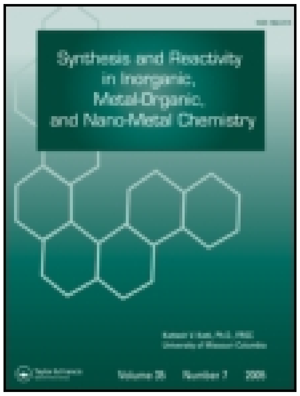

Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry

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Synthesis, Electrochemical, Spectral, and Antioxidant Properties of Complexes of Flavonoids with Metal Ions

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Synthesis, Electrochemical, Spectral, and Antioxidant Properties of Complexes of Flavonoids with Metal Ions

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ABSTRACT

Complexes of metal ions with the flavonoids quercetin (H_2L^1), rutin (H_2L^2) and 3-hydroxyflavone (HL^3) have been synthesized. From the data obtained by elemental analyses, molar conductance, atomic absorption, thermal analyses, ^{1}H NMR, IR, UV-Vis and EPR spectroscopies one can propose the compositions: $[Cu_2(L^1)(H_2O)_4]Cl_2$ (**1**),

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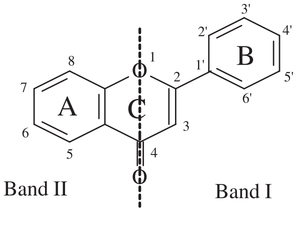
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$ (**2**), $[Cu(L^3)(H_2O)_2]Cl$ (**3**), $[Fe_2(L^1)(H_2O)_8]Cl_2$ (**4**), $[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$ (**5**) and $[Fe(L^3)(H_2O)_4]Cl$ (**6**). We have used the assumption that the electrochemical oxidation can be used as a model for antioxidant activities for the flavonoids. The cyclic voltammograms show irreversible oxidation processes at +0.58 V and +0.91 V for (**1**), +0.82 V for (**2**), +0.89 V for (**3**), +0.58 V and +0.90 V for (**4**), +0.78 V for (**5**) and + 0.86 V for (**6**) versus Ag/AgCl. These values are lower than those for the free flavonoids indicating a more favorable antioxidant activity. Furthermore, these complexes were also more effective than the uncomplexed flavonoids scavenging superoxide radicals generated by reduction of the oxygen-saturated solution. The superoxide radical scavenging activities of flavonoid complexes seemed to correlate with the redox potentials of the flavonoid.

Key Words: Flavonoids; Flavonoid complexes; Quercetin; Rutin; 3-Hydroxyflavone; Antioxidants.

INTRODUCTION

Flavonoids constitute a group of natural antioxidant substances which have been studied intensively due, in part, to their therapeutical properties, such as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral and anticancer.[1–3] The capacity of flavonoids to act as antioxidants depends on their molecular structure (Figure 1). It has been demonstrated



Quercetin (L^1): 3, 5, 7, 3', 4'-OH

Rutin (L^2): 5, 7, 3', 4'-OH; 3-Orut

rut = disaccharide glucorhamnoside

3-Hydroxyflavone (L^3): 3-OH

Figure 1. Common structure of the flavone subclass and the division of bands I and II related to the UV-Vis absorption bands.

that the flavonoids can coordinate to transition metal ions like Zn(II) and Al(III).[4–6] In this work we report the synthesis, characterization and antioxidant activities of quercetin (H_2L^1), rutin (H_2L^2) and 3-hydroxyflavone (HL^3) complexed with Cu(II) and Fe(II) ions. The antioxidant activities of the compounds were evaluated by their oxidation potentials assuming that both the electrochemical oxidation ($Fl{-}OH \rightarrow Fl{-}O^{\cdot} + e^- + H^+$) and the antioxidant activity ($Fl{-}OH \rightarrow Fl{-}O^{\cdot} + H^{\cdot}$) involve the breaking of the same O–H bond;[7] the smaller the oxidation potential the bigger will be the antioxidant activity of the compound. This suggests that the electrochemical properties of the flavonoids may be associated with their biological activities.

EXPERIMENTAL

Materials

The compounds $CuCl_2{\cdot}2H_2O$, $FeCl_2{\cdot}4H_2O$, quercetin, rutin and 3-hydroxyflavone were purchased from Aldrich Chemical Co. and were used as received. Other chemicals, from different manufacturers, were of AR grade and were purified by the usual procedures.[8]

Instrumentation and Measurement

Elemental analyses were performed using a CHNS-O CE Instruments model EA 1110 elemental analyzer. Copper and iron were determined by a model V-85 B. Braun ICP-AES-atomic emission spectrometer. Molar conductances, in MeOH, were determined in a DSS-11A Metrohm conductivity meter. The ^1H NMR spectra in DMSO-d_6 were obtained on a RMN Bruker DR X 400 9.4T spectrometer. EPR spectra at room temperature were recorded in the solid state on a K-band Bruker EP 200D spectrometer, with a microwave frequency of 24.06 GHz and a modulation frequency of 51 KHz. The IR spectra were recorded on a Nicolet FTIR 5ZDX spectrometer using KBr pellets. UV-Vis. spectra in MeOH were registered on a Hewlett-Packard 8453 Diode Array UV-Vis spectrophotometer using standard 1.00 cm quartz cells. The ε values represent the average obtained from three different solutions. Thermogravimetric analysis measurements were performed by a TA Instruments SDT 2960-Simultaneous DTA-TGA-Thermal Analysis, between room temperature and 800 °C at a heating rate of 10 °C min^{-1}. The cyclic voltammograms were obtained on a 273A EG&G Potentiostat/Galvanostat, using a one-compartment electrochemical cell equipped with a glassy carbon working electrode, a platinum wire auxiliary electrode and a Ag/AgCl reference electrode; the voltammograms were recorded in MeOH (1×10^{-3} molar solutions) with $LiClO_4$ as supporting electrolyte. Electrolysis were performed at fixed applied potentials, at 25 ± 1 °C, using a glassy carbon plate working electrode.

