecular proton transfer (ESIPT) from the phenolic hydroxyl to the carbonyl oxygen occurs upon excitation with a great change in molecular geometry. Since these molecules have acidic centers that are close to each other, studying protolytic equilibrium may also provide information about their reciprocal influence and possible interactions with the carbonyl groups.

EXPERIMENTAL

Materials. Alizarin (1,2-dihydroxyanthraquinone, 1,2-HAQ) and purpurin (1,2,4-trihydroxyanthraquinone, 1,2,4-HAQ) were purchased from Aldrich and used without further purification. Dioxane was a Fluka spectrograde product. Doubly distilled water was used to prepare the buffers for the p*K* measurements.

Apparatus. The absorption spectra were recorded with a Perkin-Elmer Lambda 16 spectrophotometer. The fluorescence spectra were recorded using a Spex Fluorolog-2 FL 112 spectrofluorimeter controlled by the Spex DM3000F spectroscopy software. This instrument gives corrected emission spectra and is equipped with an accessory for fluorescence lifetime measurements, which is based on the phase-shift method (time resolution within 30 ps). The data are processed by means of the software Global Unlimited 3.0, which allows the analysis of multiple decays up to four components.

Spectrophotometric and fluorimetric titrations. To determine the p*K*s, water–dioxane solutions (2:1, v/v) were used for solubility requirements. Britton buffers at low ionic strength ($\mu = 0.01$ mol dm^{-3}) or, in the pH 7–10 region, Tris–HCl buffers (from Aldrich), were added to the dioxane solutions of the colorants. The pH values were measured in the aqueous medium with an Orion SA-520 pH meter. Control pH measurements were also carried out in the mixed solvent and gave pH values about 0.8 units above those determined in water in the whole pH range explored. The dissociation constants of the hydroxyanthraquinones were obtained from the inflection points in the absorbance vs pH curves at the wavelengths of maximum difference in absorbance of the equilibrating species. In the fluorimetric titrations, the excitation was carried out at an isosbestic point between the spectra of the equilibrating species; the dissociation constants were obtained from the inflection points of the emission intensity vs pH curves at wavelengths where the maximum sensitivity could be obtained. Sample concentrations were of the order of 3.0×10^{-5} mol dm^{-3}. Given the low concentration of the solutions, the activity coefficient ratio of the acid–base couple was considered to be unity.

Fluorescence quantum yields and lifetimes. The emission quantum yields (Φ_F) were obtained by comparing corrected areas of the sample and the standard (quinine sulfate in 1 N H_2SO_4, $\Phi_{ST} = 0.546$)[11] emissions, using Eqn. (1) which accounts for the differences in absorbance and refraction index of the sample (A_S, n_S) and standard (A_{ST}, n_{ST}) solutions:

$$\Phi_F = \Phi_{ST}(A_{ST}/A_S)(\text{area}_S/\text{area}_{ST})(n_S{}^2/n_{ST}{}^2) \qquad (1)$$

Sample concentrations were adjusted in order to keep the absorbance within the range 0.2–0.5. The solvent contribution to the emission signal was subtracted, when necessary. The accuracy in the Φ_F values is estimated to be within 10% for $\Phi_F \geq 10^{-3}$ and within 20% for $\Phi_F < 10^{-3}$.

The emission lifetimes (τ_F) were determined by using the Pockels cell of the Spex spectrofluorimeter accessory based on the phase-shift method. The light scattered by an aqueous glycogen solution (undetectable fluorescence) was used as a standard. The accuracy of the data obtained depended on the intensity of the emission.

All measurements were performed at room temperature (20 ± 2 °C), using freshly prepared solutions.

RESULTS

Owing to the scant solubility of these molecules in water, the pH effects on the absorption and emission spectra of 1,2-HAQ and 1,2,4-HAQ were investigated in a mixed solvent [water–dioxane (2:1, v/v)]. For both molecules, three species, characterized by distinct absorption and emission spectra, were detected in the pH range explored (pH 2 –14). Each of them was stable within a limited pH interval and was the precursor for the one formed successively as the pH changed, as demonstrated by the presence of well defined isosbestic points in the absorption spectra and reversibility of the transformation. Based on these observations, the species were assigned to the neutral form (acidic solutions), mono-anion (neutral and moderately basic solutions) and di-anion (basic solutions).

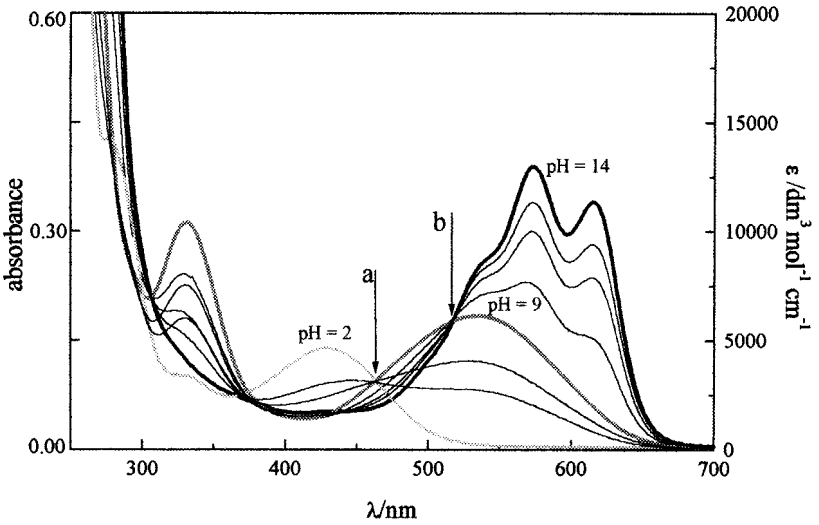


Figure 1. Evolution of the absorption spectrum of 1,2-HAQ (3.0×10^{-5} mol dm^{-3}) in water–dioxane solutions (2:1, v/v) as a function of pH. Light grey, pH = 2 (neutral form); grey, pH = 9 (mono-anion); black, pH = 14 (di-anion). Arrows indicate isosbestic points between the neutral form and mono-anion (a, 463 nm) and between the mono-anion and di-anion (b, 516 nm)

