

University of West Hungary

Thesis of Ph D Dissertation

**INVESTIGATION OF INTERACTION BETWEEN
WOOD-EXTRACTIVES AND METAL IONS**

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I. Object and aim of study

The aesthetic appearance of wood and lots of properties, which are important from the point of view of practical use, are influenced by the biochemical structure of wood-components and by the various environmental and chemical effects which happened in the life of tree or during wood-processing.

Network of organic macromolecules form varied wood structure and morphology. Countless small molecules (e.g. polyphenols, flavonoids, carbohydrates, carboxyl acids) are also bonding to the macromolecular skeleton. First of all these extractives play a significant role in the colour, odour and durability of wood, moreover influence the processes of gluing, coating, drying and chemical treatment.

Some treating metal salts have overriding importance in the wood-protecting. Metal ions using in the wood-technology also have significant affects on physical and chemical changes.

The interaction between wood surface and metal ions, in chemical respect, is not simple sorption process, but it is possible that ion exchange can take place, parallel with acid-base equilibrium, complex forming or oxidation reaction. External conditions (e.g. temperature and light) also can affect and modify highly the chemical changes. In addition to interaction between wood and metal ions, the behaviour and chemical reactions of certain components have to be studied apart from wood because of the complication and variation of processes.

The variance of different kind of timbers are caused first of all by different quality and quantity of flavonoids, which are excellent chelating agents and the most diverse components in the plant kingdom. Numerous flavonoids determine the colour of certain kind of timbers, but sometimes the colourless form of flavonoid is present in the tree and is converted into colour only by the effects of acids, alkalines, metal ions or oxidation processes. These changes account for detailed investigation of their interaction.

My research project was the investigation of interaction between three representative flavonoids from the wood-extractives and some selected metal ions.

The quercetin, rutin and robinetin were chosen as bioligand from flavonoids for studying chemical reactions. The aim of my research was the exhaustive exploration of chemical processes of metal ions in the presence of wood-components, since copper(II), chromium(III) and dichromate are important in wood-protection as well as aluminium can be accumulated in trees.

All these results of flavonoid research can be well-used not only in different fields of wood-protection and treatment, but in the chemical analysis or in the food-industry and medicine, too, by means of getting to know the properties of flavonoids, and their chelating, complex forming and structure.

II. Materials and methods

The interaction between flavonoids and metal ions in solvent and on solid surface were investigated by method of ultraviolet and visible absorption spectrophotometry.

The stock solutions of flavonoids ($\sim 3.00 \cdot 10^{-4}$ mol/dm³) were prepared in abs. ethanol by direct weighing. The check-solutions were made by addition of other ingredients and exact dilution. The flavonoid concentration of check-solution was about $6.00 \cdot 10^{-5}$ mol/dm³, and the final solution contained 50:50 volumetric mixture of ethanol and distilled water as solvent. Flavonoid stock solution was mixed with equal volume of standard electrolyte or buffer solution prepared in water. It was attended to have constant 50:50 v/v ratio of ethanol and water mixture solvent in all experiments. That is why the further reagents (e.g. reagent solutions of metal ions) were prepared in similar mixture.

Potassium chloride or potassium nitrate was dissolved in distilled water (1 mol/dm³) and the solution was used as standard electrolyte. Buffer solutions with different pH were made by mixing the solutions of potassium acetate and acetic acid or KH₂PO₄ and Na₂HPO₄.

$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ or $\text{K}_2\text{Cr}_2\text{O}_7$ and distilled water were used for making the stock solutions of metal ions. After determination their exact concentrations by volumetric analysis $1.00 \cdot 10^{-4}$ - $1.00 \cdot 10^{-2}$ mol/dm³ reagent solutions of metal ions were prepared (in ethanol:water mixture). This solution was added with precision burette into flavonoid check solution.

Occasionally sodium ethylenediaminetetraacetate (EDTA) was used for establishing the complexation processes, or demonstration of additional reactions. The flavonoid ligand of the metal complex can be converted into free by EDTA, and its concentration can be compared to the original quantity of flavonoid by spectrophotometric measurement.

The UV-VIS absorption spectra of the solutions were recorded in the range of 200 to 500 nm comparing to reference ("blank") solution.

$3.0 \cdot 10^{-5}$ mol/dm³ concentration of methanolic solution of flavonoids were used for making samples for measuring light absorption on silica gel surface. The TLC sheets were covered evenly with flavonoid solution, and then were dried on room temperature. After drying samples were cut and three sample-groups were formed. One of them remained untreated, second and third were impregnated with 1.0 g/dm³ chromium(III) chloride and potassium dichromate (in water), respectively. The absorption spectra of the silica gel plates were recorded in the range of 200 to 800 nm. Recording of the spectrums were repeated after irradiation with ultraviolet light for different length of time, and the changes demonstrated the processes of photodegradation.

