POTENTIOMETRIC AND SPECTROPHOTOMETRIC DETERMINATION OF THE STABILITY CONSTANTS OF QUERCETIN (3,3',4',5,7-PENTAHYDROXYFLAVONE) COMPLEXES WITH ALUMINIUM(III) AND IRON(II)

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ABSTRACT

In this study, the stability constants of the complexes of quercetin formed with aluminium(III) and iron(II) were determined potentiometrically by using the Calvin-Bjerrum and Irving Rossotti methods and spectrophotometrically. The evaluated protonation constants of the ligand were found: log K_1=9.63±0.11 and log K_2 = 8.32±013, at 25°C. For the aluminium and iron complexes logarithms of the stability constants were found: for Al: log K_1=14.05±0.09, log K_2=10.02±0.12, and for Iron: log K_1=9.44±0.11, log K_2=3.86±0.08.

Key words: quercetin, aluminium, iron, stability constant, potentiometri, spectrophotometri

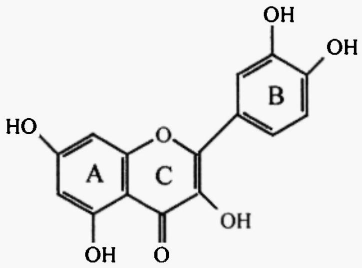
INTRODUCTION

Flavonoids are phenolic compounds widely distributed in plants whose beneficial effects on human health have gained increasing interest in recent years. Quercetin, belonging to these phenolic compounds, has been acknowledged to be an antioxidant and radical scavenger. These molecules have attracted the attention of many researchers because flavonoids display a remarkable array of biological and pharmacological activities. The antioxidant activity of flavonoids has been considered to be via possible modes of action, radical scavenging and also from their chelating properties of metal ions such as iron or copper. It has been found that flavonoids have three structural configurations that facilitate donation of hydrogen atoms in descending order of efficacy. Transition metals are strongly implicated in the generation of free radicals by decomposition of H_2O_2 or lipid hydroperoxide (LOOH) to give hydroxyl radicals or alkoxyl radicals, respectively. Metal chelating by flavonoids may sequester these metal ions and prevent free radical formation /1,2/.

The interaction of flavonoids with metal ions may change the antioxidant properties and some biological effects of the flavonoids. There are many studies, mostly using Fe ions as the transition metal, showing that flavonoids inhibit lipid peroxidation in biological systems, including low-density lipoproteins, liposomes, microsomes, and cultured hepatocytes /1/. There is a good deal of evidence that the catechol-type B ring in the flavonoids is the antioxidant active moiety, if these groups are blocked through chelation to a metallic ion. It is well known that they can also chelate other metallic ions such as Cd^{2+}, Al^{3+} and Zn^{2+}. In particular, the complexes with Al^{3+} have been used either for o-dihydroxyphenyl group or o-quinone dihydroxyphenyl group. Complexes of flavonoids with transition metal ions have been shown to be reduced by phenols after chelation and can be re-oxidized by oxygen, specially with quercetin which leads to hydroxyl radical /2/.

In nature quercetin (3,3',4',5,7-pentahydroxyflavone) which is found in the leaves, blossoms and stems /3,4/ of the plants known as plant teas has recently been used in the various areas of medicine /5,6/, food chemistry /7/ and biochemistry /8/. It is used as a natural yellow dye extract which is resistant to washing and abrasion with a lightfastness /9/. In this work one optimised the conditions required to obtain quercetin complexes with iron(II) and aluminium(III) ions /10,11/, which are used as mordants to fix colours in dyeing. There is a reference to quercetin pKa in the enclosed paper. It should be between 9.5 and 10.5 /12/, and pKa ≅ 8.7 was found for quercetin /13/. The chemical structure of quercetin (3,3',4',5,7-pentahydroxyflavone) is as shown at the top of the next page.

The flavonoids exhibit two major absorption bands in the ultraviolet/visible region. The absorptions in the 320-385 nm range 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) /3/. The spectra are related to the $\pi \rightarrow \pi^*$ transitions within the aromatic ring of the ligand molecules. In comparison with flavonoids absorption spectra, those of the complexes are shifted to the long-wavelength region.