Stoichiometric Ratios of the Complexes

Job's method[9] was used to determinate in methanol the stoichiometric ratio for the reaction between the flavonoid and the metal ion. The solutions were prepared by mixing solutions of both components of equal molar concentration (1.1×10^{-4} molar) in ratios varying from 1:9 to 9:1. Absorbances were measured at 413 nm.

Superoxide Anion Assay

The superoxide ion was generated by reduction of the oxygen-saturated solution and its concentration was determined by the anodic current in the cyclic voltammogram. Knowing that an oxygen-saturated solution in DMSO is 2.1×10^{-3} molar, we have assumed this superoxide concentration at the surface of the electrode after the reduction process. This assumption is based on the fact that the cathodic peak current for oxygen reduction has the same value as the anodic peak current for superoxide oxidation in the cyclic voltamogramm of an oxygen-saturated solution.

Preparation of the Metal Complexes

The complexes were prepared by mixing, in methanol, stoichiometric amounts of the flavonoid and metal ion, to reach the M:L molar ratio, previously determined by Job's method.

Quercetin Complexes. 0.3383 g (1 mmol) of quercetin; 0.3410 g (2 mmol) of $CuCl_2{\cdot}2H_2O$ or 0.3976 g (2 mmol) of $FeCl_2{\cdot}4H_2O$; 100 mL of methanol; reflux 2 h.

Rutin Complexes. 0.6105 g (1 mmol) of rutin; 0.2557 g (1.5 mmol) of $CuCl_2{\cdot}2H_2O$ or 0.2982 g (1.5 mmol) of $FeCl_2{\cdot}4H_2O$; 200 mL of methanol; reflux 5 h.

3-Hydroxyflavone Complexes. 0.2382 g (1 mmol) of 3-hydroxyflavone; 0.1705 g (1 mmol) of $CuCl_2{\cdot}2H_2O$ or 0.1988 g (1 mmol) of $FeCl_2{\cdot}4H_2O$; 70 mL of methanol; reflux 2 h.

In all preparations, pale yellow precipitates were formed immediately when the cooled mixtures were poured into H_2O (500 mL). The crystals were washed thrice with 1:3 EtOH/H_2O mixtures and then several times with H_2O and dried under vacuum.

RESULTS AND DISCUSSION

The syntheses of the complexes can be represented by the equations:

$$H_2L^1 + 2MCl_2{\cdot}xH_2O \rightarrow [M_2(L^1)(H_2O)_{2x}]Cl_2 + 2HCl$$

$$2H_2L^2 + 3MCl_2{\cdot}xH_2O \rightarrow [M_3(L^2)_2(H_2O)_{3x}]Cl_2 + 4HCl$$

$$HL^3 + MCl_2{\cdot}xH_2O \rightarrow [M(L^3)(H_2O)_x]Cl + HCl$$

where: M = Cu(II), x = 2; M = Fe(II), x = 4.

The solid amorphous complexes are pale yellow, stable in the air and can be stored without change of composition. They are soluble in MeOH, EtOH and DMSO, slightly soluble in Me_2CO, but scarcely soluble in H_2O and CCl_4. Table 1 shows the analytical data for the complexes. The molar conductance data in MeOH for the complexes are given in Table 2.

Stoichiometric Ratios of the Complexes

In pure methanol, results clearly show the three possible complexing sites: 3′–4′, 3–4 and 7-OH groups, in Figure 1. Figure 2 shows the absorption spectra of 3-hydroxyflavone, in methanolic solutions, with different concentrations of $CuCl_2{\cdot}2H_2O$. The disappearance of the band at 343 nm, associated with the appearance of a band at 413 nm as the mole ratio is increased, is consistent with the formation of a new species in the system. The maximum of Job's curve (Figure 3) appears at the 0.5 mole fraction indicating 1:1 stoichiometry for the reaction of Cu(II) and 3-hydroxyflavone. Table 3 summarizes the stoichiometries of the complexes.

^1H NMR of the Fe(II)-Flavonoid Complexes

The ^1H NMR spectrum of the Fe(II)-complexes has fully resolved spin–spin couplings and lies completely within the diamagnetic region,

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Table 1. Analytical data for the complexes.

