

Naringenin complexes with copper ions: potentiometric studies

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Abstract: *Flavanones belong to the group of active flavonoid compounds, rarely occurring in the plant kingdom and are found in many foodstuffs (naringenin and derivatives) and medicinal plant materials. Many studies have demonstrated a relationship between dietary intake of phenolic compounds and reduced risk of cancer and cardiovascular disease. Accordingly, during over several years, flavonoids have gained increasing interest and numerous studies have shown that they possess beneficial properties having a great influence on human health. Acid dissociation constants are believed to be the most useful physico-chemical parameters which describe the extent of ionization of functional groups as a function of pH. It is very important in research areas such as pharmaceutical drug discovery and development due to vital role in understanding the pharmacodynamic properties of new drug substances. Flavonoids are characterized by insolubility in water while they are soluble in organic solvents. Exploiting mixed solvents, especially organic solvent – water mixtures, enables a good alternative for researching on compounds which are inconspicuously soluble or insoluble in water. In this study the dissociation constant of naringenin and its complex with Cu(II) have been determined in aqueous – DMSO solvent mixtures to examine the dependence of their acid-base equilibria on number of substrates.*

Keywords: *naringenin, metal complexes, potentiometric analysis, SUPERQUAD.*

Introduction

Naringenin (4,5,7-trihydroxyflavone) is an aglycone of naringin - hydrogenated derivative of flavone, which does not contain double bond between carbon atoms C-2 and C-3 [1]. It has a broad distribution in the plant kingdom and is easily extracted from numerous of different plants. Most flavanones are presented as rutosides with part of sugar attached at position C-7 [2]. It is confirmed that small concentrations of naringenin were detected in tomatoes and its preparations. Pharmacological activity of naringenin and its derivatives include estrogenic and anti-inflammatory effect, as well as antiulcer, diastolic, skin protective and anticancer effect [3].

In addition, it has been shown that naringenin strongly reduced the level of total cholesterol and a little less HDL (*High-Density Lipoprotein*) cholesterol levels in comparison to rutin and nicotinic acid used as anti-atherosclerotic drug. What is more, studies conducted in rabbits to investigate the effects of diet rich in cholesterol, confirmed that naringenin doses cause the decrease of LDL (*Low-Density Lipoprotein*) cholesterol level in blood plasma and cholesterol on liver. Furthermore, this flavonoid has been also reported to have antioxidant properties and free radical scavenging capacity [4].

According to above statements, this aroused our interest in synthesis of its complexes with Cu(II) in view of evaluating the biological activities [5]. Majority flavonoids successfully chelate trace metals, that play an important role in oxygen metabolism. Free iron and copper elements are defined as potential enhancers of reactive oxygen species formation, for instance the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical or copper-mediated LDL (low-density lipoprotein) oxidation. It should be mentioned that these metal ions are necessary for many physiological functions as components of hemoproteins and cofactors of different enzymes, including those implicated (copper for ceruloplasmin, iron for catalase and Cu, Zn – superoxide dismutase) in the antioxidant protection [6].

Flavonoids are characterized by insolubility in water while they are soluble in organic solvents. Exploiting mixed solvents, especially organic solvent – water mixtures, enables a good alternative for researching on compounds which are inconspicuously soluble or insoluble in water. In this study the dissociation constant of naringenin and its complexes with Cu(II) have been determined in aqueous – DMSO solvent mixture to examine the dependence of their acid-base equilibria on number of complex species. This is the first report regarding the solution equilibria in Cu(II)-naringenin system.

Experimental

Materials

Naringenin [5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one] (98%), DMSO (Dimethyl sulfoxide) (anhydrous, 99.9%) and calcium chloride (CaCl₂) used in laboratory in desiccators to keep solid samples dry were purchased from Sigma-Aldrich Company (St. Louis, USA). The sodium hydroxide made of titrisol solutions and potassium hydrogen phthalate (KHP) were received from Merck (Darmstadt, Germany). Potassium nitrate (KNO₃) was purchased by Sigma-Aldrich Company (Steinheim, Switzerland). A carbonate-free sodium hydroxide solution was prepared and standardized against pure potassium hydrogen phthalate (KHP) and next was used in a potentiometric titration of the trials.

