UV-VIS SPECTROSCOPIC STUDY OF NATURAL DYES WITH ALUM AS A MORDANT

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Abstract

The formation of complexes between alum with morin and quercetin in aqueous solution with and without pH control have been studied by UV-visible spectroscopy. The stoichiometries of the complexes were evaluated using the molar ratio method. The association ratio of alum with morin and quercetin without pH control were 3 : 2 and 1 : 1, respectively. In the buffer system pH 4.5, the stoichiometry of alum with morin and quercetin were 1 : 1 and 1 : 1, respectively. The heats of formation of complex, obtained by semi-empirical PM3 method, indicated that the proposed complexes can be favorably formed.

Keywords: Morin, quercetin, spectroscopic, complexation, alum

Introduction

Flavonoids, polyphenolic pigments are widely present in plants. Morin (3,5,7,2′, 4′-pentahydroxyflavone) and quercetin (3,3′,4′,5,7-pentahydroxyflavone) are phenolic compounds derived from hydroxyl substitutions on the flavone chromophore. Figures 1(a) and 1(b) show the chemical structure of morin and quercetin, respectively. The flavone-based compounds are known to form stable complexes with metal cations. *Maclura cochinchinensis* (Lour.) Corner is widely used by villagers, especially in the northeast of Thailand, for dyeing fibres which yield a beautiful yellow colour (Moeyes, 1993). The flavonoid yellow colouring substance in the wood is morin, which is a major component of the heartwood of this
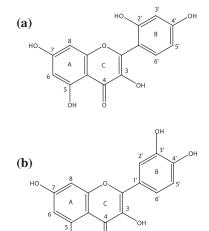

Figure 1. Chemical structure of (a) morin and (b) quercetin

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plant and quercetin is the minor substance (Lemmens and Wulijarni-Soetjipto, 1992).

Studies on morin and quercetin association with Al(III) have focused on the investigation of the stoichiometry of the complexes and the determination of possible sites of binding (Cornard and Merlin, 2001; Cornard and Merlin, 2002; Gutierrez and Gehlen, 2002).

Metal complex formation has been a prominent feature of textile dyeing from very early times, since it was recognized that the technical performance, including fastness to washing and light, of many natural dyes could be enhanced by treatment with certain metal ions, a process known as mordanting (Christie, 2001).

Alum ($KAl(SO_4)_2 \cdot 12H_2O$) is widely used as mordant for dyeing keratin fibres with polyphenolic dyes to obtain bright colours and wash-fastness.

However, the stoichiometry of complexes of alum with these dyes in aqueous solution have not been reported yet. The aim of the present work is to determine the stoichiometric composition of the complexes formed between morin and quercetin with alum in aqueous solution with and without pH control. The molar ratio method is used for the spectrophotometric determination of the complexes.

Materials and Methods

Morin [480-16-0] was purchased from Sigma. Quercetin was obtained from Acros Organics. Alum ($KAl(SO_4)_2 \cdot 12H_2O$) and methanol were obtained from Merck.

The molar ratio method has allowed us to determine the composition of the complex in solution from spectrophotometric spectra. For this method, the morin stock solution (1.0×10^{-3} M) is prepared successively in 50%(v/v) methanol. Alum solutions are prepared in distilled water. A concentration of 5.0×10^{-5} M of morin in water was diluted from stock solution and kept constant whereas alum was varied from 0 to 250 μM. Quercetin stock solution (1.0×10^{-3} M) is prepared successively in methanol. The stoichiometry of alum and quercetin were performed in a similar manner with morin. For the study in buffer system,

5%(v/v) of 1.0 M ammonium acetate-acetic acid buffer (pH 4.5) were added into mixture of alum and dyes.

In order to reach the complexation equilibrium, the absorption spectra of each solution was recorded after standing for 30 min. An Agilent 8453 UV-Vis spectrophotometer was employed for absorbance measurements using quartz cells of path length 1 cm.

For computational modeling, PC Spartan Pro (Wavefunction, Irvine, CA) was used. Lowest energy conformers were determined by molecular modeling using MMFF94 force fields. The equilibrium geometries of the lowest energy conformers were optimized using semiempirical PM3 calculations.

Results and Discussion

UV-Visible Spectra of Dyes in Aqueous Solution without pH Control and at pH 4.5

The UV-Vis spectrum of morin in aqueous solution without pH control (Figure 2(a)) is characterized by two major absorption bands with maxima at 378 nm (band I) and 261 nm (band II). Band I is considered to be associated with the absorption due to the B-ring cinnamoyl system, and band II with the absorption involving the A ring system. The absorbance of band I of morin decreases at 378 nm and a new band, which increases with the amount of added alum, appears at 415 nm.