We examined the protonation constants of quercetin and stability constants of quercetin-metal complexes. The stability constants of complexes have been determined potentiometrically using the Calvin-Bjerrum method. The calculations were performed according to Irving-Rossotti method /14/. The potentiometric results were also supported by observed spectrophotometric method. The behaviour of H^+ ion in the water-dioxane (1/1;v/v) mixture is the same as aqueous solutions /15/. Because of the insolubility of ligands in water, a dioxane-water mixture (1:1,v/v) was used instead to dissolve the ligand.

EXPERIMENTAL

Chemicals and the solutions standardization

$HClO_4$, NaOH, $Al(NO_3).9H_2O$, $NaClO_4$ and dioxane were provided by Merck, $Fe(NH_4)_2(SO_4)_2.6H_2O$ was provided by Carlo Erba. Quercetin (3,3',4',5,7-pentahydroxyflavone) was provided by Carl Roth Gmbh. All chemicals used were of analytical grade. A solution of 0.1 N NaOH was prepared from "Titrisol Merck". The concentration of Fe(II) and Al(III) ions in the solution was typically kept at 6.00×10^{-3} $molL^{-1}$ and determined accurately by titrating it with standard ethylenediamine tetraacetic acid (EDTA). A solution of 0.1 N EDTA was prepared from "Titrisol Merck". The purity and the concentrations of the prepared solution were checked by potentiometric titration method. The ionic strength of the reaction media was kept constant at 25°C (1=0.1) using $NaClO_4$ solution. Fresh solutions were prepared at time of use.

Apparatus

A Shimadzu-UV-1600 PC UV-VIS double-beam spectrophotometer was utilised for recording spectra of quercetin, aluminium(III), iron(II), quercetin-aluminium(III) and quercetin-iron(II) complexes. Radiometer TIM800 Titration Manager, ABU 901 Autoburette, amd HI 1131B Combination pH electrode were used. Computer calculations were performed on the pH-metric data.

Procedure

Determination of Protonation Constant

In order to determine the protonation constants the solutions including $HClO_4$ and ligand (quercetin) + $HClO_4$ were titrated potentiometrically using 0.1 N NaOH (Fig. 1). Average $\bar{n}_A$ values were calculated from the titration curves.

For the calculation, the following equation is given below:

$$\bar{n}_A = y + \frac{(V_1 - V_2)(N + C)}{(V_0 + V_1)C_L} \qquad (1)$$

where:

V_o = Initial volume (mL) : 50.00 mL

N = Molarity of the base(NaOH) : 0.100 $molL^{-1}$

C = $HClO_4$ concentration : 0.040 $molL^{-1}$

C_L = Ligand concentration : 0.012 $molL^{-1}$

Y = Number of protons given for quercetin : 2

V_1, V_2= NaOH volumes of V_1 and V_2 were read from the titration curves which contain HCO_4 and ligand + $HClO_4$.

$\bar{n}_A$ = $\bar{n}_A$ values which correspond to different pH values were calculated by using the volumes of V_1 and V_2 and were plotted as a function of pH, i.e. $\bar{n}_A$=f(pH).

The protonation constant of quercetin (Fig. 2) and the stability constant of quercetin-metal complexes which were used as ligands were determined in Table 1.

Determination of Stability Constant

The stability constant of the binary complexes was determined potentiometrically using the Irwing-Rossotti method /14/. Therefore the mixture which the metal ions were titrated with was standard 0.100 mol.L^{-1} NaOH solution potentiometrically and the titration curves were plotted (Fig. 1). $\bar{n}_L$ values were calculated using $\bar{n}_A$ values and the equation given below; pL values were calculated using $\bar{n}_L$ values to calculate stability constants /16/. The following equation was used to calculate $\bar{n}_L$ values:

$$\bar{n}_L = \frac{(V_o + V_2),\bar{n}_A.C_M}{(V_3 - V_2)\left[N + C + C_L\left(y - \bar{n}_a\right)\right]} \tag{2}$$

where:

V_o = Initial volume (mL) : 50.00 mL
N = Molarity of the base(NaOH) : 0.100 molL^{-1}
C_M = Metal concentration : 0.006 molL^{-1}
C_L = Quercetin(L) concentration : 0.012 molL^{-1}
C = $HClO_4$ concentration : 0.040 molL^{-1}
Y = Number of protons given for quercetin : 2

V_2, V_3 = NaOH volumes of V_2 and V_3 were read from the titration curves which contain HCO_4 + Ligand and $HClO_4$ + Ligand + Metal ions.

