

## Summary of professional accomplishments

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### 1. Personal data

Marcin Tadeusz Klepka

### 2. Scientific degrees

Ph.D. degree in physics, Institute of Physics Polish Academy of Sciences, 2009



Dissertation title: "Elemental and chemical bonds studies in disorder materials using spectroscopic techniques".

Supervisor prof. dr. hab. Krystyna Jabłońska

M.Sc. degree in experimental physics, Faculty of Physics and Chemistry, University of Lodz, 2003

Thesis title: "Dosimetric characterization of the X-ray beam in diagnostic fluoroscopic equipment"

Supervisor prof. dr. hab. Jerzy Jankowski

B.Sc. degree in medical physics, Faculty of Physics and Chemistry, University of Lodz, 2001

Thesis title: "Searching for an optimal conditions for X-ray diagnostic tests".

Supervisor prof. dr. hab. Jerzy Jankowski

### 3. Employment in scientific institutions

2018-present: assistant at the Laboratory of X-ray and Electron Microscopy Research, Institute of Physics Polish Academy of Sciences

2009-2018: adjunct at the Laboratory of X-ray and Electron Microscopy Research, Institute of Physics Polish Academy of Sciences

2007-2009: assistant at the Laboratory of X-ray and Electron Microscopy Research, Institute of Physics Polish Academy of Sciences.

4. The scientific achievement, in accordance with art.16 paragraph 2 of the Act of March 14th, 2003, concerning the scientific degrees and titles (Dz. U. item no. 882, 2016, with amendments in Dz. U. item no. 1311, 2016), being the basis of the habilitation procedure.

#### 4.1 The title and series of publications being the basis of the habilitation procedure

Applying for the habilitation degree as an scientific achievement I present the series of eight publications under common title: „Binding mechanism of metal ions in metal-organic ligand systems determined using X-ray absorption spectroscopy”, in order of appearance:

- H1 A. Drzewiecka, A.E. Koziol, M.T. Klepka, A. Wolska, H. Przybylinska, S.B. Jimenez-Pulido, K. Ostrowska, M. Struga, J. Kossakowski, T. Lis "Synthesis and structural studies of novel Cu(II) complexes with hydroxy derivatives of benzo[b]furan and coumarin" *Polyhedron* 43 (2012) 71-80;
- H2 M.T. Klepka, A. Drzewiecka, A. Wolska, W. Ferenc "XAS studies on Cu(II) complexes with derivatives of phenoxyacetic and benzoic acids" *Chem. Phys. Lett.* 553 (2012) 59-63;
- H3 A. Drzewiecka, A.E. Koziol, M.T. Klepka, A. Wolska, S.B. Jimenez-Pulido, T. Lis, K. Ostrowska, M. Struga "Two coordination modes around the Cu(II) cations in complexes with benzo[b]furancarboxylic acids" *Chem. Phys. Lett.* 559 (2013) 41-45;



- H4 A. Drzewiecka, A.E. Koziol, M.T. Klepka, A. Wolska, S.B. Jimenez-Pulido, M. Struga "Electrochemical synthesis and structural studies of zinc(II) complexes with derivatives of benzo[b]furancarboxylic acids" *Chem. Phys. Lett.* 575 (2013) 40-45;
- H5 M.T. Klepka, A. Drzewiecka-Antonik, A. Wolska, P. Rejmak, K. Ostrowska, E. Hejchman, H. Kruszewska, A. Czajkowska, I. Młynarczuk-Biały, W. Ferenc "Synthesis, structural studies and biological activity of new Cu(II) complexes with acetyl derivatives of 7-hydroxy-4-methylcoumarin" *J. Inorg. Biochem.* 145 (2015) 94-100;
- H6 M.T. Klepka, A. Wolska, A. Drzewiecka-Antonik, P. Rejmak, K. Hatada, G. Aquilanti "XAFS study of bioactive Cu(II) complexes of 7-hydroxycoumarin derivatives in organic solvents" *Chem. Phys. Lett.* 673 (2017) 113-117;
- H7 M.T. Klepka, A. Drzewiecka-Antonik, A. Wolska, P. Rejmak, M. Struga "Structural studies of Cu(II) complexes with coumarin acid derivatives obtained using direct and electrochemical synthesis" *Chem. Phys. Lett.* 691 (2018) 190-195;
- H8 M.T. Klepka, D. Kalinowska, C.A. Barboza, A. Drzewiecka-Antonika, K. Ostrowska, A. Wolska "Structural investigation of Cu(II) complexes with dibromo 7-hydroxycoumarin derivatives using methodology based on XAS" *Rad. Phys. Chem.* doi.org/10.1016/j.radphyschem.2018.11.001.

