P ' J. C e ., **75**, 1533–1541 (2001)

Spectrophotometric Studies of Acidity Constant and Cu^{2+} Ion Complexation of 1-Hydroxy-2-(prop-2′-enyl)-4-(prop-2′-enyloxy)-9,10-anthraquinone in Methanol-Water Mixtures

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(Rece' ed Ma c 5 , 2001; e ' ed a c ' Ma 14 , 2001))

The acidity constant of 1-hydroxy-2-(prop-2′-enyl)-4-(prop-2′-enyloxy)-9,10-anthraquinone in different methanol-water mixtures at 25°C has been determined spectrophotometrically. A linear reverse relationship is observed between the pK_a and mole fraction of methanol in the solvent mixtures. The complexation reaction between the anthraquinone derivative used and Cu^{2+} ion has been studied in methanol solution at 25°C by a spectrophotometric technique. The stoichiometry of the resulting complex was found to be 1:1. The formation constant of the Cu^{2+}–anthraquinone complex, evaluated from the computer fitting of the absorbance-mole ratio data at 5 different wavelengths, is log $K_f = 4.59 \pm 0.06$.

Key words: 9,10-anthraquinone derivative, pK_a, Cu^{2+} complex, stability, methanol-water, spectrophotometry

9,10-Anthraquinone derivatives, as the largest group of naturally occurring quinones, are of fundamental importance both in industry [1–4] and in medicine [5–10]. Various anthraquinones are known to possess anticancer activity [5,6,8–10], the bioreduction and redox cycling of which are supposed to play a key role in their activation as efficient drugs under aerobic conditions [5,11]. In recent years, we have been involved in the synthesis [12–14], acid-base [15–17] and electrochemical studies [18–22], some analytical applications in membrane transport [23] and solid-phase extraction of metal ions [24,25], in construction of PVC-membrane ion-selective electrodes [26–28] and solubility studies in supercritical carbon dioxide [29,30].

This work was undertaken to determine the acidity constant of a new compound 1-hydroxy-2-(prop-2′-enyl)-4-(prop-2′-enyloxy-9,10-anthraquinone (AQ), recently synthesized in our laboratories [31], in various methanol-water mixtures at 25°C by spectrophotometric measurements. The spectrophotometric behavior, stoichiometry and stability of the Cu^{2+}–AQ complex were also investigated at 25°C in methanol solution.

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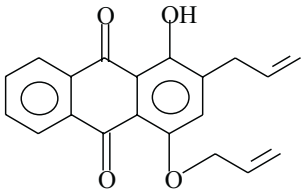

AQ

EXPERIMENTAL

Reagents: Reagent grade perchloric acid and ammonia and HPLC grade lithium hydroxide, sodium hydroxide, sodium perchlorate, copper nitrate, succinic acid, and oxalic acid (all from Merck) were of the highest purity available and used without further purification except for vacuum drying over P_2O_5. The anthraquinone AQ was synthesized and purified as described elsewhere [31].

Apparatus: The electronic spectra were recorded on a Philips PU-8750 spectrometer at 25.0 ± 0.1°C. Measurements of pH were made with a Metrohm E-603 digital pH meter using a combined glass-calomel electrode. In all experiments, the ionic strength of the solutions used was kept constant at 0.01 M using sodium perchlorate as the supporting electrolyte.