The following equation was used to calculate pL values:

$$pL = \log\left[\frac{1 + \beta_1\left[H^+\right] + \beta_2\left[H^+\right]^2 + \ldots\beta_n\left[H^+\right]^n}{C_L - \bar{n}_L.C_M}\right] \tag{3}$$

C_M = Metal concentration : 0.006 molL^{-1}
C_L = Quercetin(L) concentration : 0.012 molL^{-1}
$\bar{n}_L$ =The values were calculated using $\bar{n}_A$ values and the equation (2)
pL = pL values were calculated using β values (β values were calculated as follows).

The relation $\bar{n}_L$ = f (pL) was plotted using $\bar{n}_L$ and pL values which were calculated for each metal ion. The stability constants were determined from these graphs (Fig. 3,6 and Table 1).

The formation constant (β) was determined from stability constant metal-

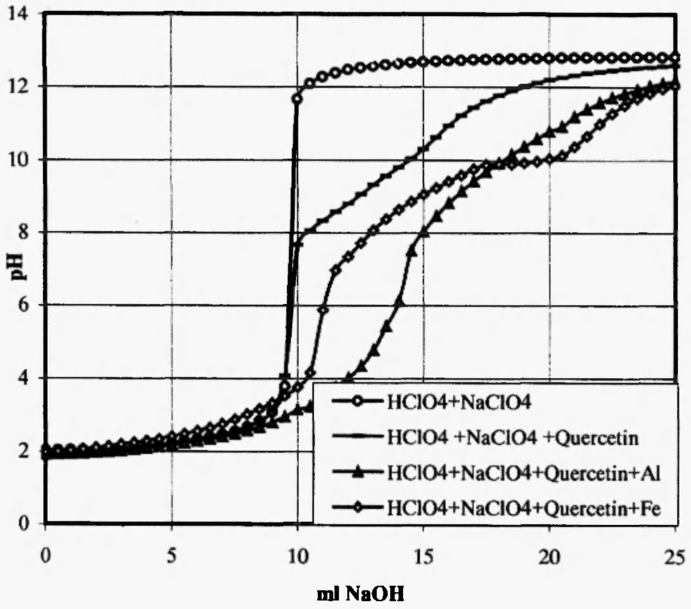


Fig. 1: Potentiometric titration curves

Table 1

The stability constants for Al(III)-quercetin and Fe(II)-quercetin complexes

	$\mathbf{LogK_1}$	$\mathbf{LogK_2}$
Quercetin	9.63 ± 0.11	8.32 ± 0.13
Al(III)-Quercetin	14.05 ± 0.09	10.02 ± 0.12
Fe(II)-Quercetin	9.44 ± 0.11	3.86 ± 0.08

ligand complexes /19/.

For quercetin-Al(III) complex ($\log K_1=14.05\pm0.09$, $\log K_2=10.02\pm0.12$):

$\beta_1 = K_1 = 1.12 . 10^{14}$

$\beta_2 = K_1 \cdot K_2 = 1.12 . 10^{14} \cdot 1.04 . 10^{10} = 1.13 . 10^{24}$

For quercetin-Fe(II) complex ($\log K_1=9.44\pm0.11$, $\log K_2=3.86\pm0.08$):

$\beta_1 = K_1 = 2.75 . 10^{9}$

$\beta_2 = K_1 \cdot K_2 = 2.75 . 10^{9} \cdot 7.24 . 10^{3} = 1.99 . 10^{13}$

The following equation was used to calculate the conditional formation constant (logK) for pH 0 to 14:

$$\log K = \log K_1^{M} \cdot \log K_2^{M} \cdot \alpha_M \cdot [(\log K_1^{L} \cdot \log K_2^{L})/[(H^+)^2 + K_1^{L} \cdot (H^+) + K_1^{L} \cdot K_2^{L}]] \quad (4)$$

$\log K_{1,2}^{L}$ = Stability constants of ligand(quercetin)
$\log K_{1,2}^{M}$ = Formation constant of metal – ligand complex
H^+ = The values at various pH 0 to 14
α_M = The fraction of α_M of the metal ion in the uncomplexed form metal may be calculated /19/.