#### 4.2 Bibliometric data

My professional achievements consists of **57** scientific publications indexed in Journal of Citation Reports. My Hirsch index is **12**. Total impact factor, according to the year of publication is **93.37**, total number of citations is **464** and without auto citations is **367\***. Moreover, I'm author and coauthor of 30 scientific reports based on synchrotron radiation studies.

#### 4.3 Introduction

After completing the doctoral thesis my main research interest has focused on the structural studies of metal-organic ligand systems, using the non-conventional in this field of research technique - X-ray absorption spectroscopy (XAS).

This technique is a unique tool which allows to study, at the atomic and molecular level, the local atomic structure around selected elements that are component of studied material. Elemental selectivity, which makes study of almost all elements possible, and high sensitivity (~ppm range) is a great advantage of this technique. XAS can be applied not only to crystals but also to disordered or partially ordered materials like: amorphous compounds, thin layers, glasses, membranes, solutions, liquids, metallo-proteins or even gasses. The physical quantity measured in XAS is the absorption coefficient  $\mu(E)$ , which describes how strongly X-rays are absorbed as a function of energy  $E$ . XAS spectra is divided into two regions: X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). Analysis of each region gives complementary information about local atomic structure around selected element.

EXAFS analysis provides information about an average coordination number, kind of near neighboring atoms and their relative structural disorder. Shape of the XANES spectra depends on geometrical arrangement of the atoms around the absorber. XANES is called a fingerprint of chemical state of element. Shift in energy of the absorption edge provides information about chemical state of the absorber. XAS technique is available mostly at synchrotron radiation facilities. Results presented below are based on measurements performed using European synchrotron sources in Germany, Sweden and Italy.

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\* Data from Web of Science dated 5th of April 2019



In my research I have focused on non-crystalline compounds with potential pharmacological significance. Research on this type of materials is important from the development of new generation of drugs point of view. In this case an important task is to learn how the organic ligand coordinate to metal. Very often it determines increased or decreased biological activity. The X-ray diffraction is the method which is commonly used in structural studies of this type of compounds. It gives direct structural information, but only for compounds in the form of monocrystals. Techniques which are commonly used for structural testing of non-crystalline compounds do not provide direct structural information. Methods such as infrared spectroscopy (FTIR), visible spectroscopy (UV-VIS) or electron paramagnetic resonance (EPR) provide information based on which a structure can only be rather speculated than directly determined. The natural compounds and their synthetic analogues arouse great interest, however, very often they do not exist in crystalline form or occur only in the form of solutions. Understanding of their structure is important due to the potential applications and at the same time difficult due to the limited number of possible experimental techniques.

For the XAS spectroscopy the challenge in studying such compounds is "light" elements of organic ligand. Usually these are oxygen, carbon or nitrogen atoms. These atoms scatters in a very similar way and it is impossible, without a realistic structural model, to distinguish between them above around 2 Å. It is the reason why EXAFS analysis in many cases is limited only to the nearest atomic environment. That part of atomic neighborhood is relatively easy to be determined (**H1-H2**), the only difficulty lies in the determination of the coordination number. The real challenge is to determine further atomic environment that allows to propose a molecular structure. The single scattering predominates in the EXAFS area, but multiple scattering, which are dominating in XANES region, allows to confirm the spatial arrangement between neighboring atoms. A model that would be stable in such analysis requires information from more than one experimental technique. In some cases, these are models of isolated molecules consisting of a metal cation and several ligands, in other polymers or other complex spatial systems are present. The key is to take into account the presence of the full organic part in the metal environment.

I have suggested that both the EXAFS and XANES areas should be used in the structural studies of the metal-organic ligand complexes. The use of these techniques allowed to provide information for constructing the initial model, as well as to verify the proposed structural model. In the literature one can find information concerning metal-organic ligand systems studies using XAS, but in almost all cases the analysis is carried out in a limited extent. This limited scope is the use of only one of the XAS regions, moreover, the proposed structural models are usually based on diffraction data. Developed methodology allows to describe the molecular structure of the organic metal-ligand complexes using the full potential of X-ray absorption spectroscopy and without the support from diffraction methods. The systems I have studied were in the form of both solid (powders) and liquid (solutions in organic solvents) (**H5-H6**). An extraordinary advantage of X-ray absorption spectroscopy is the ability to determine the local atomic environment around the absorbing atom regardless of its form or state. Combining the advantages of this technique with standard analytical, laboratory and spectroscopic methods as well as DFT calculations allowed to develop the methodology by means of which the mechanism of metal binding in non-crystalline metal-organic ligand systems have been described.