The addition of alum to quercetin aqueous solution results in significant change of the absorbance spectrum with the appearance of a new band centered on 420 nm with bathochromic shift of about 58 nm from the original band in the absence of alum. A typical example of the spectral changes observed upon addition of alum to quercetin solution is shown in Figure 2(b).

Absorption spectra of morin in aqueous solution at pH 4.5 with different concentration of alum are shown in Figure 3(a). The absorbance of the band I of morin at 355 nm decreased and a new band, which increased with the amount of alum added, appeared at 413 nm. For quercetin, the absorbance of the band I of quercetin at 368 nm decreased and a new

band, which increased with the amount of alum added, appeared at 425 nm (Figure 3(b)).

The result of the study of alum effect on the visible spectra (λ_{max}) of morin and quercetin are presented in Figures 2 and 3 which show a large bathochromic shift of morin and quercetin as the alum concentration

increases. The higher alum concentration results in higher amount of the morin and quercetin complex in solution and the intensity of the absorption band at the long wavelength is increased.

It is probable that the bathochromic shift occurs as a result of coordination by the lone
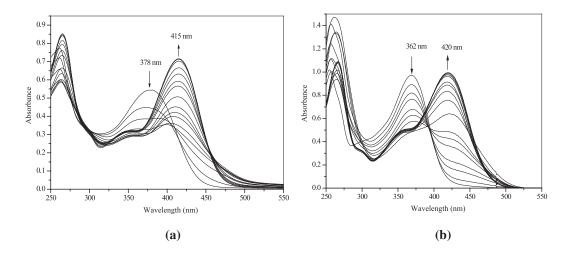

Figure 2. **Electronic absorption spectra of (a) morin (5.0×10^{-5} M) in aqueous solution in absence and in presence of alum (0 - 250 μM) without pH control, (b) quercetin (5.0×10^{-5} M) in aqueous solution absence and in presence of alum (0 - 500 μM) without pH control**
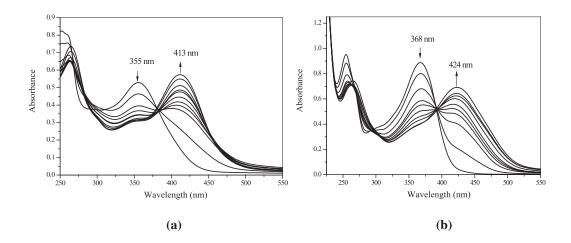

Figure 3. **Electronic absorption spectra of (a) morin (5.0×10^{-5} M) in aqueous solution absence and presence of alum (0 - 250 μM) at pH 4.5, (b) quercetin (5.0×10^{-5} M) in aqueous solution absence and presence of alum (0 - 300 μM) at pH 4.5**

pair electrons on the O donor atoms with the aluminium ion site, thus stabilizing the excited state relative to the ground state leading to longer wavelength absorption maxima (Christie, 2001 and Zollinger, 2003).

Commonly, the transition metal complexes of a coloured organic ligand exhibit light fastness which is significantly better than that of the free ligand. An explanation that has been offered for this effect is that coordination with a transition metal ion reduces the electron density at the chromophore, which in turn leads to improved resistance to photochemical oxidation (Christie, 2001).

Complex Stoichiometry of Dyes and Alum in Aqueous Solution without pH Control and at pH 4.5

The stoichiometry ratio of Al:morin complexes depends on media such as solvent and pH. The association of Al(III) ion with morin gives rise to two complexes with 1 : 1 and 1 : 2 (Al(III) : morin) stoichiometries in methanol solution and 1 : 1 in acidic methanol solution (Gutierrez and Gehlen, 2002). In this study the stoichiometry of the complex was determined using the molar ratio method. The molar ratio plots at 415 nm without pH control (λ_{max} of complex) show inflection at [alum]/[morin] 1.5, indicating a stoichiometry ratio of alum:morin, i.e. $Al_3(morin)_2$ (Figure 4(a)). The proposed structure of $Al_3(morin)_2$ is shown in Figure 6(a). In addition at pH 4.5, the molar ratio plots of [alum]/[morin] at wavelengths 355 nm and 413 nm (Figure 5(a)) show an inflection at [alum]/ [morin] 1 which indicates the formation of alum : morin 1 : 1 complex, i.e. Al(morin). The proposed structures of Al(morin) are shown in Figures 6(b) and 6(c).

Morin possesses two possible chelating sites, 3-hydroxy-4-oxo and 5-hydroxy-4-oxo systems. In addition, the 3,2′-dihydroxy system in morin is a potential chelating site, which is so placed that a seven-membered chelate ring is formed on binding Al(III) ion. An Al(III) ion bridges 2′- and 3-hydroxy groups rather than 4-keto- and 3-hydroxy groups, and makes another Al(III) ion binding with 5-hydroxy-4-oxo system possible.