The solutions for titrations were prepared as follows:

1) [0.040 $molL^{-1}$ $HClO_4$ + 0.100 $molL^{-1}$ $NaClO_4$] (in 25.0 mL dioxane-H_2O 1:1,v/v) + 25.0 mL dioxane-H_2O (1:1,v/v)
2) [0.040 $molL^{-1}$ $HClO_4$ + 0.100 $molL^{-1}$ $NaClO_4$ + 0.012 $molL^{-1}$quercetin] (in 25.0 mL dioxane-H_2O 1:1,v/v) + 25.0 mL dioxane-H_2O (1:1,v/v)
3) [0.040 $molL^{-1}$ $HClO_4$ + 0.100 $molL^{-1}$ $NaClO_4$ + 0.012 $molL^{-1}$quercetin] (in 25.0 mL dioxane-H_2O 1:1,v/v) + 0.006 mol L^{-1} Al(III) (in 25mL dioxane-H_2O (1:1,v/v))
4) [0.040$molL^{-1}$ $HClO_4$ + 0.100 $molL^{-1}$ $NaClO_4$ + 0.012 $molL^{-1}$quercetin] (in 25.0 mL dioxane-H_2O 1:1,v/v) + 0.006 $molL^{-1}$ Fe(II) (in 25mL dioxane-H_2O (1:1,v/v))

Each mixture was separately titrated potentiometrically with 0.100 mol L^{-1} NaOH (Fig. 1). The separation among ($HClO_4$), ($HClO_4$ + L) and ($HClO_4$ +L+Metal) plots in all potentiometric titration curves showed the formation of a metal-ligand complex.

For the titration of $HClO_4$ + $NaClO_4$ with the three-component mixture of $HClO_4$ + $NaClO_4$ + quercetin, one prepared the $\bar{n}A$ = f (pH) dependence ($\bar{n}A$ was calculated according to equation 1). The following protonation constants were found: $\log K_1$ = 9.63±0.11 and $\log K_2$ = 8.32±0.13 at 25°C (Fig. 2).

A similar approach involving equations 2 and 3 was applied to obtain the $\bar{n}L$=f(pL) dependence for the potentiometric titrations with $HClO_4$ + $NaClO_4$ + quercetin + [Al(III) or Fe(II)] mixtures (Fig. 3, 6).

The mol fractions of different species of metal-ligand complexes were found by means of the calculated formation constants and were plotted as a function of pH (Fig. 4, 7).