Spectral characteristics

1.2-HAQ. *Absorption spectra.* In acidic solutions (pH $\leq$5), the absorption spectrum of 1,2-HAQ showed a maximum at 430 nm, shifted to the red compared with a polar organic solvent (λ_{max} = 421 nm, in acetonitrile).[7] This absorption corresponds to the neutral molecule. The red spectral shift with respect to acetonitrile is due to hydrogen bonding with the solvent. With decreasing acidity, the absorption shifted to longer wavelengths and the solution turned from yellow to pale pink. A net isosbestic point was observed at 463 nm. In the pH 8–10 range, the spectrum did not show appreciable variations; the maximum was located at 540 nm. This spectrum is assigned to the mono-anion. A further pH increase caused an enhancement of absorbance with a further shift of the spectrum to the red (isosbestic point at 516 nm) and the color turned to blue. At pH $\geq$13, the spectral shape and intensity remained constant. This new spectrum, which is assigned to the di-anion, is vibronically structured and shows maxima located at 540, 573 and 615 nm. The spectral evolution of a 3.1×10^{-5} mol dm^{-3} solution of 1,2-HAQ in water–dioxane is shown in Fig. 1; absorption maxima and molar absorption coefficients of the three species are reported in Table 1.

Fluorescence spectra. All species derived from 1,2-HAQ by changing the acidity of the solution are fluorescent; the fluorescence quantum yield critically depends on the degree of protonation. The emission intensity increases from the neutral molecule ($\Phi_F = 5.1 \times 10^{-4}$) to the mono-anion ($\Phi_F = 1.1 \times 10^{-3}$) to the di-anion ($\Phi_F = 3.1 \times 10^{-3}$). The quantum yields are within the order of magnitude of those reported in the literature for 1,2-HAQ in organic solvents.[7,12,13] The fluorescence spectra are broad and structureless; two scarcely resolved peaks were only detected in a basic solution. Parallel to absorption, the fluorescence maxima shift to the red with increasing pH (λ_{max} = 485, 625 and 682 nm for the neutral molecule, mono-anion and di-anion, respectively). The Stokes shifts, calculated using the maxima of the absorption and fluorescence spectra, are similar for the three species. Absorption and fluorescence spectra of the three species (normalized on the maximum) are shown in Fig. 2;

Table 1. Absorption maxima (λ_{max}), molar absorption coefficients (ε), fluorescence maxima and Stokes shifts ($\Delta\nu$) of 1,2-HAQ at different protonation degrees (neutral molecule, mono-anion and di-anion) in buffered water–dioxane solutions (2:1, v/v)

Species	pH	Absorption λ_{max}/nm (ε/dm^{3}mol^{-1}cm^{-1})	$\Delta\nu$/cm^{-1}	Fluorescence λ_{max}/nm
Neutral	2.5	430 (4700)	2600	485
Mono-anion	9.0	535 (6100)	2700	625
Di-anion	14	540 (8600)		
		573 (13000)	2800	682
		615 (11300)		

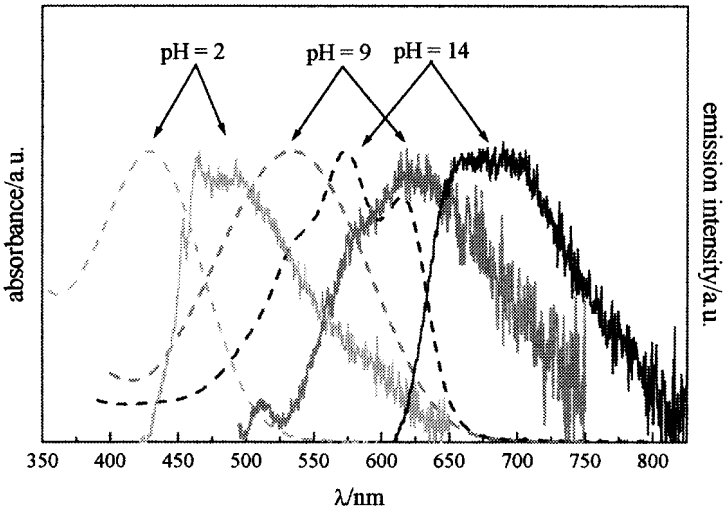


Figure 2. Absorption (dotted line) and fluorescence (full line) normalized spectra of the three species derived from 1,2-HAQ in water–dioxane solutions (2:1, v/v). Light grey, pH = 2 (neutral form); grey, pH = 9 (mono-anion); black, pH = 14 (di-anion)