Procedures: To calibrate the pH meter in various binary methanol-water mixtures used, the 0.01 M solutions of oxalate and succinate buffers were employed. The reference values of pH of these buffer solutions in different methanol-water mixtures have been reported previously [32]. A modified form of the procedure introduced by Asuero *e a* . [32] was used to determine the acidity constants. In this procedure, the absorbance of a solution of fixed concentration of AQ in a given solvent mixture was first measured in highly acidic and basic solutions. Then the absorbance measurements at three different wavelengths (including λ_{max}) of the basic form . pH of the solution were made, while the anthraquinone solution was titrated with a concentrated sodium hydroxide solution in the same solvent mixture, using a pre-calibrated micropipet. All pH values are expressed in terms of activity. Acidity constants were evaluated from the computer fitting of the absorbance-pH data to the equation that resulted from substituting the pH and absorbance values in the mass balances [33], using a non-linear least-squares program KINFIT [34]. The resulting equation for a monoprotic acid is:

$$A_{obs} = (A_o + A_1[H^+] / K_a)/(1 + [H^+]/K_a) \quad (1)$$

A_{obs} is the observed absorbance at each titration point, A_o and A_1 are the absorbance of the basic form and protonated form, respectively, and K_a is the acidity constant. The formation constant of the Cu^{2+}–AQ complex was determined by the absorbance measurements at 5 wavelengths of the spectra of solutions (with an ionic strength of 0.01 M at 25 ±0.1°C), in which varying concentrations of the metal ion (1.0×10^{-2} M) were added to a fixed concentration of AQ (4.6×10^{-5} M) until a desired metal to ligand mole ratio was achieved. Attainment of equilibrium was checked by the observation of no further change in the spectra after several hours.

When AQ reacts with Cu^{2+} ion to form a 1:1 complex

$$Cu^{2+} + AQ = Cu^{2+}.AQ \qquad K_f = [Cu^{2+}.AQ]/[Cu^{2+}[AQ], \quad (2)$$

the mass balance equations are given by:

$$C_{Cu} = [Cu^{2+}] + [Cu^{2+}.AQ], \qquad C_{AQ} = [AQ] + [Cu^{2+}.AQ] \quad (3,4)$$

where C_{Cu} and C_{AQ} are the initial analytical concentration of Cu^{2+} and AQ, respectively. The mass balance equations for the 1:1 model can be solved in order to obtain an equation for the free ligand concentration, [AQ], as:

$$K_f [AQ]^2 + (1 + K_f (C_{Cu} – C_{AQ})) [AQ] – C_{AQ} = 0 \quad (5)$$

The observed absorbance of solution is also given by:

$$A_{obs} = \varepsilon_{AQ} [AQ] + \varepsilon_{Cu.AQ} [Cu^{2+}.AQ] \quad (6)$$

where ε are the molar absorptivities of the species denoted. For the evaluation of the formation constant from the absorbance . C_{Cu}/C_{AQ} mole ratio data, the KINFIT program was also used [33]. Adjustable parameters are the K_f and ε values.

The free ligand concentration, [AQ], was calculated by a Newton-Raphson procedure. Once the value of [AQ] had been obtained, the concentrations of all other species involved are calculated from the mass balance equations (3) and (4), by using the estimated value of the formation constant at the current interaction step of the program. Refinement of the parameters was continued until the sum-of-squares of the residuals between calculated and observed absorbances for all experimental points was minimized. The output of the program KINFIT comprises the refined parameters, the sum-of-squares and the standards deviation of the data.

RESULTS AND DISCUSSION

Acidity constant of AQ: The visible absorption spectra of AQ in different methanol-water mixtures at various pH values were recorded at 25°C. Sample spectra in 80% methanol-20% water (w/w) are shown in Fig. 1. The shorter wavelength band (462 nm) that appear at low pH is due to the acid form, and the longer wavelength band (530 nm) represents the absorption by the basic form of the molecule. The occurrence of a clear isosbestic point in the resulting spectra indicates that two species (*i.e.*, AQ^- and AQ) are in equilibrium under the experimental conditions. Acidity constant of the anthraquinone AQ was investigated in four different methanol-water mixtures at 25°C spectrophotometrically at several wavelengths. Sample absorbance-pH plots in different solvent systems at 540 nm are shown in Fig. 2. Acidity constants were evaluated by fitting of the corresponding absorbance-pH data to equation (1). A sample computer fit of the absorbance-pH data is shown in Fig. 3 and all the resulting pK_a values are summarized in Table 1. It is interesting to note that, in all solvent systems studied, the pK_a values evaluated from the computer fitting of absorbance-pH data at three or four different wavelengths are in excellent agreement with each other.