Before the methodology has reached its full functionality, we have published several research papers reporting the use of the X-ray absorption spectroscopy, in structural studies of metal-organic ligand systems, at various levels. The XAS technique was not that widespread in the field of coordination chemistry as it is now. In my work, I have focused on using the full potential of XAS spectroscopy in order to determine the mechanism of metal binding in organic-metal ligand systems. In the papers **H1, H3, H4**, XAS was the main technique used in structural studies, however, only the EXAFS area was used for the analysis and only the nearest atomic environment could be determined. In the paper **H2**, the XANES region was used for the first time to determine the spatial arrangement of atoms around



copper atoms. In works **H5-H6** the shape of the methodology can be seen, in the most advanced form it was used in **H7** and **H8**.

#### 4.4 Preliminary application of XAS in the study of metal-organic ligand systems (papers **H1**, **H2**, **H3**)

Papers **H1**, **H3** and **H4** are the first in the presented cycle. The results were obtained in cooperation with a group of prof. Anna Kozioł from the Maria Curie-Skłodowska University in Lublin. This group received, using electrochemical method, a series of copper (II) and zinc (II) complexes with benzofuran and coumarin derivatives. A common part in this papers is the fact that for each metal one complex in a form of monocrystal has been received. The lack of monocrystals for the remaining complexes became the motivation for structural studies of these complexes using XAS. In order to determine the binding mechanism of Cu and Zn in these systems, the following techniques were used: (i) for initial characterization - elemental analysis, thermal analysis and infrared spectroscopy, (ii) for the main structural studies - XAS spectroscopy. The crystallized complexes were examined using the X-ray diffraction technique.

The benzofuran and coumarin systems are part in many chemical compounds of natural origin, e.g. plants. They are heterocyclic compounds with biological activity and are an important fragment of pharmacophores. Their activity can be modified by changing substituents (e.g. methyl, methoxy or hydroxyl) in the aromatic ring.

In the **H1**, structural studies of four copper (II) complexes with benzofuran derivatives (**1-4**) and one copper (II) complex with coumarin derivative (**5**) were described. In the figure 1 the schematic representation of the parent ligands is presented.

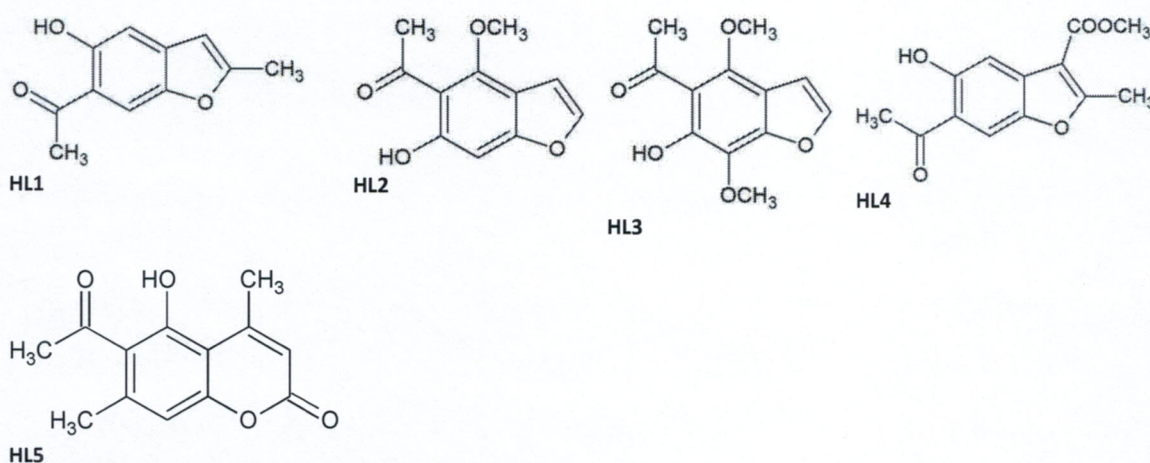


Figure 1. Schemes of parent ligand structures: derivatives of benzofuran HL1-HL4 and derivative of coumarin HL5 (paper **H1**).

Among five complexes only one was obtained in a crystalline form. This allowed to describe crystal structure of that complex using X-ray structural analysis. It was found that the molecular structure



of complex **(1)** consists of two HL1 ligands which are coordinated with metal ions in a bident manner. The coordination polyhedron is a flat square with four oxygen atoms derived from de-deprotonated hydroxyl and acetyl groups.