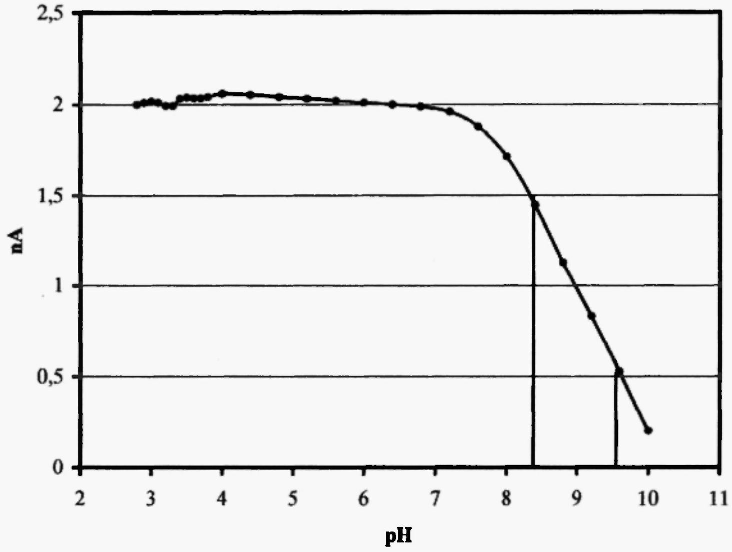


Fig. 2: $\bar{n}A = f(pH)$ curves for quercetin

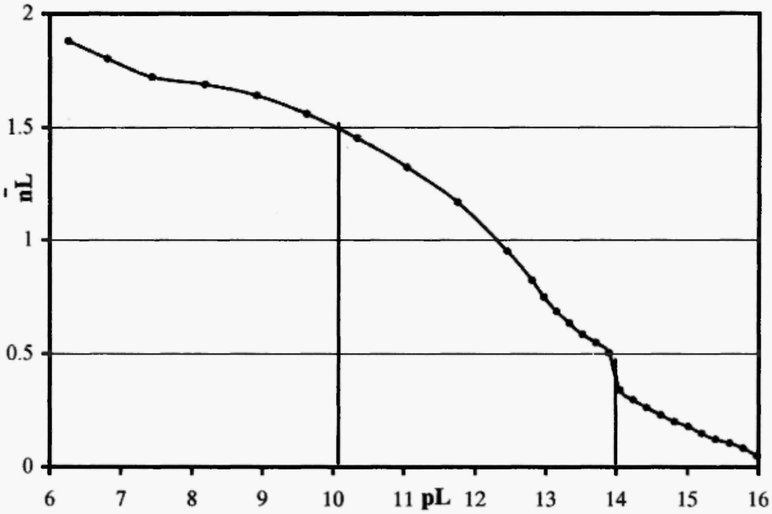


Fig. 3: $\bar{n}L = f(pL)$ curves for the Al (III)-quercetin complex

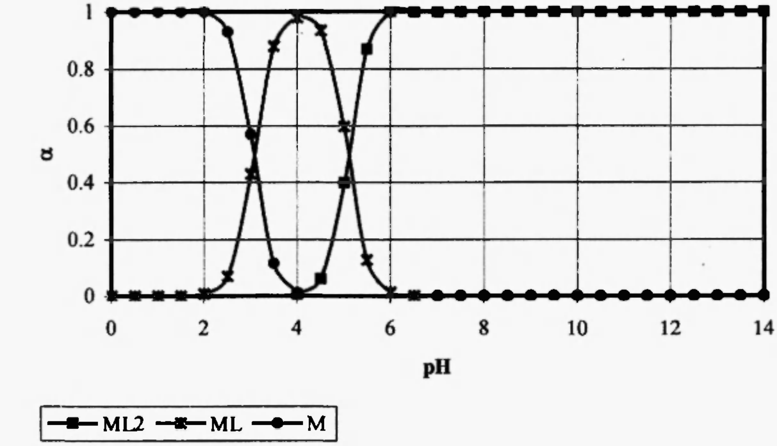


Fig. 4: Relative abundance of ionic and molecular species calculated as a function of pH for Al(III) – quercetin complex. (M + L → ML; ML + L → ML_2)

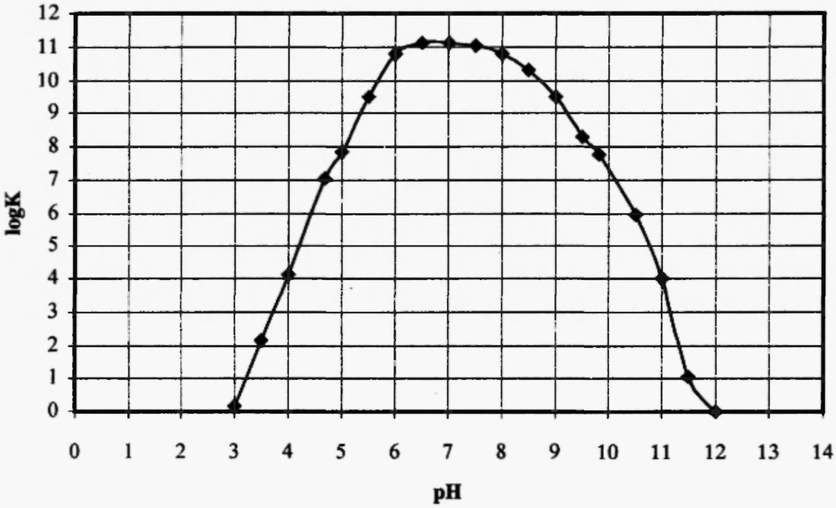


Fig. 5: Conditional formation constant as a function of pH for Al(III)-quercetin complex.

