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ANTIOXIDANT PROPERTIES OF Zn(II) AND Fe(III) COMPLEXES WITH FLAVONOLS

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The antioxidant activity of Zn(II) and Fe(III) complexes with synthetic derivatives of flavonol was evaluated by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. The Zn(II)-flavonol complexes show antioxidant properties comparable to those of the parent flavonols. It has been revealed that complexes of flavonols with ferric ions are stronger than that with zinc ions. It has been found that iron substitutes zinc in Zn(II)-flavonol complex and binding of metal-prooxidant by flavonol occurs. The Fe(III)-flavonol complexes are potential free-radical scavengers with the same activity against free stable radical as the initial flavonols.

INTRODUCTION

Flavonols are natural bioactive substances possessing the vitamin P activity and antioxidant one [1]. Antioxidant action of flavonols is stipulated by their capability to scavenge the reactive oxygen species as well as to chelate metals-prooxidants (Fe(II), Fe(III) and Cu(I)) [2, 3] and to reduce the oxidative stress which may contribute to the progression of many diseases. For these reasons flavonoid-rich diet, supplements and cosmetics are widely recommended for improving health status and prevention of chronic diseases. Many of natural and synthetic antioxidants, including flavonols, are poorly dissolved in water, this fact partially limits their experimental studies. The interaction of flavonoids with metal ions may change the antioxidant properties and biological effects of the flavonoids [4]. It is suggested that the biological activity of an organic ligand can be increased when it is co-coordinated or mixed with suitable metal ion because of its capability to act as free radical acceptor [1, 2, 4–6]. The data from literature [7] and our preliminary studies show that flavonols complexes with transition metal ions are more hydrophilic and water-soluble than the corresponding ligands. Complexes of flavonoids play an important role in limiting metal bioavailability and suppressing metal toxicity. For example, aluminum has been implicated in neurological and bone disorder. The complexation of Al(III) by quercetin reduces aluminum overload in the diet. [8]. The metal-flavonol complexes were reported to possess higher antiradical activity

than the parent flavonols [6, 9] and to act as superoxide dismutase mimics [9]. In [4] it was found that complexes of rutin, dihydroquercetin or epicatechin with Fe(II), Fe(III), Cu(II) or Zn(II) are more effective radical scavengers than the free flavonoids, due to the acquisition of additional superoxide dismutating centers.

Zn(II) and Fe(III) complexes with synthetic derivatives of 3-hydroxyflavone were the subject of present research where their structure, stability constants as well as antioxidant properties have been investigated. The choice of Zn(II)-flavonols complex is caused by the fact that zinc is the active structural component of superoxide dismutase, possesses antioxidant properties, improves the action of other antioxidants, and synthetic flavonol derivatives are more efficient and cost-effective than the natural antioxidants [10]. Besides, the use of zinc-flavonol complex may have an additional positive effect. Presence of stronger metalscomplexing ions, for example iron ions, will favour the substitution of zinc with iron. Thus, zinc ions will release into medium promoting an increase in protective functions of an organism, flavonol will bind the metal-prooxidant and convert its prooxidant properties to an antioxidant. Therefore the antioxidant behaviour of Fe(III)-flavonols complexes has been studied.

EXPERIMENTAL

Zinc acetate (Zn (CH₃COO)₂), iron (III) chloride (FeCl₃), synthetic antioxidant 3-hydroxyflavone (AO) and its derivatives

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AO1–AO3 containing in the phenyl moiety the lateral substituents with different electron-donor properties (Fig. 1) were used for investigation. Studied flavonols were synthesized according to