X-ray absorption spectroscopy was also applied to all complexes. Both XANES and EXAFS were analyzed. Analysis of XANES spectra was carried out by comparing the position in energy of the absorption edge for the studied complexes and reference compounds (CuO and Cu<sub>2</sub>O). On this basis it was found that copper in the studied complexes is at the +2 oxidation state. Additionally, based on the shape of the XANES spectra, it was found that the local atomic environment of metal ions, in all complexes with benzofuran derivatives, might have similar coordination number. Comparison of the Fourier transforms of the EXAFS oscillations confirmed that assumption. The structural data obtained from diffraction studies were used as the initial model for the EXAFS analysis. That model was used in order to determine the amplitude reduction factor ( $S_0^2$ ), this parameter is correlated with the coordination number.

In the case of all the studied complexes, it was found that in the closest vicinity of the copper atom four oxygen atoms, at two distances of about 1.89 Å and 1.95 Å, are located. It was determined that for the copper (II) complexes, with benzofuran derivatives and coumarin, flat square coordination polyhedra is the most probable. It consists of four oxygen atoms probably derived from hydroxyl and acetyl groups of the two ligands. The EXAFS analysis concerned the nearest atomic environment, so confirmation of this information was impossible at this stage. In the figure 2 the Fourier transforms (FT) of the EXAFS oscillations together with the fit of the closest vicinity of the copper (II) cation are presented.

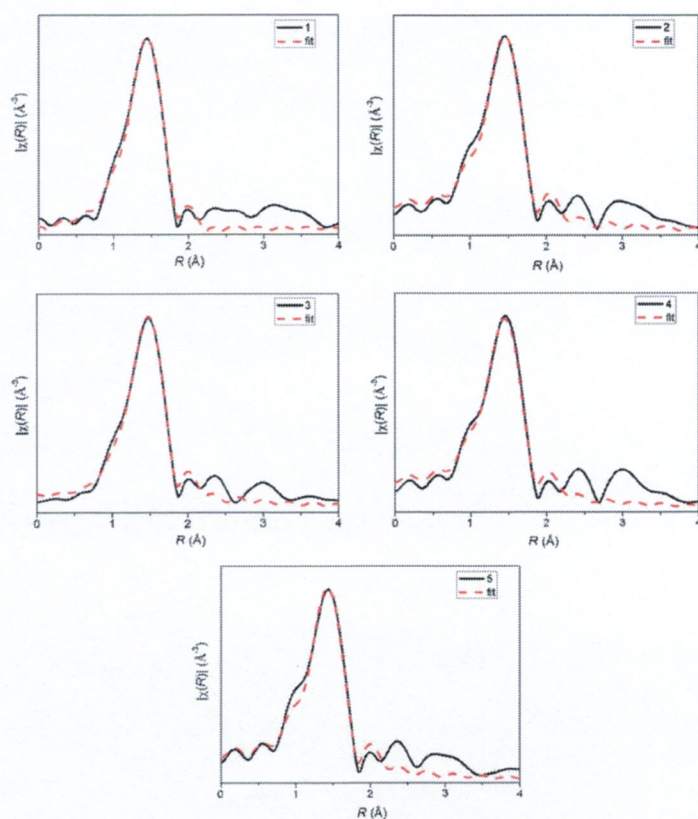




Figure 2. Fourier transforms of the EXAFS oscillation together with the fitting result for the immediate environment in the benzofuran (1-4) derivative complexes and the coumarin derivative (5) with copper (II).

In the **H3** and **H4** the structural tests of a series of three new copper (II) and zinc (II) complexes with benzofuran derivatives are presented. In the figure 3 the parent ligands used in the complexation reaction with Cu and Zn are shown. Also in case of this studies only one compound was crystallized in each of the series for both metals. The XAS analysis was performed for XANES as well as EXAFS region.

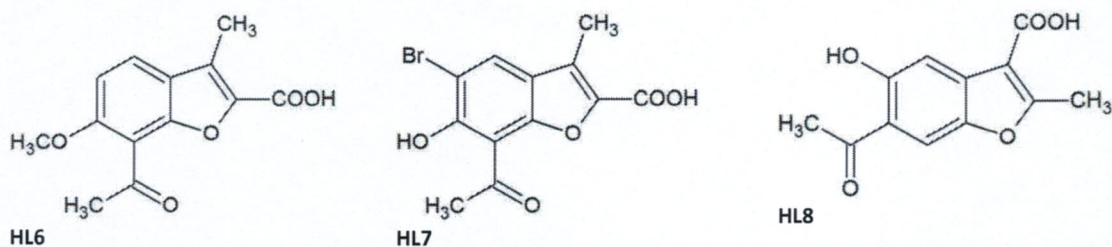


Figure 3. Schemes of parent ligand structures: derivatives of benzofuran (papers **H3** and **H4**).