Methods

Potentiometric measurements

All measurements were performed at $27(\pm 0.1)^{\circ}\text{C}$. Due to the fact that flavonoids are insoluble in water, DMSO / water mixture was prepared, in this case in the ratio 70%/30% (v/v) respectively. Properly weighed amount of naringenin has been dissolved in 6.5 mL of HNO_3 (v/v, 70%/30% DMSO/ H_2O) with constant ionic strength (0.1 M KNO_3). The volume of a sample titrated was 1.5 mL. The investigations were conducted in three different ligand to metal ratios: naringenin ligand/metal (1/1), (2/1) and (1/2). The concentrations of naringenin and Cu(II) ions were respectively, $1 \times 10^{-3}\text{ M}$ and $1 \times 10^{-3}\text{ M}$ for 1/1, $2 \times 10^{-3}\text{ M}$ and $1 \times 10^{-3}\text{ M}$ for 2/1 and $1 \times 10^{-3}\text{ M}$ and $2 \times 10^{-3}\text{ M}$ for 1/2. Titrations were carried out with three replicates in the pH range from 2.5 to 11.5 using as a titrant $0.1\text{ mol}\cdot\text{dm}^{-3}\text{ NaOH}$. Titration was conducted using automatically set Molspin pHmeter (Molspin Ltd., Newcastle-upon-Tyne, UK) equipped with a micro Molspin DSI 0.250 mL controlled by a PC computer. Measuring electrode was microelectrodes Russel CMAWL/S7. The ion product constant was determined as follows: various amounts of standard NaOH (in 70% DMSO) were added to a solution of constant ionic strength 0.1 M KNO_3 . The measured values of EMF (mV) were converted into pH, whereas the concentration of $[\text{OH}^-]$ was calculated from the amount of added base. The product of $[\text{OH}^-]$ and $[\text{H}^+]$ was taken into account in the calculation. In this way an average value of the stoichiometric autoprotolysis constant $\text{pK}_w = 15.28 \pm 0.02$ in the mixture of DMSO/water was obtained. It is in good agreement with literature data 15.26 [7].

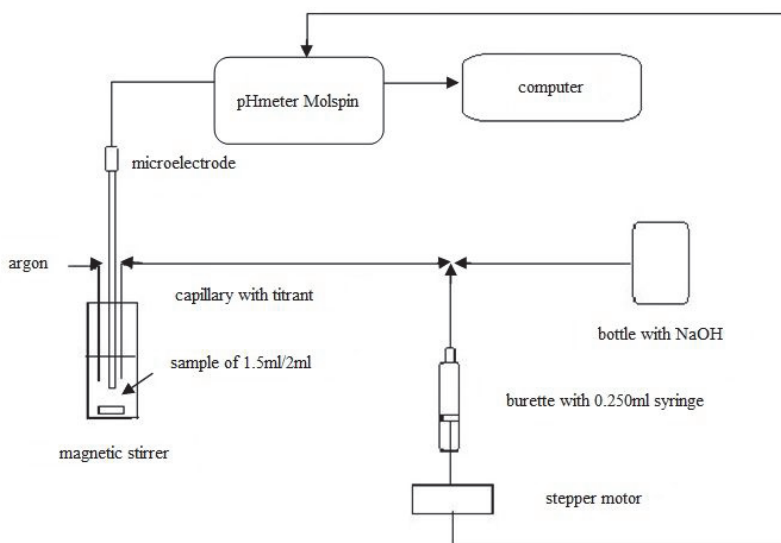


Figure 1. Titration apparatus

The equipment for potentiometric titrations consists of a 0.250 mL burette and pH meter which are clamped above the magnetic stirrer (Figure 1).

Solution equilibria analysis with the SUPERQUAD computer program

Results were analysed using SUPERQUAD – an improved general computer program for computation of formation constants from potentiometric data. SUPERQUAD is a computer program, which has been created in order to determine the formation constants from potentiometric data by minimization of an error-square sum based on measured electrode potentials. Basically, it is used to determine the equilibrium constants from titration curves. The analysis of subsequent curves gives permission for connecting particular reactions to each observed pH break point [8]. The program also permits refinement of any reactant concentration or standard electrode potential. What is more, the species distribution diagrams were also calculated using SUPERQUAD.

Hyperquad simulation and speciation

HySS (Hyperquad simulation and speciation) is a computer program which enables a system for simulating titration curves and a system for providing speciation diagrams. This program allows to calculate the equilibria in solution and form a partially soluble precipitate. The main improvement in HySS is the complete flexibility with which the conditions could be specified for speciation calculations and the simple interface to other Windows applications for the results of the calculations [9]. In this study HySS was used to initiate graphs to draw.

Results and Discussion

The titration curves of the ligand and its copper complexes at different concentrations and ligand/metal ratios are presented in Figure 2.