spectral characteristics are given in Table 1. Owing to the weakness of the emissions, the lifetimes of all three species could not be determined; this figure was measurable for the di-anion only ($\tau = 80$ ps), but with great experimental uncertainty (±50%). For the neutral form and the mono-anion, the radiative rate parameter, $k_F = 1/\tau°$, was obtained using the approximate equation[14]

$$k_F = 1.33 \times 10^{-5} n^2 \nu^2 \varepsilon(\nu) \qquad (2)$$

where n is the refractive index of the medium, $\nu(\mathrm{cm}^{-1})$ is the wavenumber and $\varepsilon(\nu)$ the molar absorption coefficient at the absorption maximum. The actual lifetime, $\tau = \Phi_F/k_F$, was estimated by dividing the experimental emission quantum yield by the calculated k_F. The τ values are shorter than the value reported in the literature for the molecule in an organic solvent ($\tau = 250$ ps).[13] The rate constant of radiationless deactivation, k_{NR}, was calculated as the difference $1/\tau - k_F$. All parameters are reported in Table 2. Even though the approximations in Eqn. (1) are very broad and allow only the orders of magnitude of the rate parameters to be obtained, the agreement between the measured and the calculated τ value of the di-anion is acceptable.

1,2,4-HAQ. *Absorption spectra.* The absorption changes observed for 1,2,4-HAQ by changing the pH occurred approximately in the same pH region as for 1,2-HAQ. A red shift was also observed for this molecule on increasing the pH of the solution but the spectra were crowded into a more restricted wavelength range (Fig. 3). The differences in molar absorption coefficients of the three species are less marked. Furthermore, the spectra are narrower and show a weak vibronic structure. With increased pH, the solution changes from yellow–orange (pH $\leq$ 3.5) to pink (pH $\approx$ 6–9) to violet (pH $\geq$ 12). Isosbestic points were observed at 480 nm for the neutral–mono-anion couple and at 500, 528 and 575 nm for the mono-anion–di-anion couple. The spectral characteristics of 1,2,4-HAQ at pH values typical of any of the three forms are reported in Table 3.

Fluorescence spectra. As already found in an organic solvent (acetonitrile),[7] the emission of 1,2,4-HAQ is far more intense than that of 1,2-HAQ. Fluorescence was observed over the whole pH range explored. Intensity and wavelength changes of the emission spectrum with changing pH were less marked than for 1,2-HAQ. The fluorescence intensity of 1,2,4-HAQ decreases slightly from the neutral molecule ($\Phi_F = 3.3 \times 10^{-3}$) to the mono-anion ($\Phi_F = 2.6 \times 10^{-3}$) and then increases again for the di-anion ($\Phi_F = 1.1 \times 10^{-2}$), which is the strongest

Table 2. Fluorescence quantum yields and dynamic parameters of 1,2-HAQ at pH values typical of the neutral form, anion and di-anion in water–dioxane (2:1, v/v) [values in parentheses were calculated (see text)]

Species	Φ_F	τ/ps	$k_F/10^7\ \mathrm{s}^{-1}$	$k_{NR}/10^9\mathrm{s}^{-1}$
Neutral form	5.1×10^{-4}	(7)	(7.5)	(140)
Mono-anion	1.1×10^{-3}	(17)	(7.0)	(60)
Di-anion	3.4×10^{-3}	80 ± 40 (30)	4.2 (11)	12 (32)

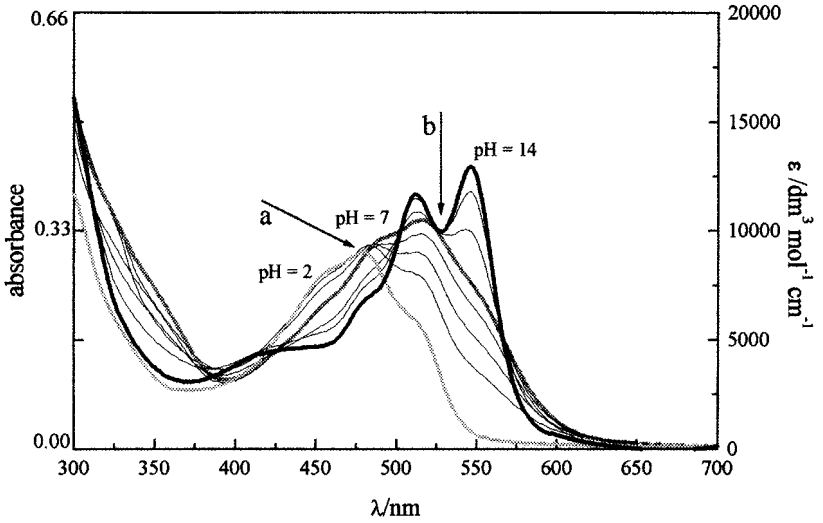


Figure 3. Evolution of the absorption spectrum of 1,2,4-HAQ (3.3×10^{-5} mol dm^{-3}) in water–dioxane solutions (2:1, v/v) as a function of pH. Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black, pH = 14 (di-anion). Arrows indicate isosbestic points between the neutral form and mono-anion (a, 480 nm) and between the mono-anion and di-anion (b, 528 nm)

fluorescing species (Fig. 4). The fluorescence spectra of the three species, which are broad and poorly structured, overlap greatly. The Stokes shifts decrease from the neutral molecule to the mono-anion and di-anion. Normalized absorption and fluorescence spectra of the three species derived from 1,2,4-HAQ are shown in Fig. 5. The fluorescence lifetimes, determined by the phase-shift method, are of the order of hundreds of picoseconds. The mono-anion is the longest-lived species ($\tau = 2600$ ps). The experimental decays fitted a bi-exponential kinetic law well, with contributions from the two species that were pH dependent. The two distinct lifetimes indicate that acidic and basic excited species do not equilibrate within the excited-state lifetime. The rate parameters for emission, $k_F = \Phi_F/\tau$, and radiationless deactivation, $k_{NR} = 1/\tau - k_F$, were determined from the experimental Φ_F and τ data; the results are reported in Table 4.