Qualitative analysis of XANES spectra allowed to determine the oxidation state of copper and zinc in complexes. It was found to be +2 for both type of metals.

The diffraction studies of the copper (II) complex with HL6 (**H3**) showed that it is a dinuclear where for each copper atom there are two monodentately coordinated ligands, and the tetragonal pyramid is a coordination polyhedron. From the comparison of the FT of the EXAFS oscillations, it was noticed that the complexes differ from each other in the local environment. In the case of the polycrystalline equivalent of the HL1 complex, four oxygen atoms were identified at two distances below 2 Å. In addition, at the distance of about 2.6 Å, the second Cu atom was identified, confirming the dinuclear nature of the complex. In the case of complexes formed with the HL7 and HL8 ligands, no additional Cu atoms were found and four oxygen atoms forms the local atomic environment. In the figure 4 the FT of the EXAFS oscillations together with the fitted closest vicinity in the form of 4 oxygen atoms is shown. The research allowed to propose coordination polyhedra in the form of a flat square for all complexes tested in powder form. The difference between the complex for which the crystal was obtained and its powder equivalent confirmed the hypothesis that the solvent molecules can be incorporated into the metal coordination sphere. Crystallization was carried out in an ethanol environment. At the top of the tetragonal pyramid, forming the coordination polyhedron of this complex, an ethanol molecule was identified.



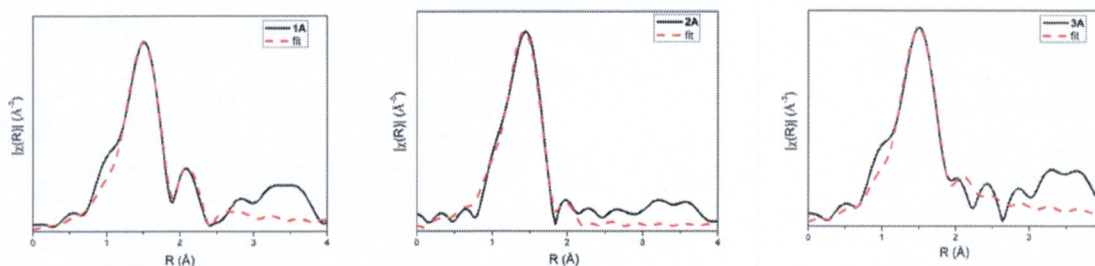


Figure 4. Fourier transforms of the EXAFS oscillation together with the result of the fit for the immediate environment in the copper (II) complexes with benzofuran derivatives.

Diffraction studies of the crystalline zinc (II) complex with the HL1 ligand (**H4**) have shown that the complex is a mononuclear with mixed monodent-bident coordination of the two ligands. The coordination polyhedron is a tetragonal pyramid. Comparison of FT EXAFS oscillations of all three complexes showed their structural similarity (see Figure 5). EXAFS analysis confirmed this observation and allowed to determine that the closest vicinity of Zn atoms consists of five oxygen atoms at the average distance of about 2 Å. Tetragonal pyramid is the most probable coordination polyhedron in these systems.

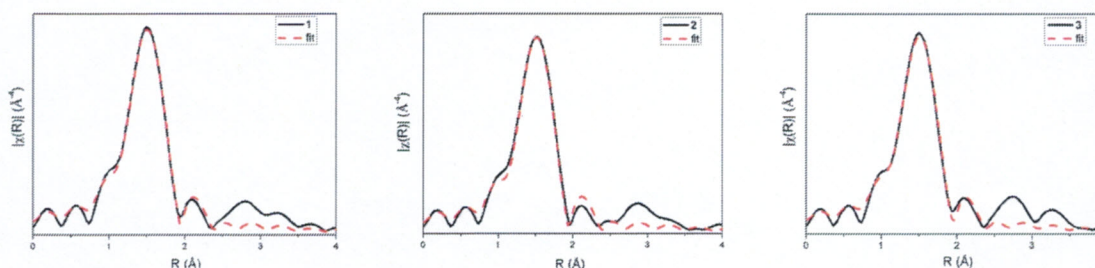


Figure 5. Fourier transforms of the EXAFS oscillation together with the result of fitting the immediate environment in the zinc (II) complexes with benzofuran derivatives.