The acidic dissociation constant, pK_a is an important parameter in absorption, distribution, metabolism, excretion, toxicity researches due to that fact it helps to explain chemical phenomena such as absorption, distribution and elimination of substances. Potentiometry is a useful method to evaluate pK_a values of biologically active compounds, even when they are not sufficiently soluble in water. It does not require the presence of any chromophore (as different spectroscopies do) When compound is sparingly soluble in water, the pK_a determination is commonly done in organic/water mixtures. DMSO/water mixtures are often used because they have lower polarity than pure water, but keep a similar environment. DMSO is only weakly acidic, it tolerates relatively strong bases and has been extensively used in many studies. The aqueous pK_a determination of most compounds with a pK_a value between 4 and 10 is usually straightforward but when the compound has very low or very high pK_a or it shows several and close pK_a values the determination of pK_a may be more complex.

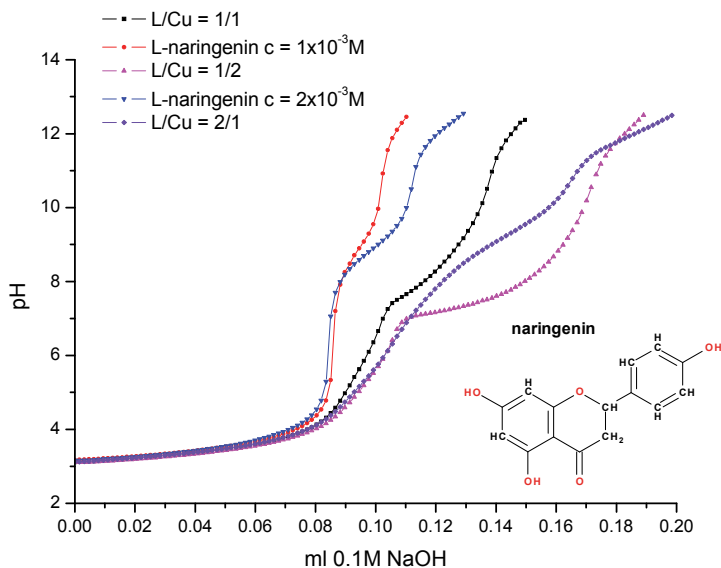


Figure 2. Curves of potentiometric titrations of naringenin and its Cu(II) complexes

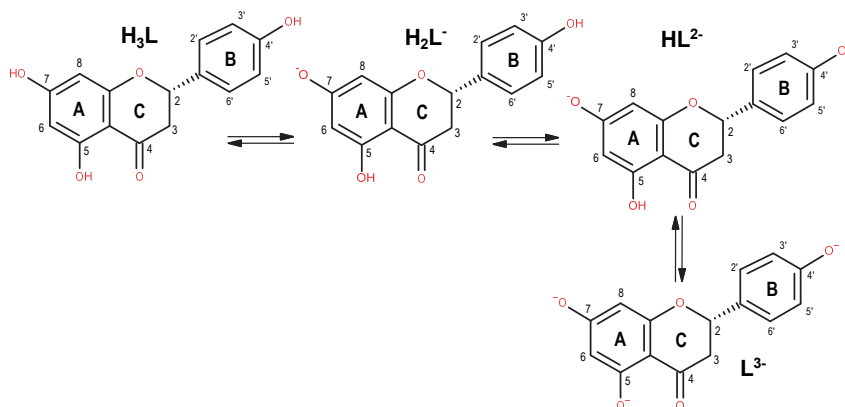


Figure 3. Structures of naringenin (2*S*)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one) species

The role of flavonoids is related to their chemical structure, for instance their antioxidant efficiency is related to their hydrogen radical donating abilities and the number of hydroxyl groups in the molecule. The hydroxyl moiety deprotonation has influence in the intrinsic antioxidant potential of flavonoid. Generally, deprotonation enhances the antioxidant action of the flavonoid [10], thus the knowledge of their physicochemical parameters such as pK_a is important to predict their antioxidant capacity.

In addition, the pK_a values determine the precise species presented in a biological medium and their knowledge is fundamental in bioclinical and pharmacological studies. In naringenin there are three hydroxyl groups positioned at 5, 7 and 4' carbons able to deprotonate consecutively with increasing pH (Figure 3).

According to NMR studies successive dissociations occur in the following sequence: 7-OH, 4'-OH, 5-OH [11]. The calculated pK_a values of naringenin are listed in Table 1 together with values reported in the literature for comparison [7, 12, 13, 14].