Acid–base properties

The pKs relative to the protolytic dissociation of 1,2-HAQ and 1,2,4-HAQ were determined in water–dioxane (2:1, v/v) by spectrophotometric titration. For both molecules, two inflection points were found in the pH range explored (pH 2–14). For 1,2,4-HAQ, in which a third hydroxyl group is present, a third inflection point would be expected; however, owing to the double negative charge, the protolytic dissociation of the di-anion into the tri-anion takes place at pH values much higher than 14. On the other hand, the acid–base equilibrium of the anthraquinonic carbonyl occurs at pH values (p$K_a = -8.5$, for anthraquinone)[15] below the explored range. The pK values determined are reported in Table 5. The differences between pK_2 and pK_1 (5.8 for 1,2-HAQ and 4.8 for 1,2,4-HAQ) indicate a lower acidity of the second OH, which is mainly due to the effect of

Table 3. Absorption maxima (λ_{max}), molar absorption coefficients (ε), fluorescence maxima and Stokes shifts ($\Delta\nu$) of 1,2,4-HAQ at different protonation degrees (neutral molecule, mono-anion and di-anion) in buffered water–dioxane solutions (2:1, v/v)

Species	pH	Absorption λ_{max}/nm (ε/dm^{3}mol^{-1}cm^{-1})	$\Delta\nu$/cm^{-1}	Fluorescence λ_{max}/nm
Neutral	2.5	455 (8000) 480 (9000) 510 (6000)	3400	575
Mono-anion	7.0	515 (10400)	2300	585
Di-anion	14	485 (6700) 513 (11700) 547 (13000)	1600	600

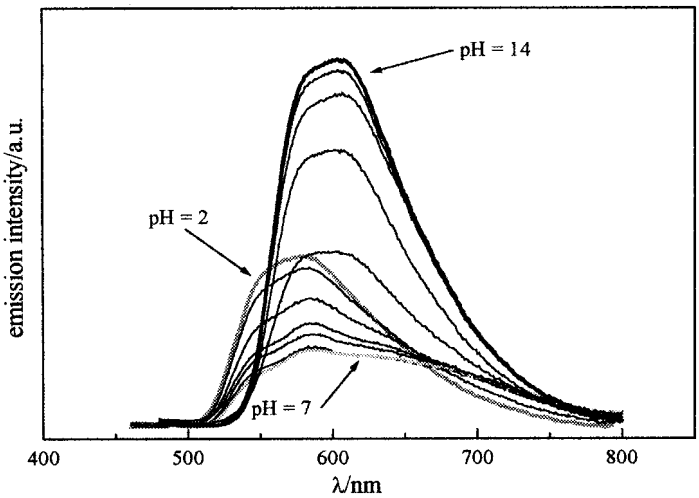


Figure 4. Emission changes of 1,2,4-HAQ (2.3×10^{-5} mol dm^{-3}) in water–dioxane (2:1 v/v) solutions as a function of pH; λ_{exc} = 377 nm.). Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black, pH = 14 (di-anion)

electrostatic interaction opposing deprotonation of the mono-anion. It can be seen from Table 5 that 1,2,4-HAQ is a stronger acid than 1,2-HAQ, in both the first and second dissociation steps.

Fluorimetric titrations should provide information about the excited-state pK*, if the protolytic equilibrium in the excited state is established within the lifetime of that state.[16] However, for the molecules under study, the fluorimetric titration curves showed inflection points at the same pH values as the spectrophotometric ones (Fig. 6, Table 5), that is, they also gave the ground-state pKs. An alternative way to determine pK* is provided by the thermodynamic Förster cycle,[17] which is based on measurements of frequency shifts accompanying protolytic dissociation. By assuming that $\Delta S°$, the entropy change of the protolytic reaction, does not change appreciably upon excitation, that is, $\Delta H° \sim \Delta G°$, the following equation is obtained:

$$\mathrm{p}K^* = \mathrm{p}K + (N_A h/2.303RT)(\nu_{A^-} - \nu_{HA}) \qquad (3)$$

where N_A is Avogadro's number, h is Planck's constant, R is the gas constant and T is absolute temperature; HA and A^- indicate the equilibrating acid–base couple. To determine the frequency differences ($\nu_{A^-} - \nu_{HA}$) between 0–0 levels, the intersections of normalized absorption and