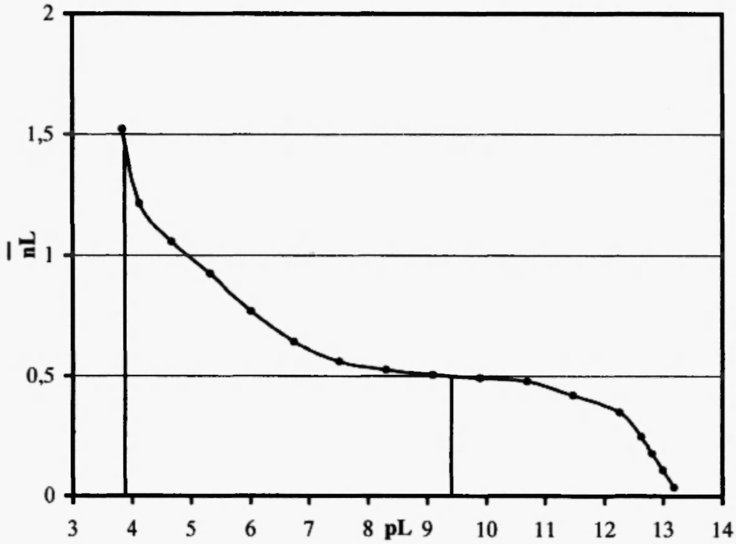


Fig. 6: $\overline{n}L = f(pL)$ curves for the Fe (II)-quercetin complex

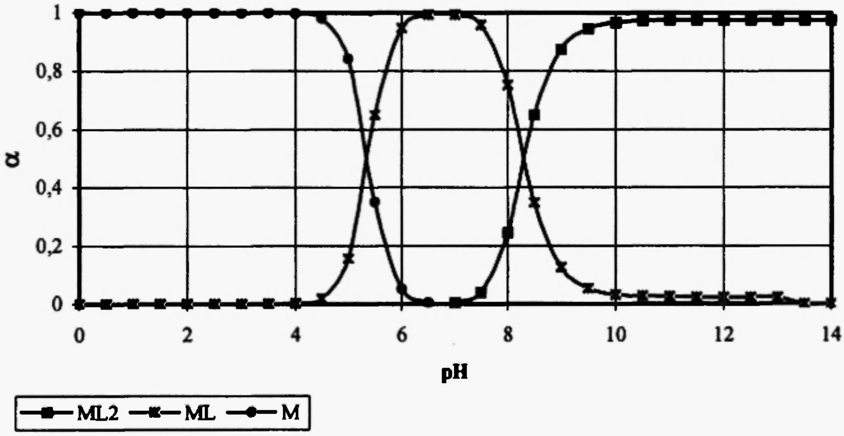


Fig. 7: Relative abundance of ionic and molecular species calculated as a function of pH for Fe(II) – quercetin complex. (M + L → ML; ML + L → ML_2)

In addition, the conditional formation constants were calculated according to equation 4 and were plotted as a function of pH (Fig. 5, 8) /14/.

In the spectrophotometric measurements the $HClO_4$ + $NaClO_4$ + dioxane + H_2O mixture served as a reference, since it was of the same composition as the mixtures used for the potentiometric titration. To this mixture we added quercetin solution and recorded the absorption spectra. Next, the additions of aluminium(III) and iron(II) solutions were made separately and the absorption spectra were recorded again (Fig. 9, 10). A similar procedure for metal ions was performed in the absence of quercetin (Fig. 9 and 10).

RESULTS AND DISCUSSION

The aluminium(III) and iron(II) salts were used as mordant in the dyeing process with natural dyes consisting of flavonoids. In this study, the protonation constants of quercetin with a weak acidity were determined by Irwing-Rosotti method by potentiometry. The conditional formation constants were calculated and these constants were found to be in agreement with the formation constants of binary systems. In this calculation, the pK values of ligands and the formation constants of complexes which they formed with metals were used as data. The formation constants of complexes were found from the formation curves $\bar{n}L=f\ (pL)$ which were drawn for aluminium(III) and iron(II) complexes. The conditional formation constants equal the "β values" of the complex. The formation constants of metal-quercetin complexes were found in this work to be in agreement with the calculated conditional formation constants of $\beta_2=K_1.K_2$. The conditional formation constants, namely the stability constants of complexes can also be calculated. The behaviour of iron(II) and aluminium(III) ions have been examined in this work.