From the data given in Table 1, it is immediately obvious that the nature of solvent plays a fundamental role in the acid-base equilibria. In fact, there is a considerable decrease acidity of molecule by increasing mole fraction of methanol in the binary methanol-water mixtures. It is well known that the energy required for the separation of charges in acid dissociation, which is inversely proportional to the dielectric constant of the medium, is compensated by the solvation of the resulting ions [32]. Thus, due to much lower dielectric constant, D, and solvating ability (as expressed by the Gutmann donor number, DN [35]) of methanol (D = 32.6 and DN = 19) than those of water (D = 78.3 and DN = 33) [36], it is not surprising to observe such a decrease in the extent of ionization of AQ by an increase of methanol in the solvent mixtures used.

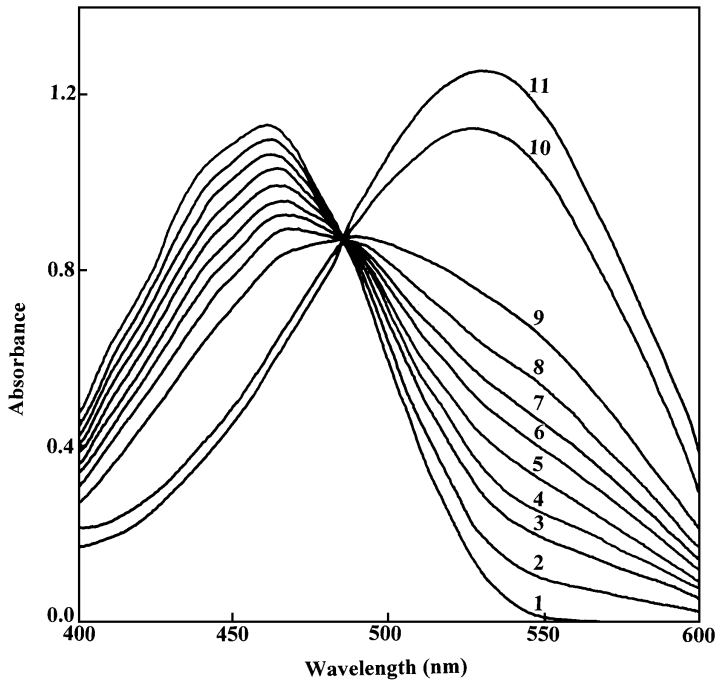

Figure 1. Absorption spectra of AQ in 80% methanol-20% water mixture at different pH values: (1) acidic, (2) 10.98, (3) 11.23, (4) 11.38, (5) 11.53, (6) 11.64, (7) 11.73, (8) 11.83, (9) 12.0, (10) 12.66, (11) basic.