Complex	Molecular formula	M.p. (°C)	M. wt.	Anal. found (calcd.) %			Yield (%)
				C	H	M	
$[Cu_2(L^1)(H_2O)_4]Cl_2$	$C_{15}H_{16}Cl_2Cu_2O_{11}$	190	577.27	31.30 (31.21)	2.84 (2.79)	22.27 (22.71)	82
$[Fe_2(L^1)(H_2O)_8]Cl_2$	$C_{15}H_{24}Cl_2Fe_2O_{15}$	182	628.86	28.79 (28.65)	3.80 (3.85)	17.84 (17.76)	84
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	$C_{52}H_{72}Cl_2Cu_3O_{36}$	210	1540.66	40.67 (40.54)	4.65 (4.71)	12.82 (13.41)	74
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	$C_{52}H_{84}Cl_2Fe_3O_{45}$	225	1670.45	37.52 (37.39)	5.14 (5.07)	10.13 (10.02)	78
$[Cu(L^3)(H_2O)_2]Cl$	$C_{15}H_{13}ClCuO_5$	165	372.27	48.46 (48.40)	3.50 (3.53)	17.11 (17.06)	85
$[Fe(L^3)(H_2O)_4]Cl$	$C_{15}H_{19}ClFeO_7$	175	402.62	44.69 (44.75)	4.81 (4.76)	13.80 (13.85)	88

Table 2. Molar conductance data in MeOH for the complexes.

Complex [1 × 10^{-3} molar]	Λ_M (Ω^{-1} cm^2 mol^{-1})	Electrolyte type[10]
$[Cu_2(L^1)(H_2O)_4]Cl_2$	204.21	1:2
$[Fe_2(L^1)(H_2O)_8]Cl_2$	194.13	1:2
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	208.85	1:2
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	191.56	1:2
$[Cu(L^3)(H_2O)_2]Cl$	102.48	1:1
$[Fe(L^3)(H_2O)_4]Cl$	99.25	1:1

indicating a low-spin Fe(II) center. The ^{1}H NMR data for the complexes, as well as those for the free ligands are listed below.

Quercetin (H_2L^1): δ 12.50 (s, 1H, 5-OH), 10.76 (s, 1H, 7-OH), 9.60 (s, 1H, 3-OH), 9.39 (s, 1H, 4′-OH), 9.31 (s, 1H, 3′-OH), 7.68 (d, $J_{H2'/H6'}$ = 1.8 Hz, 1H, 2′-H), 7.55 (dd, 1H, 6′-H), 6.89 (d, $J_{H5'/H6'}$ = 8.5 Hz, 1H, 5′-H), 6.43 (d, $J_{H8/H6}$ = 1.5 Hz, 1H, 8-H), 6.22 (d, 1H, 6-H).

$[Fe_2(L^1)(H_2O)_4]Cl_2$: δ 12.48 (s, 1H, 5-OH), 10.74 (s, 1H, 7-OH), 9.27 (s, 4′-OH), 9.21 (s, 1H, 3′-OH), 7.63 (d, $J_{H2'/H6'}$ = 2.0 Hz, 1H, 2′-H), 7.46 (dd, 1H, 6′-H), 6.81 (d, $J_{H5'/H6'}$ = 8.2 Hz, 1H, 5′-H), 6.41 (d, $J_{H8/H6}$ = 1.7 Hz, 1H, 8-H), 6.21 (d, 1H, 6-H).

Rutin (H_2L^2): δ 12.63 (s, 1H, 5-OH), 10.82 (s, 1H, 7-OH), 9.69 (s, 1H, 4′-OH), 9.28 (s, 1H, 3′-OH), 7.49 (d, $J_{H2'/H6'}$ = 1.9 Hz, 1H, 2′-H), 7.52 (d,

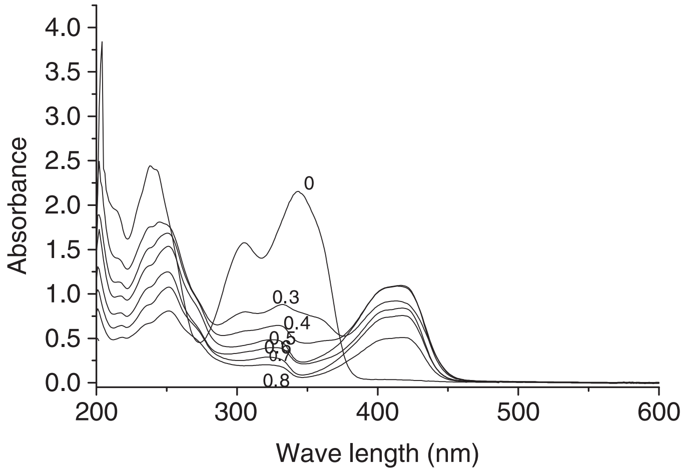


Figure 2. UV-Vis spectra of 3-hydroxyflavone in the presence of Cu(II) ions in methanol. The molar ratios M/L are indicated for each spectrum.

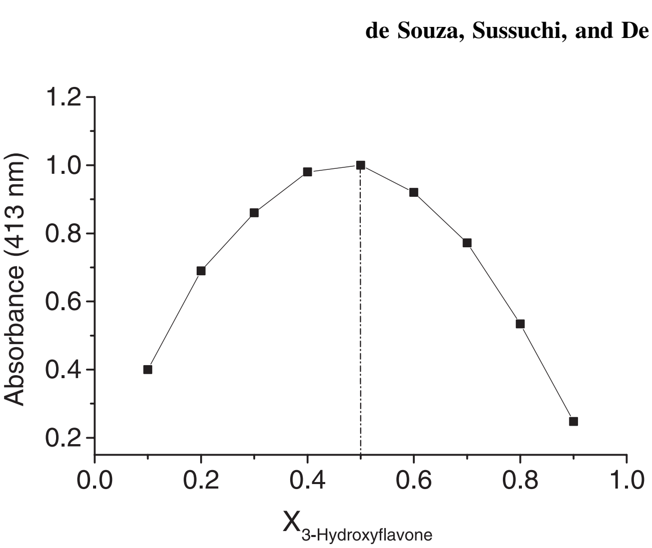


Figure 3. Job's curve: plot of absorbance (at 413 nm) versus mole fraction (X) of 3-hydroxyflavone.