From the titration results we can conclude that quercetin-metal complexes 2:1 (quercetin:metal ratio) both Al(III) and Fe(II). Since the coordination number = 6 of both metals, the proposed structures of quercetin-metal complexes are given in Figure 5 /2, 13/ The complexes have two water molecules /13/. As the 3-hydroxy group has a more acidic proton, the 3',4'-dihydroxy groups bind a metal ion /13, 18/. It was found by the two methods that the quercetin and rutin are oxidized by a two-electron process /2/.

The conditional formation constant for Al(III)-quercetin complex was found as the maximum value logK=11.13±0.10 at the range of pH=6.50 (Fig. 5). This value is in accordance with the relative abundance (Fig. 4). The

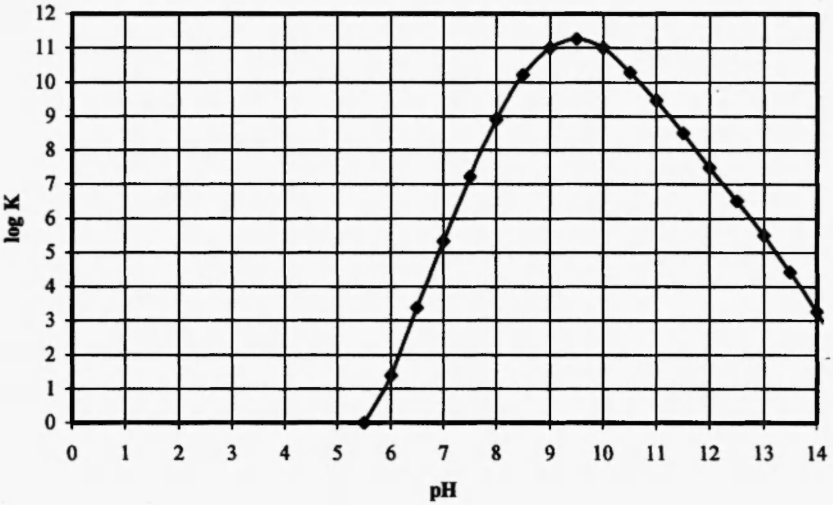


Fig. 8: Conditional formation constant as a function of pH for Fe(II)-quercetin complex.

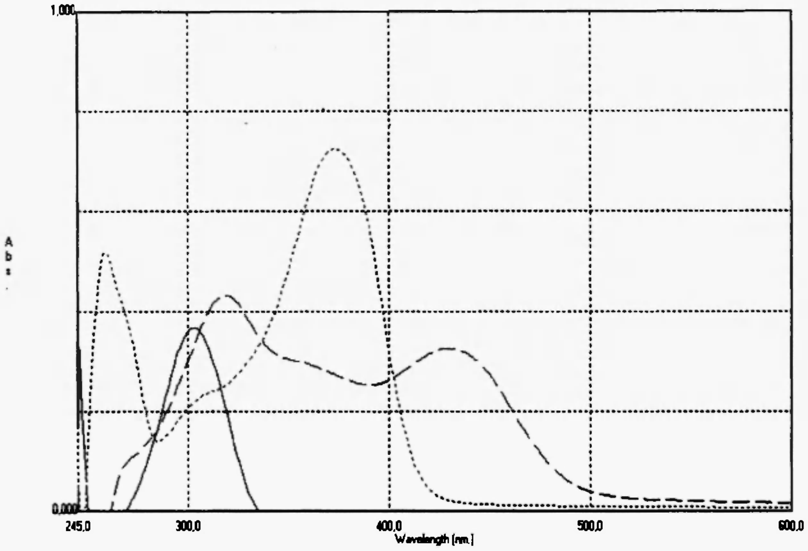


Fig. 9: UV-vis spectra of Al(III) ions, quercetin and Al(III)-quercetin complex in 1:1 dioxane+H_2O+$HClO_4$+$NaClO_4$ (50 μM at pH 6.50). (.....Quercetin; ____Al(III); -------Al(III)-quercetin)