In summary, the use of X-ray absorption spectroscopy allowed from the one hand to confirm data obtained from X-ray structural studies and from the other showed differences related to the incorporation of solvent molecule into the coordination sphere in the crystallization process. Thanks to application of the XAS, it was possible to determine the local atomic surroundings for all Cu (II) and Zn (II) O-donor complexes with organic ligands. The fact that the use of XAS gave only information about the nearest atomic environment, and allowed for the conclusion of multi-core nature of the complex, left the impression that some additional information are missing. At this stage, I decided that the XANES region, sensitive to geometry, should be implemented more broadly than just to determine the oxidation state of the metal.

#### 4.5 XANES in structural studies of metal-organic ligand complexes (paper H2)



In the paper **H2**, the XAS technique was used in a more advanced manner. XANES calculations were for the first time used, by us, to identify the spatial arrangement of atoms in structural studies of the metal-organic ligand complexes. Models used for XANES calculations were proposed on the basis of elemental, thermal analyses and EXAFS analysis for the nearest environment.

Presented in **H2** results were performed in cooperation with prof. Wiesława Ferenc from UMCS in Lublin. It was conducted in the manner of structural studies of copper complexes with herbicides. Three copper (II) complexes with derivatives of phenoxyacetic and benzoic acids were studied (see Figure 6). It was proved that these types of compounds are absorbed in the soil. In addition, they are able to form complexes with metal atoms, which might increase their biological activity. So they can have a dual function, biological activators that allow to protect plants, as well as purify the soil from metals such as copper. Understanding the mechanism of copper binding in these compounds is important from the environmental protection point of view. Analytical methods used in the research were: elemental, thermo-gravimetric analyses and magnetic methods.

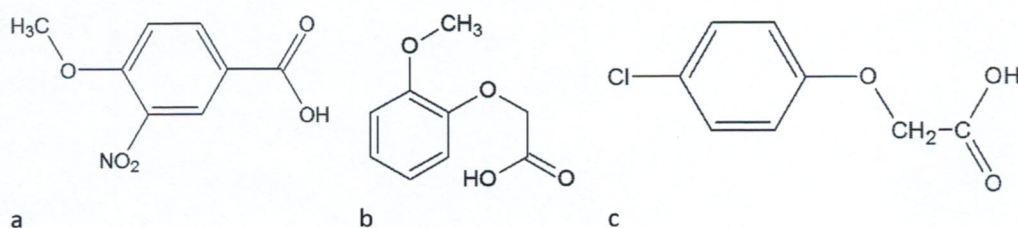


Figure 6. Schemes of parent ligands: a) 4-methoxy-3-nitrobenzoic acid - Cu4m3nb, b) 2-methoxyphenoxyacetic acid - Cu2mpa, c) 4-chlorophenoxyacetic acid - Cu4cpa (paper **H2**).

In the **H2**, both qualitative and quantitative analysis of XANES spectra was applied. The former allowed to determine the oxidation state of copper ions that it is +2. The results of the elemental analysis allowed to estimate the metal to ligand ratio which is 1:2 and suggested the presence of the water molecule in the system. EXAFS analysis for each of the complexes indicated presence of different number of oxygen atoms in the nearest neighborhood of the copper atom. It suggested that for each of the studied complexes different coordination polyhedron was formed. In order to confirm this observation a quantitative analysis of XANES spectra was used by mean of comparison of experimental and theoretical spectra with each other. Based on the data, from above mentioned techniques, a number of structures were proposed and their theoretical XANES spectra were calculated and then compared with the experimental ones. As the best were chosen those which shape was not in contradiction with the shape of the experimental spectrum. It was found that in the investigated copper (II) complexes with benzoic and phenoxyacetic acids derivatives different molecular structures and coordination polyhedron were formed (see Figure 8c). Thanks to the implementation of XAS into the study, there were revealed three different copper complexes formation of three different coordination polyhedrons: a flat square, a tetragonal pyramid, and a tetragonal bipyramid (see Figure 7c). The results of the EXAFS, XANES analysis and the proposed models of molecular structures are shown in Figure 7a-c.

In the **H2** paper for the first time, in our studies, XAS spectroscopy was used in an advanced way. The analysis of both the EXAFS and XANES regions showed usefulness of XAS in studies of such systems. However, the way in which it was carried out required optimization, because all models calculated



theoretically were modified manually. At this stage, the idea of using theoretical calculations (DFT) seemed to be a good way of supporting searching for and indicating possible structural models.