In the study of the stoichiometry of alum and quercetin in aqueous solution without pH control, a molar ratio plot is presented in Figure 4(b). It was found that alum : quercetin 1 : 1. As Figure 5(b) ratio was showed the molar ratio plots at control pH 4.5 of [alum]/[quercetin] in aqueous solution at 368 nm and 425 nm. It was also showed [alum]/[quercetin] ratio 1, noted Al(quercetin).

Quercetin possesses three possible chelating sites in competition: 3-hydroxychrom-one, 5-hydroxychromone and the 3′,4′-dihy-droxy group (Cornard and Merlin, 2002). The proposed structures of Al(III) complexation with quercetin are shown in Figures 6(d) and 6(e).

Al(III) ion has a coordination number of six and forms a complex with an octahedral configuration. It can form complexes with six monodentate ligands, or three bidentate ligands. We propose a possible structure of Al–dye complexes in Figure 6.

Computational Modeling

Molecular modeling was carried out on the structures of the proposed complexes by semiempirical PM3 calculations to determine the heat of formation of the complexes. Water molecules have been added to obtain an octahedral environment of the complex ions which are the most likely structure of Al(III) complexes. Table 1 shows heat of formation values of complexes with H_2O molecule. It was found that the heat of formation showed negative values for all the proposed structures.

Conclusion

Applying the molar ratio method, it was determined that stoichiometric composition of complex formed is $Al_3(morin)_2$, Al(morin), and Al(quercetin) in aqueous solution. Alum caused a bathochromic shift of the visible absorption bands in morin and quercetin. The bathochromic shifts observed are consistent with the lone pair electrons in the donor atoms (O in the dyes) participating in metal ion coordination and stabilizing the excited state relative to the ground state.
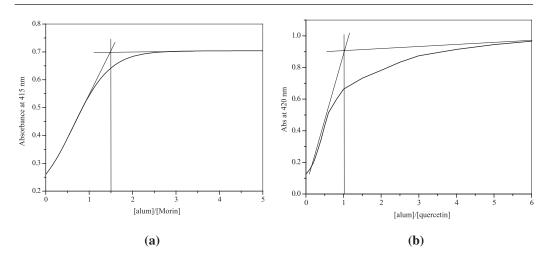
Figure 4. Absorbance versus (a) [alum]/[morin] molar ratios plots at 415 nm (b) [alum]/ [quercetin] molar ratios plots at 420 nm without pH control
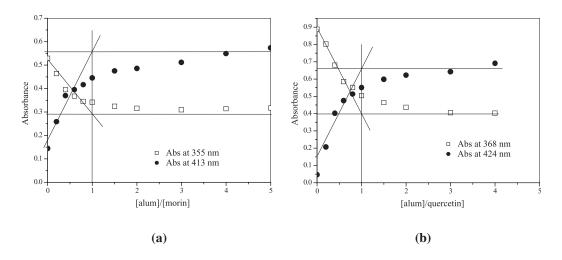

Figure 5. Absorbance versus (a) [alum]/[morin] (b) [alum]/[quercetin] molar ratios plots at pH 4.5

Table 1. Heat of formation of proposed complexes by using semiempirical PM3 calculations

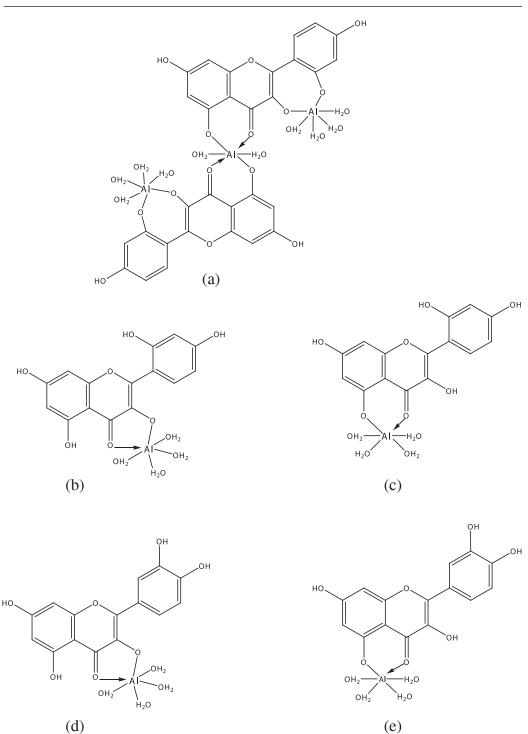| Complex structure	Heat of formation (kcal/mol)
(a)	-764.358
(b)	-212.079
(c)	-209.255
(d)	-206.514
(e)	-207.029

Figure 6.   The proposed structures of Al(III) complexation (a) Al$_3$(morin)$_2$, (b), (c) Al(morin) and (d), (e) Al(quercetin)

Acknowledgements

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