Table 1. The pK_a values of naringenin determined in DMSO/water mixture at $I = \text{const}$ (0.1 M KNO_3) and at $25 \pm 0.1^\circ C$ and other ones taken from the literature for comparison

Ligand	Species	Log β	pK_{a5} 5-OH	$pK_{a4'}$ 4'-OH	pK_{a7} 7-OH	Reference
Naringenin	HL	11.12 \pm 0.03				Present work
	H ₂ L	19.61 \pm 0.02	11.12 \pm 0.03	8.49 \pm 0.02	7.47 \pm 0.01	
	H ₃ L	27.08 \pm 0.01				
70%/30% DMSO/water			11.70	10.91	8.64	[7]
50%/50% DMSO/water			10.19	8.31	7.06	[12]
Rutin			11.90	8.80	7.35	[13]
50%/50% ethanol/water			11.85	8.90	7.49	[14]

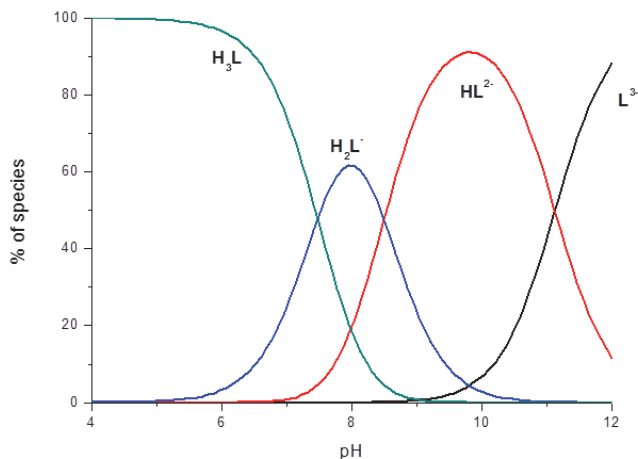


Figure 4. Distribution diagram of naringenin species in 70% (v/v) DMSO

The results show that the values of pK_a are similar to those reported in the literature. The log β values obtained in DMSO/water mixture increase with the

increasing number of protons in naringenin species. It should be mentioned that presented data are only preliminary. Rutin was selected due to the structurally related hydroxyl groups. Some differences are derived from different experimental conditions such as ionic strength or solvent type. In Figure 4 the equilibrium distribution of different protonated species of naringenin is shown as a function of pH calculated from the recorded potential values.

When pH increases, the protonated ligand loses protons and it is converted to the other forms as seen in Figure 4. Because of low acidic constants of the compound, the HL^{2-} and L^{3-} species are present at high pH values. As Figure 4 shows, H_3L is the main species in low pH range while H_2L^- dominates at neutral range.

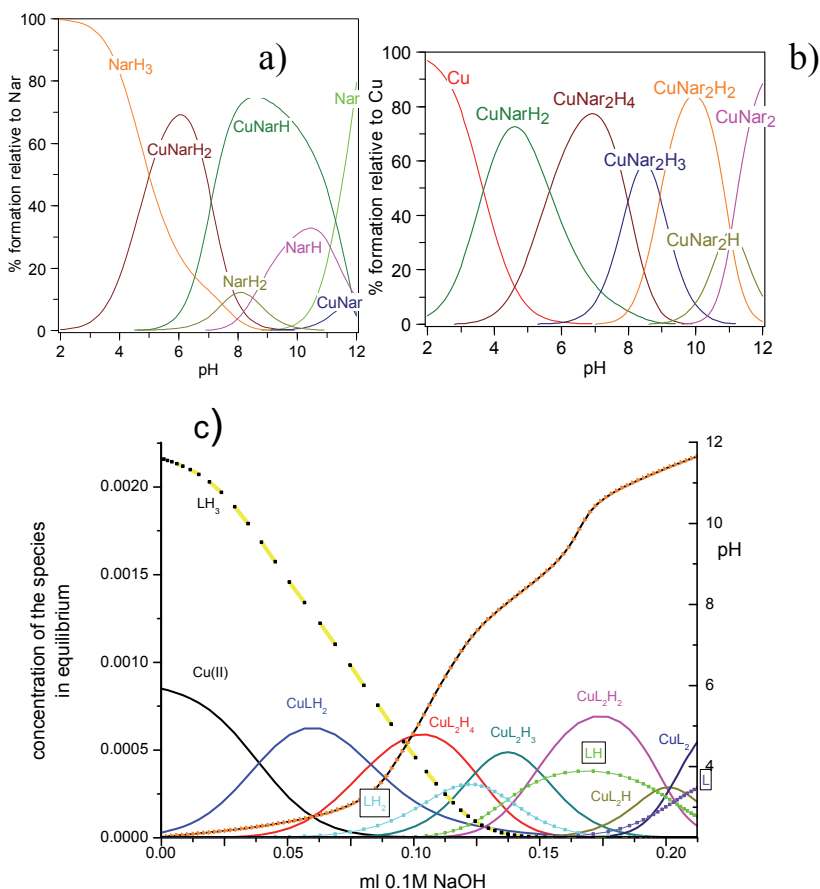


Figure 5. Species distribution diagrams in Cu-naringenin system for the ratios M/L: a)1:1, b) 1:2 and c) the relationship between the species in equilibrium and the amount of the base for M/L = 1/2 system