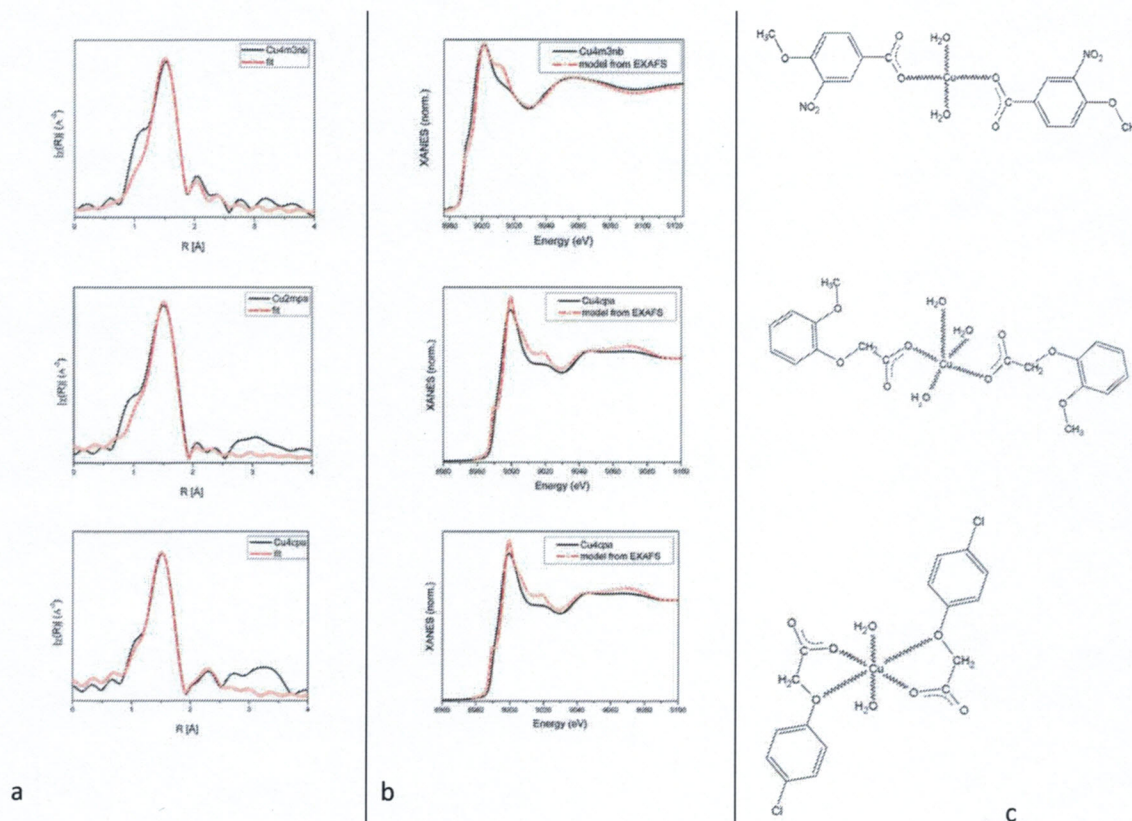


Figure 7. Fourier transforms of the EXAFS oscillations together with the result of fitting for the immediate oxygen environment in the phenoxycetic and benzoic acid complexes (a), comparison of the experimental and theoretical XANES spectra of the studied complexes (b), structural models of the studied complexes proposed on the basis of XAS analysis (c).

#### 4.6 Investigations of copper complexes with coumarin derivatives (papers H5-H8)

The potential of using XAS for structural studies of non-crystalline metal-organic ligand systems, presented in the papers H1 - H4, was the motivation for developing this technique in studies of similar systems but with potential pharmacological significance. Cooperation with the Medical University of Warsaw, which provided organic ligands with microbial activity primarily coumarin derivatives, was established. Within the grant of the National Science Center (NCN), of which I was the manager, a metal binding mechanism of copper (II) complexes with the mentioned bioactive ligands was described. The motivation was to observe that metal complexation of biologically active ligands often leads to the increased activity and reduced toxicity. The complexes were obtained by electrochemical method and classical chemical synthesis in solution at the Institute of Physics of the Polish Academy of Sciences. It was possible thanks to the creation of a "unique scientific workshop" within the framework of the mentioned grant. The results of the structural analysis presented in the H5-H8 papers were conducted under motivation of search for a new generation of drugs.