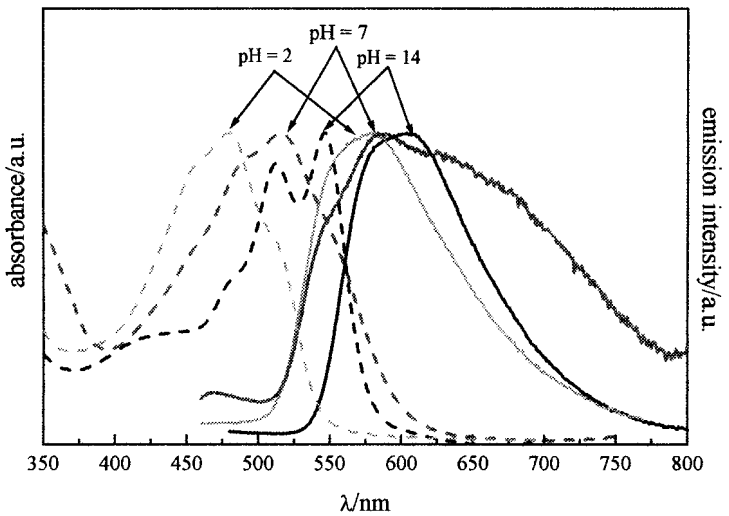


Figure 5. Absorption (dotted line) and fluorescence (full line) normalized spectra of the three species of 1,2,4-HAQ in water–dioxane solutions (2:1, v/v). Light grey, pH = 2 (neutral form); grey, pH = 7 (mono-anion); black pH = 14 (di-anion)

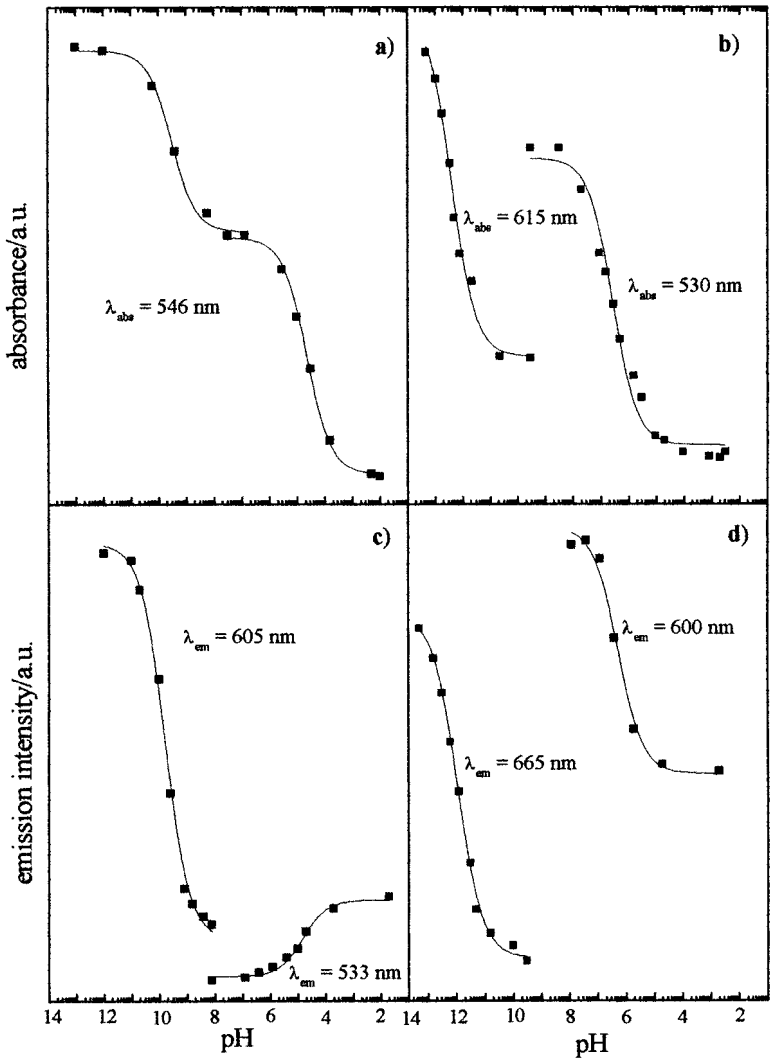


Figure 6. Spectrophotometric and fluorimetric titration curves: (a) changes of 1,2-HAQ absorbance as a function of pH; (b) changes of 1,2,4-HAQ absorbance as a function of pH; (c) changes of 1,2-HAQ emission as a function of pH; (d) changes of 1,2,4-HAQ emission as a function of pH

fluorescence spectra were taken (see Figs 2 and 5). This method gave the best results.[18] The excited-state p*K**s obtained for 1,2-HAQ and 1,2,4-HAQ are reported in Table 5. It can be seen that these molecules are stronger acids in the excited state than in the ground state in both dissociation steps. However, the acidity increase upon excitation is much more marked for 1,2-HAQ ($\Delta pK^*_1 = 10$ and $\Delta pK^*_2 = 2.6$) than for 1,2,4-HAQ ($\Delta pK^*_1 = 1.2$ and $\Delta pK^*_2 = 0.9$).

DISCUSSION

The spectral behavior of alizarin has been the subject of

Table 4. Fluorescence quantum yields and dynamic parameters of 1,2,4-HAQ at pH values typical of the neutral form, mono-anion and di-anion in water–dioxane (2:1, v/v)

Species	Φ_F	τ/ps	$k_F/10^7$ s^{-1}	$k_{NR}/10^9$s^{-1}
Neutral form	3.3×10^{-3}	150 ± 10	2.2	6.7
Mono-anion	2.6×10^{-3}	2600 ± 40	0.1	0.4
Di-anion	1.2×10^{-2}	380 ± 10	3.1	2.6

Table 5. Dissociation constants (pK_1 and pK_2) of 1,2-HAQ and 1,2,4-HAQ in water–dioxane (2:1, v/v) obtained from (I) spectrophotometric and (II) fluorimetric titrations. Excited-state pK*s calculated by the Förster cycle. ΔpK_1 *and ΔpK_2* represent the differences between the ground state pK and the corresponding excited state pK*.