Spectrophotometric measurements were carried out with a Shimadzu UV-3101PC type UV-VIS-NIR Scanning Spectrophotometer with using 2, 5, 10 and 20 mm quartz cuvetts or reflection supplement. The spectra were evaluated by version 2.2 of UV-2101/3101PC Personal Spectroscopy Software.

OP-274 pH/ION Meter (with OP0808-P combined glass electrode) and OP-930 automatic burette of Radelkis Ltd. (Hungary) were used for potentiometric determinations. The pH meter was fitted to standard buffer solutions. Values of pH Reference Value Standards for the 0.05 m potassium hydrogen phthalate buffer in various aqueous organic solvent mixtures at different temperatures in Table 3.5.1. of “Compendium of Analytical Nomenclature – Definitive Rules 1997” (IUPAC) were used for definition of the real pH data of solutions having 50:50 volumetric ratio of ethanol and water mixture.

Finnigan LASERMAT 2000 MALDI-TOF Spectrometer was used for mass-spectrometric measurements. Copper and chromium content of wood-extractives were measured by GBC Integra XM type ICP-Atomic Absorption Spectrometer, too. Photodegradation was carried out by SUNTEST (Hanau No. 7011) apparatus equipped with sunlight filter.

Equilibrium constants were calculated by the help of SCIENTIST for Windows computer program (MicroMath Scientific Software, USA, 1994).

III. Summary of the results of the research

In consequence of the complicated interaction between wood-extractives and metal ions my studies were confined the investigations of quercetin, rutin and robinetin from the characteristic flavonoids of wood. These compounds were impregnated on solid silica gel surface or were dissolved in ethanol-water solvent mixture with various acidity or alkalinity, and their spectrophotometric properties were studied. Furthermore the processes of their interaction were investigated with aluminium, copper(II), chromium(III) or dichromate ions.

III.1. Investigation of acid-base properties of flavonoids

[4, 5, 10, 12, 15, 19, 20]

1. 50:50 v/v ratio of ethanol and water solvent mixture was selected for research, which could be both proton acceptor and proton donor, and had less oxidative feature for testing of

flavonoid metal interactions. The most important spectral data of quercetin, rutin and robinetin and the validity of Lambert-Beer-law have been controlled in this solvent mixture [19].

2. On the evidence of the absorption spectra plotted against pH of the solutions it has been realized that quercetin seems to be a monoprotic acid in contrast to rutin, which dissociates in two steps. Both computer program and graphic representation were used for determination of the dissociation constants of these flavonoids. The numerical values of their dissociation equilibria have been only slightly different from the data of bibliography because of the different composition of solvents [12].

3. Essential structural transformation of quercetin and robinetin were detected during the examination. These changes were appeared after their dissociation processes even though in weak basic solution, but only dissociation equilibrium of rutin was observable in the same conditions. The irreversible transformation of quercetin was concluded from the spectral changes, and the apparent rate constant of the process has been determined, too [19].

4. The probable issue of the structural changes of quercetin and robinetin molecules were established by the investigation of spectra recording in different circumstances. These reaction steps has been included the processes of dissociation, tautomer and mezomer transformations, as well as addition reaction of any component of solvent. Even different reaction steps of structural changes were recognized in strong alkaline and weak basic solutions, but the products has been the same protonated forms [15].

III.2. Coordination of Al(III) to quercetin or Al(III) to rutin [4, 12, 15, 17, 18, 20]

5. Formation of aluminium-quercetin and aluminium-rutin complexes were studied in ethanol-water solvent mixture containing potassium chloride standard electrolyte or potassium acetate and acetic acid buffer solution [4, 18].

6. It was established that mainly binuclear complexes of the flavonoids have been developed, if the metal ion and flavonoid ratio is high, and pointed out that the Al(III)s coordination were

not passed off on the ways published by others. Innuendo the metal ions have not coordinated to the two different side of the ligand, but the dimeric form of aluminium hydroxyl particle has been connected to quercetin or rutin [17].

7. The apparent stability constants of aluminium-quercetin and aluminium-rutin complex formation have been determined by computational iteration method with using the measured absorbance at several wavelengths in buffered medium. The coordination of dimeric Al(III) form was presumed for the calculation. In accordance with my previous supposition the results have been proved the complex forming of Al_2Q or Al_2Ru in one step [17].

III.3. Interaction of Cu(II) and quercetin or rutin

[4, 5, 10, 17]

8. The reactions of quercetin and rutin were studied and the investigations were carried out in ethanol-water solvent mixture containing three types of standard electrolytes. It has been observed that the electrolytes have significant influence in the possibility of complexation processes even in case of same medium composition. Fundamental differences of reactions were demonstrated in presence of electrolytes or buffer solution [4].