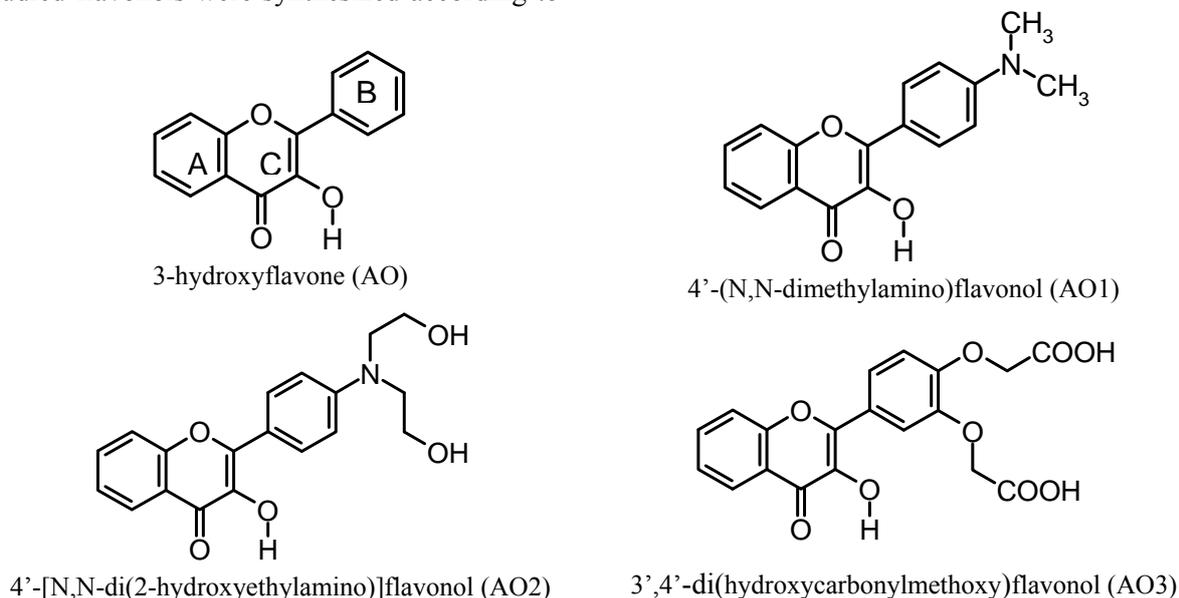


Fig. 1. Structures of the flavonols

The method of isomolar series [12] was used to determine, in 70 % ethanol, the stoichiometric ratios for the reactions between the flavonols and zinc ions. For this method, a solution of zinc acetate (0.5 mM) in 70 % ethanol was mixed in the different volume ratios (1:9–9:1) with the solution of flavonol (0.5 mM) in the same solvent. After mixing during the stirring at room temperature the changes in color of the solutions were observed indicating that complexation occurred. The kinetics of the formation of the complexes between flavonols and zinc ions was measured by monitoring the changes in UV/Vis spectrum.

UV/Vis spectra were recorded on a Perkin Elmer Lambda 35 UV–visible double beam Spectrophotometer at 25 °C (scanning speed = 480 nm/min) using quartz cuvette (pathlength of cuvette (Perkin-Elmer) $d = 10$ mm).

The stoichiometric composition of Fe(III)–flavonol complexes was determined by spectrophotometric titration [13]. 0.04 M solution of flavonol in KH_2PO_4 (KBP) buffer (pH 6.86) and 1 M solution of ferric chloride in 0.1 M HCl were prepared. The titration experiments were performed by sequential additions of 8–40 μL of metal ion solution to the same 2 mL of flavonol solution in a quartz

[11]. Their purity of not less than 99 % was confirmed by chromatographic and $^1\text{H-NMR}$ spectroscopy [11].

cuvette (ratio of Fe^{3+} /flavonol in reaction varied in the range of 0.2–2.5). The mixture was equilibrated at 25 °C until no further spectroscopic change was observed (ca. 2 to 10 min) and UV/Vis spectra were recorded. To determine the apparent binding constants of the metal and flavonol, the approach described in [13] was used.

Flavonol concentration in the solution was determined from optical density values in the absorption maxima at 340, 407, 403 and 357 nm (compounds AO, AO1, AO2, AO3 respectively). Metal complexes for further study were prepared by mixing of flavonols and salt solutions of the same concentration (0.5 mM) in a 1:1 ratio.

Stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH $^\bullet$) (Merck, Germany) was used to evaluate the antiradical activity [14]. The solution of diphenylpicrylhydrazyl was added to a solution of the substance in 70 % ethanol, with the ratio of $[\text{Flavonol}]/[\text{DPPH}^\bullet]$ being varied in the range of 0.33–8. The concentration of stable radicals at various times after the start of the reaction was determined spectrophotometrically from optical density at the absorption maximum of 520 nm. As control, the solution with the same concentration of DPPH $^\bullet$ but without flavonol was used.

RESULTS AND DISCUSSION

UV-absorption spectrum of flavonol (see the structure in Fig. 1) has broad bands, 220–270 and 270–370 nm (Fig. 2, *a*). The peak with a maximum around 340 nm (“Band I”) is attributed to the cinnamoylic portion of the flavonol (B ring); that with a maximum around 240 nm (“Band II”) is related to the benzylic moiety (A ring); the absorption band with a maximum around 305 nm corresponds to the pyronic ring (C ring) [15]. The presence of electron donor substituents ($-\text{N}(\text{CH}_3)_2$, $-\text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$, $-(\text{OCH}_2\text{COOH})_2$) in the B ring induces the shift of a maximum at 340 nm to the long-wave region (360–410 nm) and appearance of a new peak with a maximum around 280 nm (Fig. 2, *b, c, d*). In all the cases the complexation causes a bathochromic shift of absorption band I. This shift can be explained by the extension of the conjugated system due to the complexation [5].

The isomolar series and UV/Vis titration methods give the 1:1 stoichiometry for AO, AO1 and AO2 complexes with both Fe^{3+} and Zn^{2+}

ions. The $\text{Zn}(\text{II})\text{:AO3}$ and $\text{Fe}(\text{III})\text{:AO3}$ complexes have stoichiometry of 2:1 and 1:2 respectively. These features are in agreement with the results obtained by other researchers for flavonol– $\text{Zn}(\text{II})$ [16] and flavonol– $\text{Fe}(\text{III})$ [6] complexes. The formation of $\text{Zn}(\text{II})\text{:AO3} = 2:1$ may be due to the presence of additional binding metal sites-carboxyl groups of lateral substituents in B ring in the AO3 molecule. The carboxyl groups are known to have lower values of ionization constants ($\text{pK} \sim 4$) compared with the 3-hydroxy groups of flavonols ($\text{pK} \sim 9$, [17]), so we can expect that the compound will bind iron ions within a wider pH range.

Taking into account the stoichiometry of the complexes and the data on the concentration of the components at equilibrium, the apparent binding constants K_{app} between the flavonols and metal ions were estimated (Table).