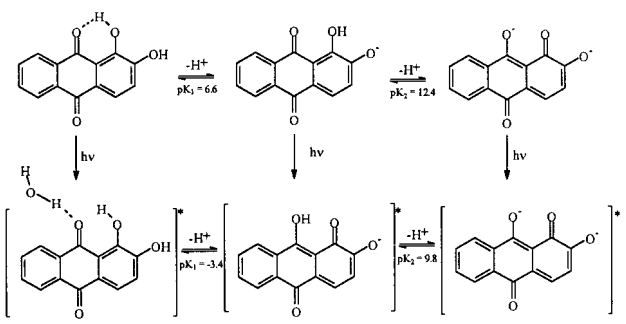
	1,2-HAQ		1,2,4-HAQ	
pK	(I)	(II)	(I)	(II)
pK_1	6.57 ± 0.05 (7.4)[a]	6.3 ± 0.1	4.70 ± 0.05	5.0 ± 0.1
pK_2	12.36 ± 0.05 (11.8)[a]	12.0 ± 0.1	9.51 ± 0.05	9.8 ± 0.1
pK_1*	−3.4		3.5	
ΔpK_1*	10		1.2	
pK_2*	9.8		8.6	
ΔpK_2*	2.6		0.9	

[a] Data taken from Ref. 22.

several investigations,[3,12,19–21] whereas little has been reported about purpurin.[3,21] Ground-state protolytic equilibrium constants of some dihydroxyanthraquinones, including 1,2-HAQ, in mixed aqueous solvents have also been reported.[22]

In this work, the study of the pH effect on absorption and fluorescence spectra of 1,2-HAQ and 1,2,4-HAQ allowed their ground- and excited-state pKs to be determined; the assignments of the neutral, mono-anionic and di-anionic ground-state species are consistent with spectral changes previously observed with similar molecules and the pH of the solutions where they occurred.[18,22] It is worth noting that the pKs determined here, and also as those from the literature, have to be treated with caution, since they were measured in mixed solvents, where the dielectric constant is reduced compared with pure water. However, the dielectric constant decrease for the mixture used [water–dioxane (2:1, v/v)], calculated by the empirical equation reported by Anderson,[23] is relatively modest, hence the effect on the pKs does not exceed one pK unit.

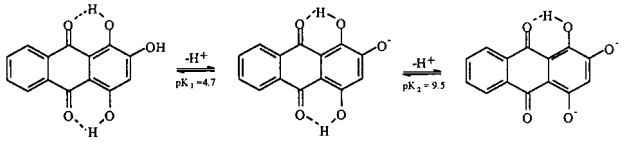
Acid–base properties provide information about the charge distribution in the molecule. If acidity constants are known in both the ground and excited states, information about the change in electronic distribution upon excitation is available. An excited-state molecule generally possesses properties which are different from those of the same molecule in the ground state, owing to the redistribution of the electronic density. Acidity may increase or decrease upon excitation. For the molecules studied here, the red shift of the absorption spectra, accompanying deprotonation, denotes that the electronically excited neutral form has a higher energy level than the excited deprotonated species, compared with the ground state, and therefore exhibits a propensity to dissociate at lower pH values than the ground state, that is, it becomes a stronger acid.



Scheme 2

For the 1,2-HAQ, the pK_1 and pK_2 determined here are in qualitative agreement with those reported in the literature[22] (see Table 5), even though the latter were obtained using a lower dioxane percentage (1% vs 33%). Compared with monohydroxyanthraquinones (1-HAQ and 2-HAQ),[18] for which pK measurements were carried out under the same conditions (33% dioxane) as in this work, both alizarin and purpurin are stronger acids. This is due, as previously suggested,[22] to the inductive electron-attracting effect of an OH group on the nearby one, which increases the intrinsic acidity of both. Since the H^+ mobility of the 1-OH is reduced by hydrogen bonding with the carbonyl in 1,2-HAQ, the 2-OH group is the first to dissociate. In the mono-anion, the negative charge decreases the intrinsic acidity of the other OH group in the molecule, as well as its hydrogen bonding capability. Since the spatial distance between the two negative charges should be as large as possible to stabilize the di-anionic species, the second deprotonation is probably followed by charge transfer (CT) from the 1-O^- to the 9-CO, yielding the 1,10-keto form. This species shows an extended conjugated system, which is responsible for the large, red shift of the absorption spectrum. Thus, we believe that the deprotonation steps occur as shown in Scheme 2.

In 1,2,4-HAQ, the first dissociation step also occurs at the 2-OH group which is by far the most acidic one due to the inductive effects of the other *ortho* (1-OH) and *meta* (4-OH) hydroxyl groups, both hydrogen-bonded to carbonyls. The noticeable difference in pK_1 between 1,2-HAQ and 1,2,4-HAQ (approximately 2 pK units) is due to a further acidity increase of purpurin induced by the 4-OH in the *meta* position with respect to the 2-OH



Scheme 3

undergoing deprotonation [$\sigma_{meta}(OH) = 0.121$].[24] Regarding the site of the second deprotonation step, it should be considered that the negative charge at the mono-anion decreases the acidity of both the 1- and 4-OH. This effect, which is mainly electrostatic in nature, is less important on the 4-OH than on the 1-OH because it decreases with distance. It can be concluded that the second deprotonation step occurs at the 4-OH; this is also consistent with the greater acidity of the mono-anion of purpurin compared with that derived from alizarin.