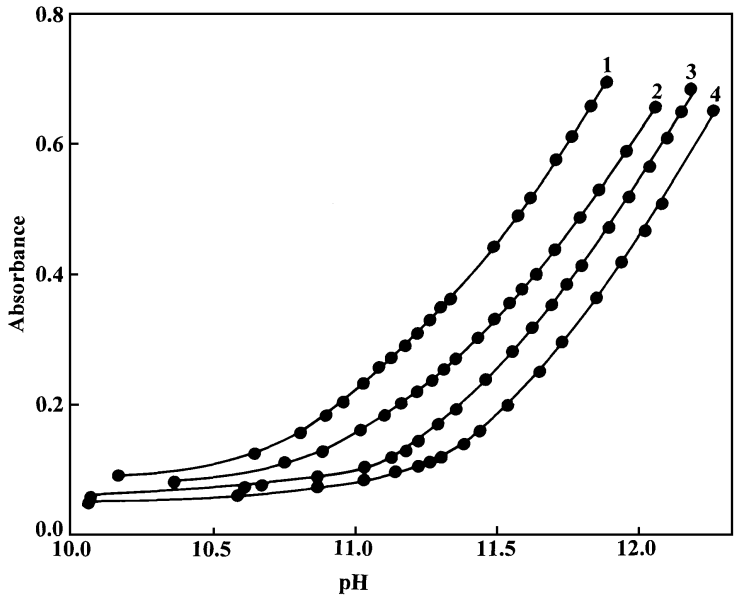

Figure 2. Absorbance-pH plots of AQ in different methanol-water mixtures. Weight percent of methanol in the binary mixture is (1) 60, (2) 70, (3) 80, (4) 90.

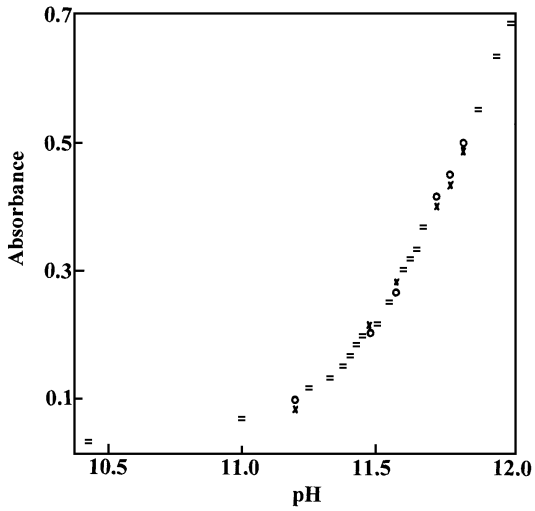

Figure 3. Computer fit of the absorbance-pH data for AQ in 70% methanol-30% water mixture: (×) experimental point; (o) calculated point; (=) experimental and calculated points are the same within the resolution of the plot.

Table 1. Acidity constant of AQ in different methanol-water mixtures at various wavelengths.

wt% Methanol	Wavelength (nm)		pK_a
60	522		11.74 ± 0.02
	530		11.75 ± 0.02
	540		11.77 ± 0.02
		Mean value	11.75 ± 0.02
70	522		11.94 ± 0.02
	530		11.95 ± 0.02
	540		11.96 ± 0.02
		Mean value	11.95 ± 0.02
80	522		12.14 ± 0.03
	530		12.14 ± 0.02
	540		12.16 ±0.02
		Mean value	12.15 ± 0.02
90	522		12.37 ± 0.03
	530		12.39 ± 0.02
	540		12.40 ± 0.02
		Mean value	12.39 ± 0.02

There is actually a linear relationship between pK_a of AQ molecule and mole fraction of methanol, X_{MeOH}, in the binary mixtures (Fig. 4). A similar trend has already been reported for different acid-base [15–17] and complexation equilibria [37–40] in different binary solvent mixtures. It seems reasonable to assume that the preferential hydration of the resulting conjugated base, AQ^-, is mainly responsible for such monotonic dependence of pK_a upon the solvent composition.

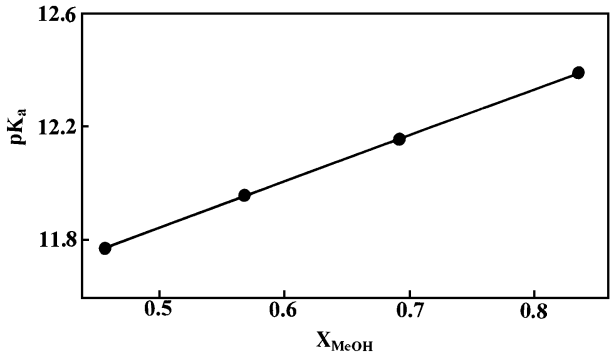

Figure 4. Variation of pK_f values of AQ with X_{MeOH} in the binary mixtures.