The naringenin has the donor atoms on adjacent A and C rings with the most strongly chelating ability towards metal ions as it was reported for many other

flavanone ligands. It is a single 5-hydroxychromone (5-hydroxy-4-keto position) metal-binding site. In comparison, rutin ring system contains two very efficient binding sites for metal ions, such as: {C(3')-OH, C(4')-OH} and {C(4)=O, C(5)-OH} donor pairs [14].

The calculations of potentiometric data were performed for two metal/ligand ratios 1/1 and 1/2. The species distribution diagrams obtained by HySS program are presented in Fig. 5 (a, b, and c). One can see that the Cu(II) ions start the coordination at pH above 2 and the first species found is the CuNarH₂ with a very likely [C(5)-O⁻, C(4)=O] donor atom set as it was observed in other similar flavonoid's system [14]. The two hydroxyl groups localized at C(7) and C(4') are protonated. The formation of the next species depends on the metal to ligand ratio. The CuNarH and CuNar are found in 1/1 system. One can assume that the coordination mode does not change only the protonation sites of naringenin is different, in CuNarH C(7)-OH protonated while in CuNar C(7)-O⁻ deprotonated, respectively. The other model of complex species can be attributed for the 1:2 ratio system. Apart from the CuNarH₂ dominating at pH 4, the species with the stoichiometries: CuNar₂H₄, followed by CuNar₂H₃, CuNar₂H₂, CuNar₂H, and CuNar₂ can be formed with increasing pH. The 2×[C(5)-O⁻, C(4)=O] donor atom sets can be expected in the complexes containing two molecules of naringenin. The only difference between them derives from the protonation form of the ligand as it can be seen in Fig. 5c where the concentration of the species in equilibrium is presented. The overall stability constants of the complexes as logβ together with the values for Cu(II)-rutin system for comparison are reported in Table 2.

Table 2. The overall stability constants of the complexes found in the system studied and the other for comparison

Ligand	Species	Coordination mode	logβ
Naringenin (this work)	CuNarH ₂	[C(5)-O ⁻ , C(4)=O]	25.3±0.01
	CuNarH		18.31±0.03
	CuNar		6.25±0.05
	CuNar ₂ H ₄	2×[C(5)-O ⁻ , C(4)=O]	49.15±0.01
	CuNar ₂ H ₃	2×[C(5)-O ⁻ , C(4)=O]	41.17±0.01
	CuNar ₂ H ₂	2×[C(5)-O ⁻ , C(4)=O]	32.21±0.01
	CuNar ₂ H	2×[C(5)-O ⁻ , C(4)=O]	21.2±0.02
	CuNar ₂	2×[C(5)-O ⁻ , C(4)=O]	10.13±0.02
	CuRutH ₂	[C(5)-O ⁻ , C(4)=O]	27.67±0.04
	CuRutH	[C(5)-O ⁻ , C(4)=O]	23.00±0.06
Rutin [14]	CuRut	[C(5)-O ⁻ , C(4)=O] or	17.13±0.07
	CuRut ₂ H ₂	[C(3'-O ⁻ , C(4')-O ⁻]	42.06±0.10
	CuRut ₂ H	[C(3'-O ⁻ , C(4')-O ⁻]	33.92±0.12
	CuRut ₂	[C(3'-O ⁻ , C(4')-O ⁻]	26.20±0.12

logβ, ± SD calculated by SUPERQUAD

The logβ values of CuLH₂ complexes rutin and naringenin are comparable, while all other constants are higher for the Cu(II)-rutin system. This can result from the fact that in the CuLH₂ is the same coordination mode ([C(5)-O⁻, C(4)=O]) for the

both naringenin and rutin while in the others is different especially in the species with the 1:2 stoichiometry where the catecholic atom donors [C(3'-O⁻), C(4'-O⁻)] of rutin dominate. The another effect influencing the stability could be arisen from a different electron densities of C and B rings of naringenin and rutin.

Conclusions

Application of potentiometry is possible to measure the proton, metal binding constants and correlate the constants and observations of chelating ability of flavonoids in order to present antioxidant efficiency data for structure activity relationships and mechanism elucidation. Furthermore, it is quite useful to study the ligand forms in solution prior to the complex formation process. This study was an introduction to later experiments. Obtained results will be used for further investigations.

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