In the excited state, the phenolic OH is more acidic than in the ground state, while the carbonyl oxygen is more basic. The electronic excitation, which is of CT character, shortens the distance between the oxygen of the carbonyl and the hydroxyl group by about 0.1 Å and strengthens the hydrogen bond.[25] This favors intramolecular hydrogen atom transfer (or proton transfer, depending upon the degree of CT)[9] from the 1-OH to the carbonyl. If ESIPT occurs, the 9,10-keto form rearranges into the 1,10-keto form, lowering the energy of the excited state.[2] For molecules similar to those under study, it has been suggested that the potential functions along the proton transfer coordinate in the ground and excited state are double-minimum energy surfaces.[9,10,26,27] In the ground state, the lowest minimum is related to the 9,10-keto form, while the minima invert their positions in the excited state. Whether proton transfer is fast enough to observe emission from the keto form depends on the hydrogen-bond strength and the stabilization energy of the 1,10-keto excited state. In some dihydroxyanthraquinones (e.g. 1,5-HAQ)[10,26] ESIPT was irreversible and occurred so fast ($k > 10^{12}$ s^{-1})[28] that fluorescence, showing a large Stokes shift, was only observed from the 1,10-keto form. The energy barrier to ESTP increases with the polarity of the solvent and also with changes in the relative OH positions;[7] for example, it does not occur for 1,4-HAQ.[28]

Referring to the molecules studied here, alizarin and purpurin exhibit a different behavior in organic solvents.[7] For 1,2-HAQ, proton transfer occurs in the excited state and the emission shifts to the red. For 1,2,4-HAQ, the intramolecular hydrogen bond is maintained in the excited state, similarly to 1,4-HAQ,[28] and fluorescence was observed from the intramolecularly hydrogen-bonded 9,10-keto form.[7] A single exponential decay ($\tau = 1.5 \pm 0.3$ ns) was found for purpurin emission in benzene solution, whereas for 1,2-HAQ the fluorescence decay followed a bi-exponential law. The longer lived species ($\tau_1 = 4.0 \pm 0.3$ ns) was assigned to the 1,10-keto form, and the shorter-lived one ($\tau_2 = 0.2 \pm 0.1$ ns) was assigned to the 9,10-keto form for which proton transfer competes with fluorescence emission. The τ value reported for alizarin in the literature (0.25 ns in acetonitrile)[13] is in excellent agreement with τ_2.

In acidic solution, the fluorescence of 1,2-HAQ (neutral molecule) shows a small Stokes shift (Table 1), which indicates that the geometry of the S_1 excited state is not significantly displaced from that of the ground state. The emission comes from the relaxed 9,10-keto excited state and is weak and short-lived possibly owing to competition with ESIPT or, more likely, to intermolecular solvent hydrogen-bonding perturbation (Scheme 2). Emission from the 1,10-keto excited state was not detected in aqueous solution.

The relatively longer lived emission of 1,2,4-HAQ in aqueous solution is in agreement with the lack of competitive processes while maintaining intramolecular hydrogen bonding. This explains the higher emission intensity of this molecule which was held rigid and quasi-planar by the intramolecular hydrogen bonds in both neutral and mono-anionic forms. The further increase in emission quantum yield of the di-anionic form is probably related to the greater compactness of the ion, where the contribution to deactivation through OH vibrations is weak. Both neutral and mono-anionic excited states, being so similar to the ground state, deprotonate at pH values close to those of ground-state deprotonations. For this molecule, both acid–base equilibria in the excited state involve intramolecular hydrogen-bonded forms, as in the ground state; deactivation through solvent interaction is not very effective.

In the case of 1,2-HAQ, we believe that the intermolecular hydrogen bond with the solvent contributes in large part to fast deactivation of the excited state. The rate coefficient for the non-radiative processes, k_{NR}, is given by the sum of the internal conversion (k_{IC}) and intersystem crossing (k_{ISC}) rate constants. It is known that 1,2-HAQ undergoes fairly efficient intersystem crossing ($\Phi_{ISC} = 0.16$) at a rate of 0.6×10^9 s^{-1} in acetonitrile;[19] since k_{NR} is much greater in aqueous medium, it is likely that internal conversion dominates excited-state deactivation. A comparison of Tables 2 and 4 indicates that the greatest difference between the two molecules is that alizarin has a faster radiationless deactivation than purpurin, while the radiative rate parameters are almost of the same order of magnitude. This indicates that, upon excitation, the aqueous hydrogen-bonding environment affects them differently. The greatly increased acidity of 1,2-HAQ in the excited state ($pK_1 - pK_1^* = 10$) denotes that the excited-state molecule is substantially different from the ground-state molecule. In the excited state, the hydrogen bonding with the solvent is strengthened considerably by the increased electron density on the carbonyl oxygen, owing to the charge-transfer nature of the excited state. Consequently, the hydroxyl acidity increases.

Finally, it is worth noting that, for both molecules, excited-state decay occurs faster than the establishment of protolytic equilibrium, over the whole pH range explored. In contrast, excited-state fluorimetric titration was feasible for 1- and 2-monohydroxyanthraquinones.[18] The reason why structurally similar molecules, studied under comparable conditions, exhibit such different behaviors is still unclear.