Complexation of Cu^{2+} with AQ: In preliminary experiments, it was found that addition of an equivalent amount of Cu^{2+} to a methanol solution of AQ (some 10^{-4} M) results in a very fast change in the color of solution from yellow to violet, while the presence of an excess amount of other transition metal ions (*i.e.*, 100–200 times) such as Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+} showed no observable color change in the AQ solution. This is most possibly indicative of a selective complex formation of the anthraquinone derivative with Cu^{2+} ion in methanol solution. It is noteworthy that we have recently used AQ as a very suitable reagent for the highly selective solid phase extraction and determination of ultra trace amounts of Cu^{2+} ions in aqueous media [41].

In order to determine the stoichiometry and stability of the resulting AQ complex with Cu^{2+} ion in methanol solution, the spectra of a series of solutions containing a constant concentration of the ligand (4.6×10^{-5} M) at a fixed ionic strength of 0.01 M (maintained by $NaClO_4$) and 25°C and varying amounts of the metal ion were obtained (shown in Fig. 5). As it is seen, the complexation is accompanied by a relatively strong shift of the absorption band on AQ, with a λ_{max} = 462 nm in methanol solution, towards longer wavelengths (λ_{max} = 492, 525 and 560 nm). Generally, electrostatic interaction of a bound metal ion would not be able to produce such pronounced effects on the electronic structure of a dye molecule, and hence its spectrum [42]. It seems reasonable that a large change in the conjugation of the AQ molecule, brought about by copper ion complexation, is responsible for the observed spectral changes.

The stoichiometry of the complex was determined by the continuous variations method. The resulting plots obtained at two different wavelengths are shown in Fig. 6. The metal ion to ligand mole ratio in the Cu^{2+}. AQ complex is 1:1. Moreover, the existence of a well-defined isosbestic point in the spectra of AQ titration with copper ion (Fig. 5) is also a good indication for simple 1:1 complexation equilibrium in solution. The formation constant of the resulting 1:1 complex in methanol solution was evalu-

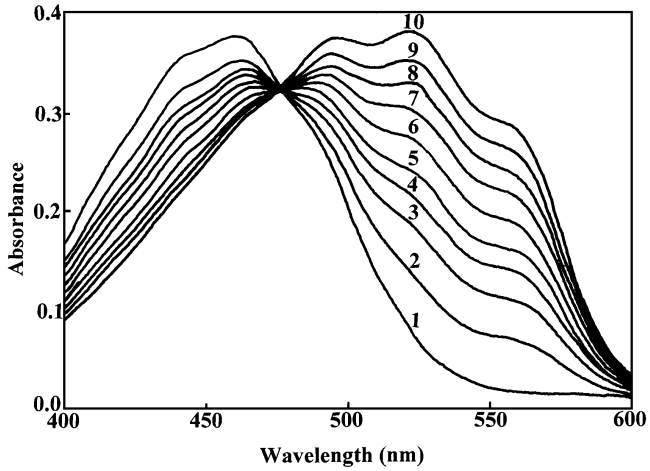

Figure 5. Visible spectra for titration of 4.6×10^{-5} M AQ with Cu^{2+} ion in methanol solution. The C_{Cu}/C_{AQ} mole ratio is: (1) 0.00, (2) 0.26, (3) 0.52, (4) 0.78, (5) 1.04, (6) 1.51, (7) 2.16, (8) 3.03, (9) 3.39, (10) 6.05.