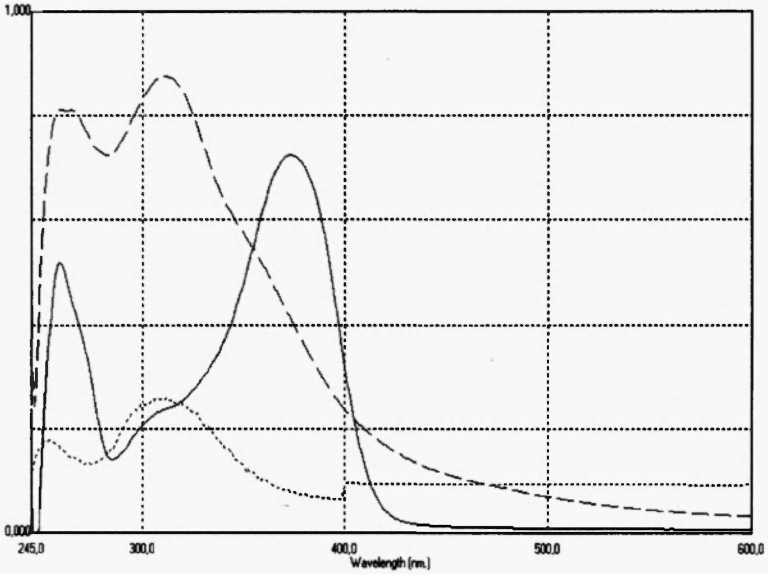


Fig. 10: UV-vis spectra of Fe(II) ions, quercetin and Fe(II)-quercetin complex in 1:1 dioxane+H_2O+$HClO_4$+$NaClO_4$ (50 μM at pH 9.50).
(___Quercetin; ....Fe(II); __ __ Fe(II)-Quercetin)

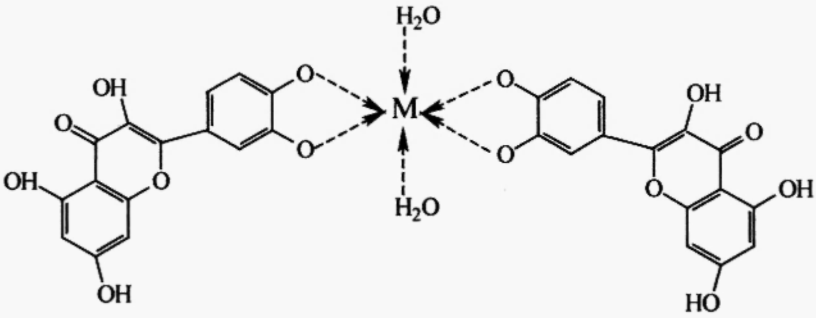


Fig. 11: Proposed structure of quercetin-metal complexes: (M = Al(III) or Fe(II) ions).

conditional formation constant for Fe(II)-quercetin complex was found as the maximum value logK = 11.26±0.13 at the range of pH = 9.50 (Fig. 8). This value is in accordance with the relative abundance (Fig. 7).

The complex formation shows a maximum in a narrow pH range and by decreasing rapidly with the increase of pH, the Al(III)-quercetin complex disappears completely at pH = 12 (Fig. 5). In spite of this, Fe(II)-quercetin complex still partially exists even at pH = 14 (Fig. 8).

Table 2

Maximum absorption wavelengths.

	Band I(nm)	**Band II(nm)**
Quercetin	258.50	373.50
Al(III)	303.00	-
Al(III)-Quercetin	318.00	429.00
Fe(II)	310.00	-
Fe(II)-Quercetin	264.00	310.00

According to the spectra in Figure 9 Al(III) ions, quercetin and Al(III)-quercetin complex exhibit maxima at the wavelength of 303, 258, 373 and 318, 429 nm respectively (Table 2). At 429 nm absorption of Al(III) ions and quercetin decrease, while absorption of the Al(III)-quercetin is increased. After the reaction was completed, no absorption signal originating either from aluminium(III), or quercetin was observed /13/. Similar conclusions could be drawn from the spectrum of Fe(II)-quercetin complex. Although iron(II) ions absorb at 310 nm, the iron(II)-quercetin complex absorbs at 264 and 310 nm in Table 2. Therefore, with the progress of the complexation, absorption signals of quercetin and Fe(II) ions decreased, while absorption of Fe(II)-quercetin complex was enhanced (Fig. 10). After the reaction was completed, one could observe only the latter /17/.

The results presented are in good agreement with the previous ones for quercetin (258 nm;373 nm) and the quercetin /$AlCl_3$ mixture (430 nm) obtained by Markham /3/.

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