CONCLUSIONS

The results of this work indicate that both 1,2-HAQ and 1,2,4-HAQ behave as acidichromic molecules since their aqueous solutions change color as the pH changes. Emission is less sensitive to changes in the pH than absorption, especially for purpurin, for which the fluorescence spectra overlap widely. The fluorescence quantum yields and the lifetimes are markedly dependent on the pH of the medium. The Φ_F values increase approximately by one order of magnitude from the neutral molecule to the di-anion; the τ values are on a picosecond time-scale; for the purpurin-derived mono-anion only the lifetime is fairly long ($\tau = 2.6$ ns). Radiationless processes (k_{NR}, internal conversion and intersystem crossing) contribute greatly to excited singlet deactivation. Dissipation of the electronic energy through hydrogen bonding with the solvent is much more efficient for 1,2-HAQ than for 1,2,4-HAQ.

The p*K* values determined in the ground state are in line with the positional effects of the hydroxyl groups. The excited-state p*K**s indicate that both molecules become stronger acids upon excitation. However, the acidity increases much more for alizarin, in agreement with the greater change in electronic distribution upon excitation.

Regarding the aim of defining the limits of acidity in atmospheric pollutants (acid rain in particular), that may introduce chromatic changes on surfaces painted with these colorants, it can be concluded that the mono-anionic forms (pink color) are stable in neutral and moderately alkaline media; an increase in acidity (pH <6) turns alizarin yellow, while a lower pH value (pH <4.5) is needed to change the color of purpurin. In the alkaline range, the color changes only at high pH values (pH >9 for 1,2,4-HAQ and pH >12 for 1,2-HAQ).

Another interesting aspect of this study of the pH effect on colorants used in artistic painting resides in its potential to interpret interactions of colorants with the chemical environment in which they are embedded. Results in this field will be published in specialized journals.

Acknowledgements

We thank the Italian Consiglio Nazionale delle Ricerche and Ministero dell'Università e della Ricerca Scientifica e Tecnologica for financial support. C.M. is grateful to ENEA for a PhD fellowship.

REFERENCES

1. Arcamone F, Penco S. *Pontif. Acad. Sci. Scr. Varia.* 1986; **70**: 225–241.
2. Nagaoka S, Nagashima U. *Chem. Phys.* 1996; **206**: 353–362.
3. Navas Diaz A. *J. Photochem. Photobiol., A: Chem.* 1990; **53**: 141–167 and references cited therein.
4. Gollnik K, Held S. *J. Photochem. Photobiol., A: Chem.* 1993; **70**: 135–145.
5. Renè de la Rie E. *Stud. Conserv.* 1982; **27**: 1–7, 65–69, 102–108.
6. Matteini M, Moles A. *La Chimica nel Restauro*. Nardini: Florence, 1986.
7. Miliani C, Romani A, Favaro G. *Spectrochim. Acta, Part A* 1998; **54**: 581–588.
8. Palit DK, Pal H, Mukherjee T, Mittal JP. *J. Chem., Soc., Faraday Trans.* 1990; **86**: 3861–3869 and references cited therein.
9. Barbara PF, Walsh PK, Brus LE. *J. Phys Chem.* 1988; **93**: 29–34.
10. Van Benthem MH, Gillispie GD. *J. Phys. Chem.* 1984; **88**: 2954–2960.
11. Meech SR, Phillips D. *J. Photochem.* 1983; **23**: 193–217.
12. Allen NS, McKellar JF. *J. Photochem.* 1976; **5**: 317–320.
13. André JC, Kawenoki I, Kossanyi J, Valat P. *J. Photochem.* 1982; **19**: 139–150.
14. Calvert JG, Pitts JN Jr. *Photochemistry*. John Wiley: New York, 1967; 172.
15. Grieg CC, Johnston CD. *J. Am. Chem. Soc.* 1968; **90**: 6453–6457.
16. Ireland JF, Wyatt PAH. *Adv. Phys. Org. Chem.* 1976; **12**: 131–221.
17. Förster T. *Z. Electrochem.* 1950; **54**: 531.
18. Richtol HH, Fitch BR. *Anal. Chem.* 1974; **46**: 1749–1754.
19. Gollnick K, Held S, Màrtire DO, Braslavsky SE. *J. Photochem. Photobiol., A: Chem.* 1992; **69**: 155–165.
20. Kawenoki I, Keita B, Kossanyi J, Nadjo L. *Nouv. J. Chim.* 1982; **6**: 387–393.
21. El Ezaby MS, Salem TM, Zewail AH, Issa R. *J. Chem. Soc. B* 1970; 1293–1296.
22. Gillet H, Pariaud JC. *Bull. Soc. Chim. Fr.* 1966; 2624–2631.
23. Anderson JE. *J. Phys. Chem.* 1991; **95**: 7062–7064.
24. McDaniel DH, Brown HC. *J. Org. Chem.* 1958; **23**: 420–427.
25. Marzocchi MP, Mantini AR, Casu M, Smulevich G. *J. Chem. Phys.* 1998; **108**: 534–549.
26. Smulevich G, Foggi P. *J. Chem. Phys.* 1987; **87**: 5657–5663.
27. Smulevich G, Foggi P, Feis A, Marzocchi MP. *J. Chem. Phys.* 1987; **87**: 5664–5669.
28. Flom SR, Barbara PF. *J. Phys. Chem.* 1985; **89**: 4489–4494.