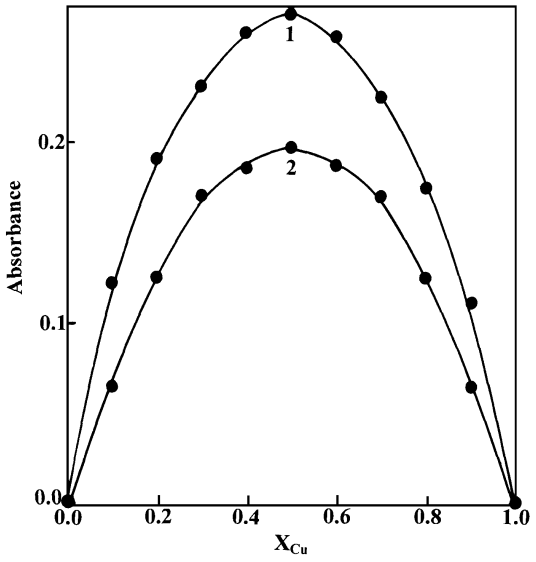

Figure 6. Continuous variations plot for Cu^{2+}–AQ system in methanol solution at two different wavelengths. Conditions: total concentration, 8.9×10^{-5} M; wavelength (1) 530 nm and (2) 560 nm.

ated at 25° C by absorbance measurements at five different wavelengths of the corresponding visible spectra, of solutions in which varying concentrations of the metal ion were added to a fixed amount of AQ in solution (4.6×10^{-5} M). The resulting absorbance . C_{Cu}/C_{AQ} mole ratio data are given in Fig. 7. The log K_f and the corresponding molar absorptivities, obtained from the KINFIT program, are summarized in Table 2. Once again, there is an excellent agreement between the log K_f values obtained at five different wavelengths (Table 2), emphasizing that highly reliable equilibrium constants can be obtained by the method employed. As seen, the mean value obtained for the formation constant of Cu^{2+}. AQ complex is log $K_f = 4.59 \pm 0.06$.

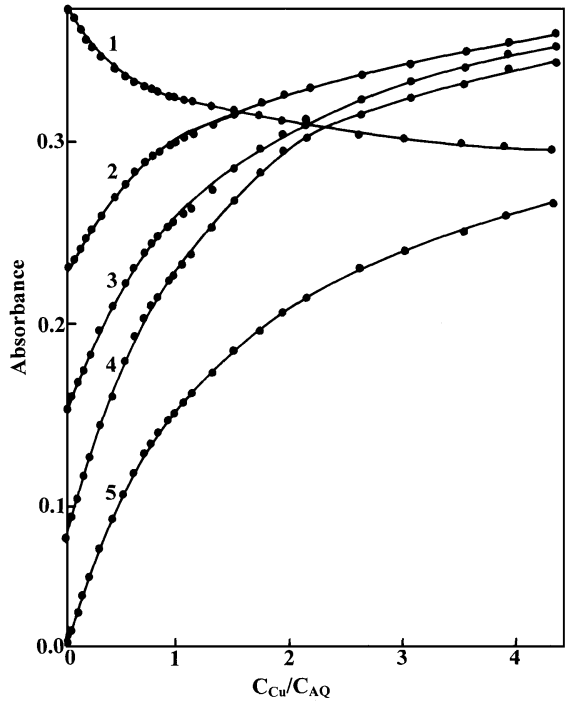

Figure 7. Absorbance-mole ratio plots for Cu^{2+}–AQ system in methanol solution at different wavelengths: (1) 461, (2) 496, (3) 507, (4) 522, (5) 556.

Table 2. Formation constants and molar absorptivities of Cu^{2+}.AQ complex at different wavelengths.

Wavelength (nm)	log K_f	ε_{AQ} ($M^{-1}cm^{-1}$)	$e_{Cu.AQ}$ ($M^{-1}cm^{-1}$)
641	4.61 ± 0.04	7911 ± 16	5871 ± 46
496	4.53 ± 0.03	5042 ± 20	8325 ± 65
507	4.57 ± 0.03	3347 ± 28	8304 ± 85
522	4.68 ± 0.07	1569 ± 91	8545 ± 244
558	4.57 ± 0.03	466 ± 34	6749 ± 104
Mean value	4.59 ± 0.06		

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