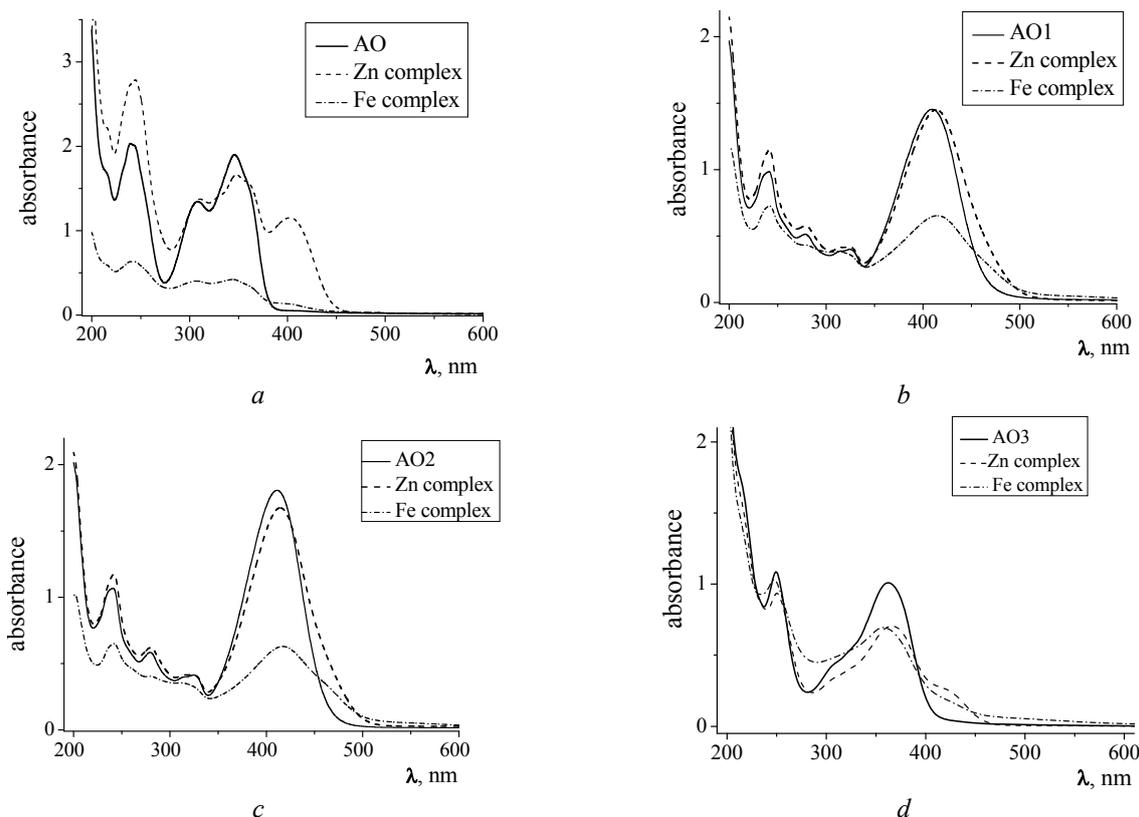


Fig. 2. UV-vis spectra of AO (*a*), AO1 (*b*), AO2 (*c*), AO3 (*d*) in aqueous-alcoholic solution and their corresponding Zn and Fe complexes (metal–ligand molar ratio 1:1)

Table. Estimated apparent binding constants with metal ions

Flavonol	Apparent binding constants $K_{app} \cdot 10^4$, l/mol		
	Zn(II)–flavonol in 70 % ethanol (pH 6.6)	Zn(II)–flavonol in KPB (pH 6.86)	flavonol–Fe(III) in KPB (pH 6.86)
AO (1:1 complex)	~0.5	not determined	~2
AO1 (1:1 complex)	~0.1	not determined	~3
AO2 (1:1 complex)	~0.3	not determined	~5
AO3 (2:1 complex)*	~3	not determined	~3

*For the reaction of AO3 and Zn^{2+} / Fe^{3+} (the second order in reactant AO3) the constant addition of the first ligand can be estimated as $\sqrt{K_{app}} = \sqrt{1 \times 10^9 \text{ l}^2/\text{mol}^2} \approx 3 \times 10^4 \text{ l/mol}$

As one can see, the studied compounds form more strong complexes with iron ions than those with zinc ones. It was not possible to estimate the values of K_{app} for Zn(II)–flavonol complexes in KPB (pH 6.86) because formation of these complexes in water was not observed. Comparing the values of K_{app} for the flavonols studied one can conclude that the flavonol derivatives AO1–AO3 have an increased capability to chelate Fe^{3+} ion in comparison with the initial substance. For all the flavonols, the complexation constant with Fe(III) was found to be higher than that with Zn(II), therefore iron will substitute zinc from its complex with flavonol.

The capability of iron to substitute zinc in Zn(II)–flavonol complex and, consequently, the additional antioxidant effects due to binding metal-prooxidant are confirmed by the data presented in Fig. 3.

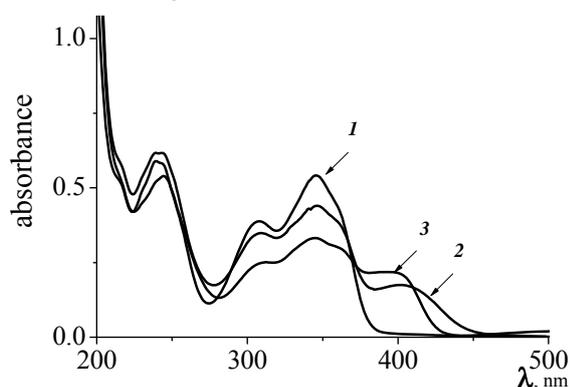


Fig. 3. UV-vis spectra for AO (1), AO– Zn^{2+} (2) and AO– Zn^{2+} – Fe^{3+} solutions (3). $[AO]/[Zn^{2+}]=1$, $[AO]/[Fe^{3+}]=0.5$

As can be seen from the spectra, addition of ferric cations to a solution of the Zn(II)–AO complex, even at the ratio of AO/Fe = 0.5, leads to the disappearance of the band at 403 nm corresponding to the complex Zn(II)–AO (curve 2) and to the appearance of a new band at

394 nm due to formation of the complex Fe(III)–AO (curve 3).