$J_{H5'/H6'}$ = 8.8 Hz, 1H, 5′-H), 7.85 (dd, 1H, 6′-H), 6.41 (d, $J_{H8/H6}$ = 1.5 Hz, 1H, 8-H), 6.24 (d, 1H, 6-H).

$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$: δ 12.58 (s, 1H, 5-OH), 9.57 (s, 4′-OH), 9.17 (s, 1H, 3′-OH), 7.43 (d, $J_{H2'/H6'}$ = 1.6 Hz, 1H, 2′-H), 7.39 (d, $J_{H5'/H6'}$ = 8.4 Hz, 1H, 5′-H) ,6.79 (dd, 1H, 6′-H), 6.36 (d, $J_{H8/H6}$ = 1.8 Hz, 1H, 8-H), 6.18 (d, 1H, 6-H).

3-Hydroxyflavone (HL^3): δ 9.81 (s, 1H, 3-OH), 8.37 (s, 1H, 5-H), 6.82 (d, $J_{H6/H7}$ = 8.2 Hz, 1H, 6-H), 7.55 (dd, 1H, 7-H), 7.00 (d, $J_{H8/H7}$ = 8.4 Hz, 1H, 8-H), 8.30 (m, 2H, 2′, 6′-H), 7.63 (m, 2H, 3′, 5′-H), 7.54 (m, 1H, 4′-H).

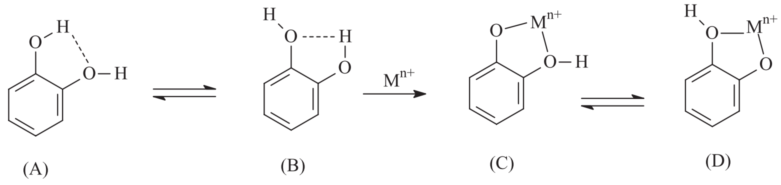
$[Fe(L^3)(H_2O)_4]Cl$: 8.32 (s, 1H, 5-H), 6.77 (d, $J_{H6/H7}$ = 7.4 Hz, 1H, 6-H), 7.47 (dd, 1H, 7-H), 6.97 (d, $J_{H8/H7}$ = 7.5 Hz, 1H, 8-H), 8.28 (m, 2H, 2′, 6′-H), 7.61 (m, 2H, 3′, 5′-H), 7.51 (m, 1H, 4′-H).

Quercetin and rutin are able to chelate metal ions via the phenolic groups 3′ and 4′. Upon complexation, the metal ion displaces 1 proton from

Table 3. Stoichiometries (M:L) of the complexes of metal ions with quercetin, rutin, and 3-hydroxyflavone in methanol.

Flavonoids	Cu(II)	Fe(II)
Quercetin	2:1	2:1
Rutin	3:2	3:2
3-Hydroxyflavone	1:1	1:1

the flavonoid nucleus. In solution, the flavonoids are in a relatively rapid equilibrium between structures (A) and (B) (Eq. 1).[11] Both structures have an easily removable H atom, whereas the other H atom is intramolecularly bonded. From the integration of the ^{1}H NMR signals, it was found that in each complex, the ratio of structures (C) and (D) is 3:1. The ^{1}H NMR spectra of the Fe(II) complexes also reveal that the resonances of the hydrogen of the 3-OH groups are not present in quercetin and 3-hydroxyflavone complexes and that the hydrogens of the 7-OH groups are not present in the rutin complexes indicating the loss of the OH protons due to complexation. The proton signals of the complexes are shifted to lower frequency relative to the free flavonoids. This result is probably due to the increase of the conjugation caused by the effect of coordination when the complex is formed.



(1)

Quercetin and rutin deprotonation and complexation of the 3′ and 4′ groups.

IR Spectra

Ligands and complexes IR spectra present evidence of the coordination between the transition metal ions and flavonoids. The data are summarized in Table 4. It can be noted that: 1) ν(C–O–C) and ν_{ring}(C=C)

Table 4. IR data[a] (cm^{-1}) for ligands and complexes.

Compound	ν(M–O)	ν(C=O)	ν(C–O–C)	ν(C=C)	ν(O–H)
Quercetin	–	1661 s	1260 s	1589 s	3625–3375 b, m
$[Cu_2(L^1)(H_2O)_4]Cl_2$	608 w	1609 s	1258 s	1587 s	3630–2390 b, m
$[Fe_2(L^1)(H_2O)_8]Cl_2$	561 w	1606 s	1256 s	1586 s	3672–2397 b, m
Rutin	–	1653 s	1257 s	1590 s	3667–3030 b, m
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	610 w	1651 s	1252 s	1586 s	3690–2896 b, m
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	559 w	1649 s	1254 s	1588 s	3640–2896 b, m
3-Hydroxyflavone	–	1649 s	1259 s	1589 s	3324–2987 b, m
$[Cu(L^3)(H_2O)_2]Cl$	610 w	1598 s	1261 s	1589 s	3344–3035 b, m
$[Fe(L^3)(H_2O)_4]Cl$	562 w	1596 s	1260 s	1587 s	3300–2983 b, m