9. The complex formation of rutin and copper(II) has been found to be 1:1 = metal ion : ligand compound by molar ratio method in presence of potassium nitrate electrolyte or potassium acetate and acetic acid buffer solution. While Cu_2Q complex formation was typical of coordination process of quercetin. All of them have been supported by the calculation of apparent stability constants [10].

10. Such peculiarity of quercetin was observed in presence of copper(II) and potassium chloride, which had not been described earlier in the literature. The well-founded inquiry of the reaction has been supposed that similar product was arisen in this process, which was observed in alkaline solution presence of potassium hydroxide. However verification of this supposition is demanded further investigations [5].

11. The presentation of complex forming between copper(II) and quercetin in potassium acetate and acetic acid buffer was not to be found in the literature, that is why it was investigated in detail. Only chelating process has been detected at low concentration of Cu(II), but complexation and oxidation reaction has been observed successively at high ratio of metal ion and ligand. The equilibrium constant of the first period has been calculated, too [17].

III.4. Interaction between Cr(III)/Cr(VI) and quercetin or robinetin

[6, 7, 11, 13, 21]

12. Some different degree of dichromate ion reduction was measured in the leaching of beech wood and spruce treated with preservations, which has been caused first of all by the difference of wood-extractives in the two types of wood-species [21].

13. Complexation processes on solid surface have been unequivocally documented by the results of investigation of interaction between chromium(III) and quercetin as well as robinetin in contradiction to the missing reaction in solutions where the ligand change reactions of Cr(III) have been hindered [6].

14. It was established that the quercetin and the robinetin have been oxidized by dichromate ion and the nascent Cr(III) could be chelating to the oxidized product of the ligands on silica gel surface [11].

III.5. Investigation of photodegradation processes

[6, 7, 8, 11, 13, 14, 18]

15. The results of the spectral changes of the flavonoids have proved that the molecular structure of quercetin or robinetin has been assumed only transitional quinone form in the case of ultraviolet light irradiating, after which the decomposition of flavonoid turns into complete on silica gel surface [6].

16. The differential spectra were indicated that flavonoids treated with chromium(III) or dichromate have been characteristically and differently modified after irradiating [11].

17. The formation of chromium(III) complexes evoked significant light filter effects of quercetin and robinetin, but Cr(VI) oxidized

the flavonoids and caused only a little retardation in the process of their photodegradation and the photoresistance of the surface have been depended first of all on the quantity of dichromate [7].

IV. Proposals for utilization of the results

The duty of my research, which included investigation of spectrophotometric properties and interactions of quercetin, rutin and robinetin with metal ions, presented some new scientific results in wood-chemistry.

The observations in the course of detailed studying of acid-base properties of flavonoids can be used for explanation of contradiction perceived in measuring of buffer capacity, total acidity or pH of wood, as well as can be reminded us of inaccuracy and irregularity of used analytical procedure or circumstances. Further investigations are required to study the transformation of quercetin and robinetin in alkaline solution and to specify the kinetic parameters of processes. For the explanation of structural changes it is necessary to identify the particles produced in the alkaline interaction.

On the basis of study results of quercetin and rutin chelating with aluminium ion can be explained the high value of Al(III) concentration taken up by living tree. Since the flavonoids are able to coordinate less soluble aluminium species, can form stable complexes with them, therefore possible the aluminium intake from soil.

It will be suitable to apply special analytical methods in the investigation for studying of different kind of Al(III) particles. Performing of additional experiments in various solvent mixtures can make an opportunity of selecting the right ligands for increasing of Al(III) evacuation of human body.

The knowledge of details of interaction between copper(II) and flavonoids is essential to work out technology for decrease of leachable copper(II) of wood preservatives. Besides the kinetic measurements, it would be important to verify the alkaline analogous process, to identify the sour cherry coloured transitional

product, and to explore the possibility of Cu(II)/Cu(I) transformation.

The investigations of properties of quercetin and robinetin in solution were completed with studying of their photodegradation processes on silicagel surface, and the results have provided explanation of colour changes of wood surface, too. Further examinations are demanded for identification of particles forming in the reaction, as well as for kinetic and structural interpretation of changes. The different flavonoid structures are needed comparative analysis.

The results of the studies of interaction between chromium(III) and quercetin or robinetin can explain the strong bonding of Cr(III) in the root of plants. The development of technical working-out for reinforcement of photoresistance calls for additional investigation of chromium(III)-flavonoid complexes or rather needs the determination of the most effective ratio of Cr(III) and flavonoid.

The reaction of the interaction between dichromate ions and flavonoids gives us information on possible reduction of wood-protective chromates to chromium(III). However the scientifically established results demand specifying of concentration of components on surface, and identifying of products in the steps of oxidation and complexation.

In conclusion all results of my flavonoid research can be well-used not only in different fields of wood-protection and treatment, but in the chemical analysis or in the food-industry and medicine, too.

V. Publications and presentations

Articles and publications

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