Kinetic curves of the reaction between DPPH• radical and flavonols or their complexes with zinc show no significant differences in the kinetics of radical decay in the solution containing individual flavonol or its complex with zinc (Fig. 4). A decrease in the reaction rate for the case of radical – complex interaction is probably due to the fact that for all the time some part of flavonol molecules is in a "bound" state.

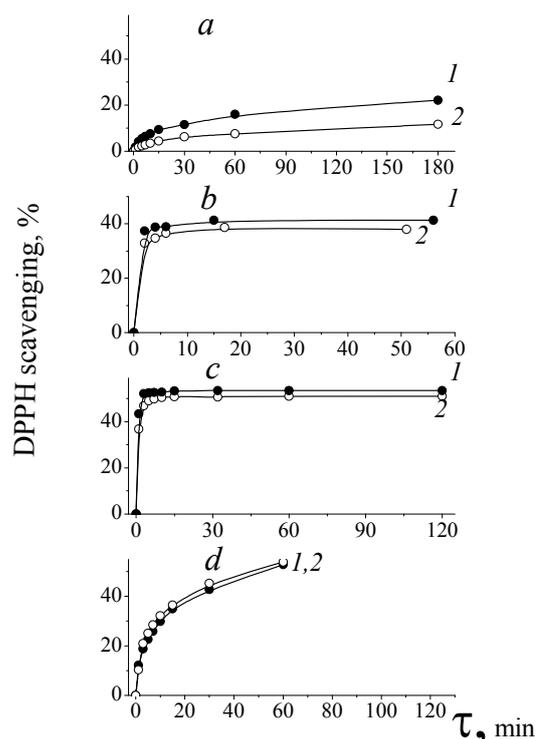


Fig. 4. Effect of flavonols (1) AO (a), AO1 (b), AO2 (c), AO3 (d) and their complexes with Zn^{2+} (2) on reduction of DPPH• radical. $[Flavonol]/[Zn^{2+}]=1$, $[Flavonol]/[DPPH^{\bullet}]=1$

As was mentioned above, the capability of flavonols to chelate Fe^{3+} is very important for their antioxidant activity because a "site-specific scavenging" may occur [6]. This means that if

the Fe^{3+} is still catalytically active, radicals are formed in the vicinity of the flavonol, which surrounds the Fe^{3+} , and can be scavenged immediately. In that case, the flavonol would have a double, synergistic action which would make it an extremely powerful antioxidant [6].