[a] b = Broad, m = medium, s = strong, w = weak.

frequencies[12] are slightly shifted upon complexation indicating that the ring oxygen (position 1, Figure 1) is not involved in the complexation, 2) the characteristic band of carbonyl groups in the quercetin and 3-hydroxyflavone complexes appears at 1600 cm^{-1}, while in the free flavonoids[13] these bands are at 1660–1650 cm^{-1}. The ~50 cm^{-1} displacements suggest that the coordination of quercetin and 3-hydroxyflavone involves the oxygen atoms of the carbonyl and 3-OH groups, 3) for the rutin complexes no shifts of the carbonyl bands were observed, presumably because the 3-OH group of the rutin is blocked by the sugar moiety (disaccharide glucorhamnoside), 4) ν(M–O) frequencies appear in the 560–610 cm^{-1} range,[13] 5) ν(O–H) frequencies appear as broad bands (3690–2390 cm^{-1}) indicating the presence of water. The presence of coordinated water is also supported by thermal analyses.

EPR Spectra

The K-band EPR spectrum of the $[Cu_2(L^1)(H_2O)_4]Cl_2$ complex is shown in Figure 4. The g tensor values, presented in Table 5, can be used to derive the ground state. The $g_{\parallel} > g_{\perp} > 2.0023$ values and the $g_{\parallel}/A_{\parallel}$ ratio suggest square-planar geometry of the complexes. Square-planar complexes have a $g_{\parallel}/A_{\parallel}$ quotient[14] ranging from 113 to 150 cm; this ratio increases with the increase of tetrahedral distortion. This geometry is also supported by the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital with $^2B_{1g}$ as the ground state (see discussion of UV-Vis

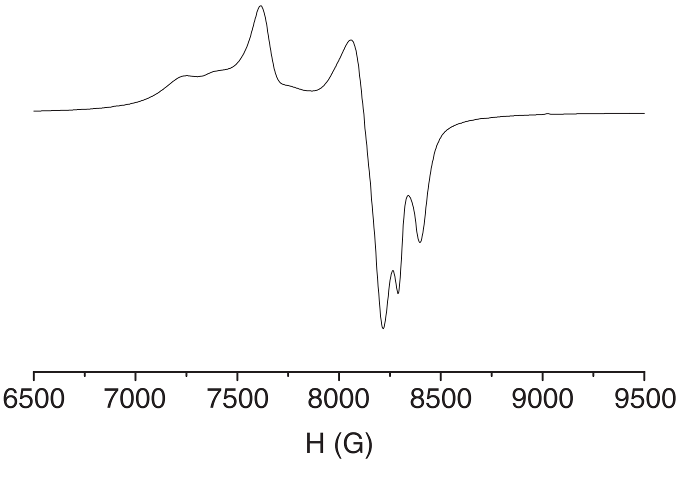


Figure 4. The K-band EPR spectrum of $[Cu_2(L^1)(H_2O)_4]Cl_2$.

Table 5. EPR spectral properties of Cu(II) complexes.

Complex	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$ (10^{-4} cm^{-1})	$A_{\perp}$ (10^{-4} cm^{-1})	$g_{\parallel}/A_{\parallel}$ (cm)	g_{iso}	A_{iso} (10^{-4} cm^{-1})	α^2
$[Cu_2(L^1)$-$(H_2O)_8]Cl_2$	2.27	2.06	163	72	139	2.13	102	0.81
$[Cu_3(L^2)_2$-$(H_2O)_{12}]Cl_2$	2.24	2.05	168	76	133	2.11	107	0.76
$[Cu(L^3)$-$(H_2O)_4]Cl$	2.25	2.10	157	71	143	2.15	100	0.66

spectra). The molecular coefficients α^2 were calculated by using the following equation.[15]

$$\alpha^2 = -(A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04$$

The α^2 values (Table 5) indicate the fairly covalent nature of the metal-ligand bonding. No EPR signals appeared for the iron complexes at room temperature as expected for low-spin Fe(II) complexes.

UV-Vis Spectra

The flavonoids exhibit two major absorption bands in the ultraviolet/visible region (Figure 1); The absorptions in the 320–385 nm range

Table 6. UV-Vis data of the ligands and complexes.

	λ_{max} (nm), (ε in dm^3 mol^{-1} cm^{-1})		
Compound [50×10^{-6} molar]	Band I ($\pi \rightarrow \pi^*$)	Band II ($\pi \rightarrow \pi^*$)	d–d
Quercetin	372 (14414)	256 (13706)	
$[Cu_2(L^1)(H_2O)_4]Cl_2$	451 (11054)	268 (31036)	590 (147)
$[Fe_2(L^1)(H_2O)_8]Cl_2$	430 (21794)	268 (31394)	605 (115)
Rutin	358 (17700)	259 (21400)	
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	354 (18760)	267 (27472)	598 (128)
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	365 (10200)	265 (14400)	602 (102)
3-Hydroxyflavone	343 (22410)	238 (26512)	
$[Cu(L^3)(H_2O)_2]Cl$	418 (7168)	248 (23160)	590 (178)
$[Fe(L^3)(H_2O)_4]Cl$	400 (29950)	246 (42256)	601 (118)

Table 7. Thermal analytical data for ligands and complexes.

	Water loss temperature (°C)			Decomposition temperature (°C)		
	Endothermic process			Exothermic process		
Compound	T_1 (°C)	T_2 (°C)	Total wt. loss (%)	T_3 (°C)	T_4 (°C)	Total wt. loss (%)
Quercetin	–	–	–	393	638	100.00
$[Cu_2(L^1)(H_2O)_4]Cl_2$	128	210	12.68	405	662	71.58
$[Fe_2(L^1)(H_2O)_8]Cl_2$	131	219	23.06	408	673	76.99
Rutin	–	–	–	398	678	100.00
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	132	215	7.14	412	687	83.98
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	135	218	13.09	420	693	86.96
3-Hydroxyflavone	–	–	–	381	624	100.00
$[Cu(L^3)(H_2O)_2]Cl$	122	189	9.73	392	627	78.72
$[Fe(L^3)(H_2O)_4]Cl$	127	196	17.96	402	631	84.26

T_1–T_2 temperature range corresponding to complex dehydration; T_3–T_4 temperature range corresponding to complex decomposition.

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correspond to the B ring portion (cinnamoyl system, band I), and the absorptions in the 240–280 nm range correspond to the A ring portion (benzoyl system, band II).[16,17] The spectra are related to the $\pi \rightarrow \pi^*$ transitions in the ligand molecules. These bands are shifted to higher wave length in the spectra of the complexes as shown in Table 6. The d–d bands around 590 nm ($\varepsilon \sim 150\ M^{-1}\ cm^{-1}$) of the Cu(II) complexes can be assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ and $^2B_{1g} \rightarrow {}^2E_g$ transitions as observed for copper compounds having square-planar geometry;[18] the band at ~605 nm ($\varepsilon \sim 115\ M^{-1}\ cm^{-1}$) in the Fe(II) complexes can be attributed to $^1A_{1g} \rightarrow {}^1T_{1g}$ for a Fe(II) center with $S = 0$.[18] The spectra give significant information about the structures of the complexes. As the 3-hydroxy group has a more acidic proton,[19] the 3-OH and 4-oxo groups are the first sites involved in the complexation process. The 3′,4′-dihydroxy groups bind a second metal ion. The 5-OH group is not involved due to lesser proton acidity and the steric hindrance caused by the first complexation.

Thermal Analysis

Thermal data for the dehydration and decomposition of the complexes are given in Table 7. These data show that all complexes contain coordinated water. The dehydration temperature (120–220 °C) and the corresponding weight loss indicate the number of water molecules. The complexes decompose in the 381–589 °C temperature range causing

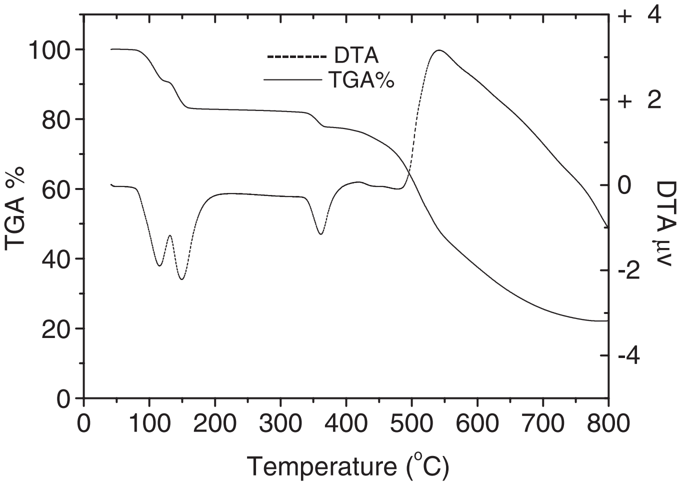

Figure 5. DTA and TGA diagram of the $[Cu_2(L^1)(H_2O)_4]Cl_2$ complex.

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ca. 80% weight loss. The final products are the metal oxides. The thermogravimetric curve of the $[Cu_2(L^1)(H_2O)_4]Cl_2$ complex is shown in Figure 5. From these sets of data one can propose the complex structures shown in Figure 6.

Electrochemical Study

The cyclic voltammograms of ligands and complexes show well-defined oxidation peaks and practically no reverse reduction peaks indicating EC processes involving two electrons.[20,21] The Ep_a data are shown in Table 8.