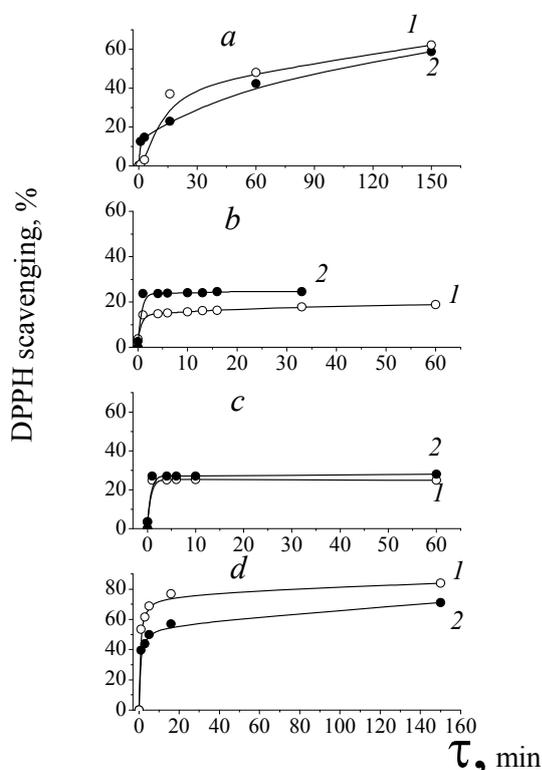


Fig. 5. Effect of flavonols (1) AO (a), AO1 (b), AO2 (c), AO3 (d) and their complexes with Fe^{3+} (2) on reduction of DPPH^{\bullet} radical. [Flavonol]/[Fe^{3+}]=1 for AO–AO3, [Flavonol]:[DPPH^{\bullet}]=3:1 for AO, AO3, [Flavonol]:[DPPH^{\bullet}]=1:8 for AO1, AO2

As shown in Fig. 5, Fe(III) –flavonol complexes exhibit antiradical activity comparable to those of the parent flavonols, with the exception of Fe(III) –AO1 complex. Some increase in antioxidant properties of Fe(III) –AO1 complex may be caused by increase in AO1 solubility in presence of Fe^{3+} ions. Thus, these results show that 3-hydroxyflavone and its derivatives are capable to chelate Fe^{3+} ions, the complexes formed being capable to inhibit DPPH^{\bullet} radical.

CONCLUSIONS

The stoichiometric ratios for the complexation of synthetic flavonols and Zn(II) and Fe(III) metal ions were determined. It has

been found that the stoichiometric composition of the complex is 1:1 for AO, AO1, AO2 and 2:1 and 1:2 for AO3 with Zn(II) and Fe(III) respectively.

The calculated binding constants for formation of metal–flavonol complexes indicate that the compounds studied form stronger complexes with ferric ions than those with zinc ions. It has been revealed that ferric cation substitutes zinc in Zn(II) –flavonol complex and the binding of metal–prooxidant by flavonol occurred.

Radical scavenging activities of 3-hydroxyflavone and 4'-(N,N-dimethylamino), 4'-[N,N-di(2-hydroxyethylamino)] (3',4'-di(hydroxycarbonylmethoxy) derivatives of 3-hydroxyflavone as well as activities of the flavonols complexes with transition metal (Zn^{2+} and Fe^{3+}) towards DPPH^{\bullet} were examined. All the Zn(II) –flavonol complexes show the antioxidant properties comparable to those of the parent flavonols.

Inhibitory effect of Fe(III) –flavonols against the DPPH^{\bullet} radical shows that these complexes are potential free-radical scavengers, and that their activity against the DPPH^{\bullet} radical is the same as that of for the initial flavonols. These results suggest that Zn(II) –flavonols complex could convert Fe^{3+} from a prooxidant to an antioxidant in radical-generating system.

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Антиоксидантні властивості комплексів Zn(II) та Fe(III) з флавонолами

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Вивчено антиоксидантну активність комплексів Zn(II) та Fe(III) з флавонолами щодо стабільного радикала 2,2-дифеніл-1-пікрилгідразилу. Встановлено, що антиоксидантні властивості вихідних флавонолів та їх комплексів з Zn(II) практично однакові. З'ясовано, що флавоноли з іонами заліза утворюють більш стійкі комплекси ніж з іонами цинку. Доведено, що залізо може заміщувати цинк в його комплексі, приводячи до зв'язування металу-прооксиданту. Комплекси з Fe(III) є потенційними інгібіторами вільних радикалів з активністю, що не перевищує активність вихідних флавонолів.

Антиоксидантные свойства комплексов Zn(II) и Fe(III) с флавонолами

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Изучена антиоксидантная активность комплексов Zn(II) и Fe(III) с флавонолами по отношению к стабильному радикалу 2,2-дифеніл-1-пікрилгідразилу. Установлено, что антиоксидантные свойства исходных флавонолов и их комплексов с Zn(II) практически одинаковы. Выявлено, что флавонолы с ионами железа образуют более прочные комплексы, чем с ионами цинка. Показано, что железо может замещать цинк в его комплексе, приводя к связыванию металла-прооксиданта. Комплексы с Fe(III) являются потенциальными ингибиторами свободных радикалов с активностью, которая не превышает активность исходных флавонолов.