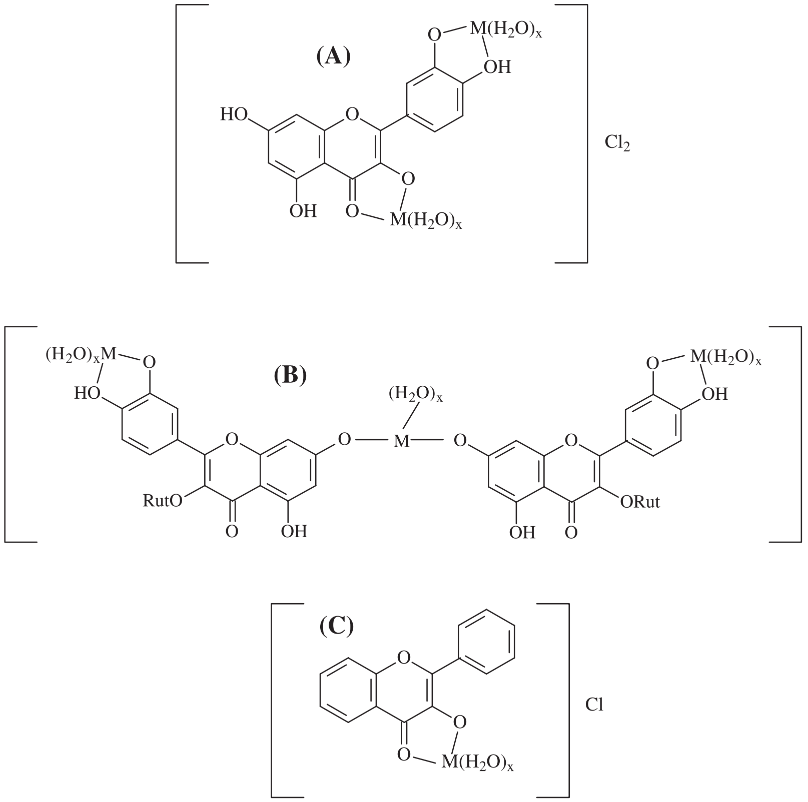

Figure 6. Tentative structures of the complexes: (**A**) quercetin complexes, (**B**) rutin complexes and (**C**) 3-hydroxyflavone complexes. M = Cu(II) or Fe(II), x = 2 for Cu(II) and x = 4 for Fe(II).

Table 8. Electrochemical data of the ligands and complexes.

Compound [1×10^{-3} molar]	Ep_a versus Ag/AgCl (V)
Quercetin	+0.62 and +0.93
$[Cu_2(L^1)(H_2O)_4]Cl_2$	+0.58 and +0.91
$[Fe_2(L^1)(H_2O)_8]Cl_2$	+0.58 and +0.91
Rutin	+1.10
$[Cu_3(L^2)_2(H_2O)_6]Cl_2$	+0.82
$[Fe_3(L^2)_2(H_2O)_{12}]Cl_2$	+0.78
3-Hydroxyflavone	+0.91
$[Cu(L^3)(H_2O)_2]Cl$	+0.89
$[Fe(L^3)(H_2O)_4]Cl$	+0.86

Quercetin by itself presents two oxidation processes at +0.62 V and +0.93 V (Figure 7). Controlled-potential electrolysis of a solution of quercetin at +0.75 V shows that residual current is attained when a number of coulombs equivalent to a two-electron reaction is reached; successively, under a fixed applied potential of +1.2 V, the second anodic peak disappears when additional number of coulombs corresponding to a two-electron reaction is reached. These processes are outlined in Figure 8.

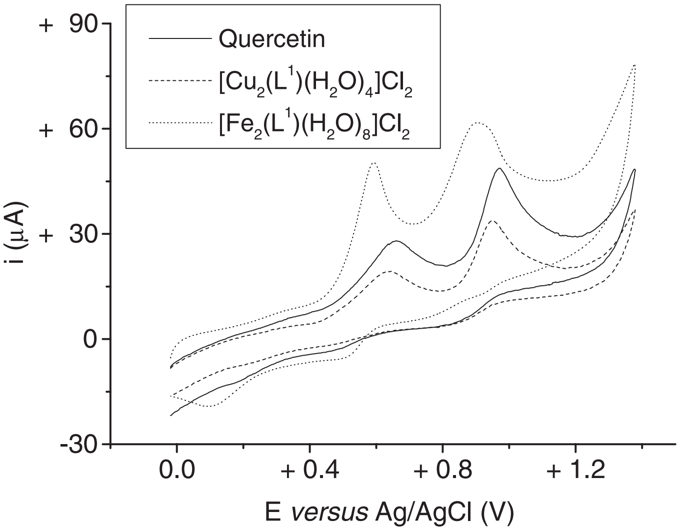

Figure 7. Cyclic voltammograms of quercetin (H_2L^1) and Cu(II) and Fe(II) complexes (1×10^{-3} molar); 0.1 molar $LiClO_4$/MeOH; scan rate 0.1 V.s^{-1}.

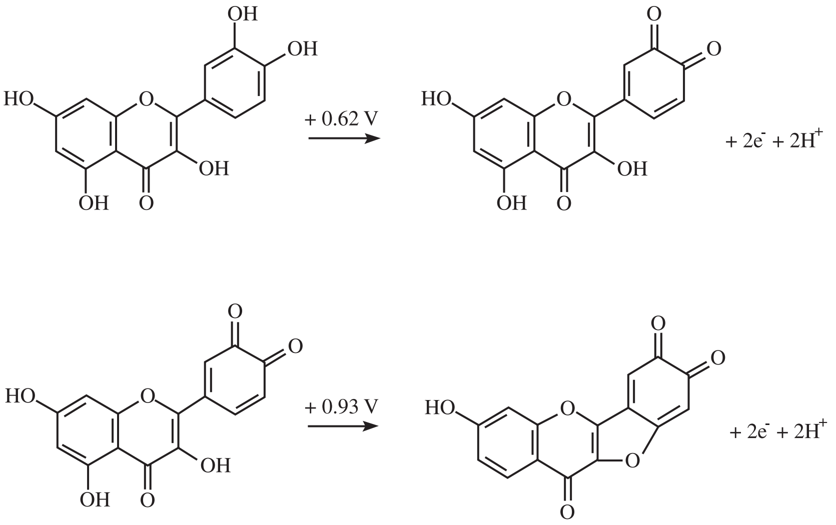

Figure 8. Oxidized structures proposed for quercetin.

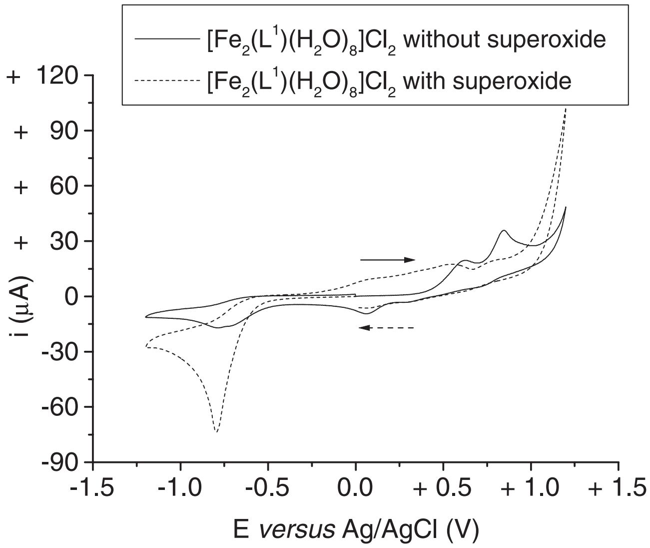

Figure 9. Cyclic voltammograms of $[Fe_2(L^1)(H_2O)_8]Cl_2$, L^1 = quercetin, 1×10^{-3} molar, in the absence (—) and presence (---) of 1 equivalent superoxide ions; 0.1 molar Bu_4NClO_4/DMSO; scan rate 0.1 $V.s^{-1}$.

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The data in Table 8 show that there was an appreciable difference in the oxidation potentials of the flavonoids investigated, and that, as expected, extended conjugation and an increasing number of hydroxyl groups lowered the oxidation potential. The cyclic voltammetric data also show a considerable decrease of the oxidation potentials of the complexes related to that of the free flavonoids. Thus, flavonoid-metal complexes have been found to be more effective antioxidant than the free flavonoids.

Superoxide Ion Scavenging

Various low-molecular-weight superoxide dismutase (SOD)-like complexes of iron and copper have also been reported to have SOD mimic activity.[22,23] The complexation with metal ions decreases the oxidation potential of the flavonoid/semiquinone redox pair (see Table 8). As a consequence, the complexed flavonoids are relatively more readily oxidized by superoxide ion than the uncomplexed ones. The interaction between $[Fe_2(L^1)(H_2O)_8]Cl_2$ and 1 equivalent of superoxide ion was studied by cyclic voltammetry (Figure 9). The absence of the oxidation peaks of

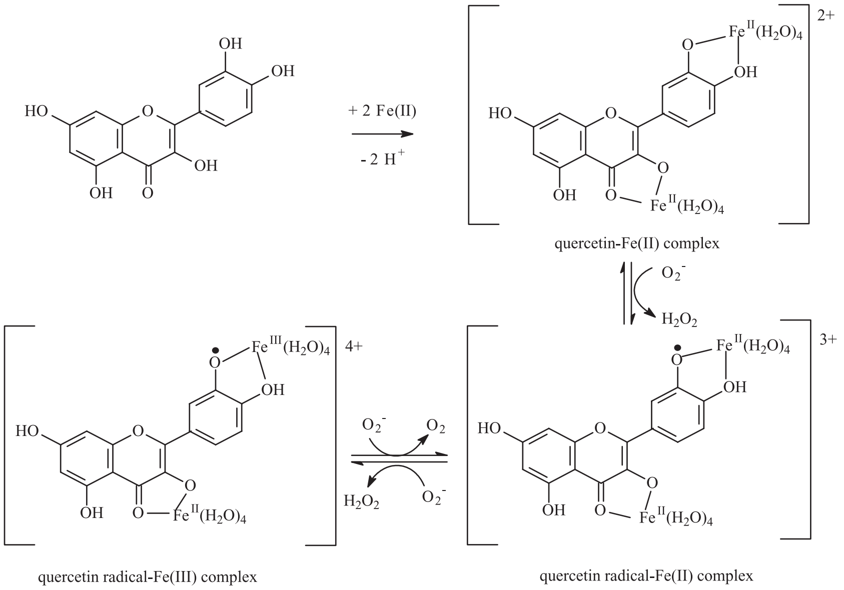

Figure 10. Mechanisms of superoxide scavenging by flavonoid complexes.

the $[Fe_2(L^1)(H_2O)_8]Cl_2$ complex and of the superoxide ion reveals the ability of these compounds to scavenge radicals. The dismutation of superoxide ion by flavonoid complexes could be explained by the redox cycling of the metal and the ligand as shown in the Figure 10: the metal complex can be oxidized to a Fe(II)-semiquinone complex by superoxide radical, which is oxidized by another superoxide radical to form the Fe(III)-semiquinone complex.

CONCLUSION

The results obtained in this work show that metal ion-flavonoid complexes, in the solid state, contain coordinated water. The spectroscopic data show the importance of the 3-OH group as coordination site. The electrochemical data clearly show that the antioxidant activity depends on the number and positions of OH groups in the flavonoid structures. Metal ions increase the flavonoids antioxidant activities as shown by the lower complexes oxidation potentials relative to those of the free flavonoids. In addition, the free radical scavenging activities are maintained in the complexes.

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