The long-term gap in the distribution of new pharmaceuticals and the adaptive capacity of pathogenic pathogens has drawn the way back to pre-antibiotics era. Historically, the invention of antibiotics was a milestone in the fight against diseases that pose a deadly threat. Currently, a biofilm, a multicellular structure that prevents the penetration not only of immune cells, but also therapeutic compounds, as well as other mechanisms of drug resistance of microorganisms are a serious threat. According to WHO predictions, mortality due to bacterial and viruses infectious will become the world's number one in the next few decades, surpassing those caused by cancer. The creation of a new generation of the drug is possible thanks to the cooperation of groups from the borderline of chemistry, biology and physics. From the targeted therapy point of view, it is important to understand the molecular structure of a potential pharmacophore. Among the many widely studied metal-organic ligand systems, more and more attention is paid to compounds that do not form crystalline systems or are present as a solution.

#### 4.6.1 Methodology

The use of XAS in the study of this type of systems allowed to plan the order of research using both standard analytical and spectroscopic methods. The whole has been arranged into a methodology, the scheme of which is shown in the figure 8.

The methodology is divided into three main blocks: (i) basic characterization, (ii) an initial structural model, (iii) complexes' molecular structure. Each block assumes the use of appropriate analytical, spectroscopic technique or theoretical calculations. The common feature for all blocks is the use of XAS. This allows the study of the mechanism of metal bonding with the organic part in the absence of crystallographic data.

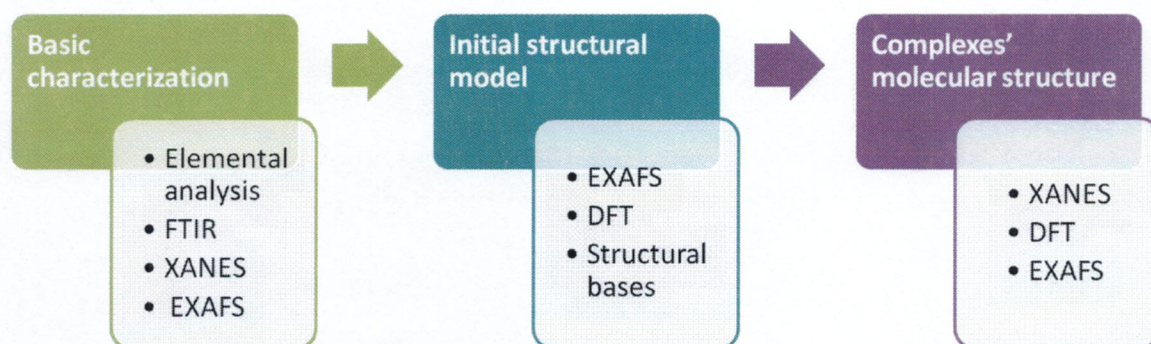


Figure 8. Diagram of the methodology developed as a part of ongoing research.

In the first block, the methodology assumes the use of elemental analysis, which allows determining the ratio of metal to the organic part, and estimating the amount of water molecules or solvent in the studied system. Infrared spectroscopy (FTIR) provides information whether the complexation reaction was effective and complex was formed. The assessment is made by measuring the spectra of the ligand and the complex, in the case of a mutual shift of the spectra superimposed on each other, this is evidence of the complex formation. Further analysis of FTIR spectra allows to indicate the functional groups of ligands potentially active in the binding process to the metallic center. Qualitative analysis of XANES spectra allows to determine the oxidation state of the metal in the tested compound. However, the EXAFS analysis provides information about the nearest atomic environment which allows to determine what kind of atoms are present in the absorber's surroundings, how many of them and how far they are. In order to minimize the number of models, which will then be refined, information obtained from the above techniques is used. In addition, the search for the optimal initial model is



supported by databases where structures of similar organic systems with metals are sought. Also the EXAFS analysis is extended to identify atoms in the farther distance around the absorber. In the case of the studied systems these are usually carbon atoms, the amount of which often suggests a way of coordination the organic part to the metal (monodent or bident). For the proposed models, energy optimization using DFT methods is performed. The preliminary model obtained at this stage is checked by comparing the XANES spectrum measured experimentally with the theoretically calculated one. XANES spectra are sensitive to the spatial arrangement of atoms (geometry) due to the predominant multiple scattering effects in this area. In the absence of shape correlation, the model is modified, optimized by means of DFT and again compared in the XANES region. A model whose XANES spectrum, calculated theoretically, is not in conflict with the experimental one, and confirmed in the DFT calculations, is used for a full EXAFS analysis. The full EXAFS analysis assumes the use of the full structure of the proposed compound, including the inclusion of geometrically sensitive multiple scattering paths together with the single scattering paths, to confirm not only the presence of individual atoms and their distances, but also their spatial orientation. Before the methodology reached its full functionality, it went through several stages.

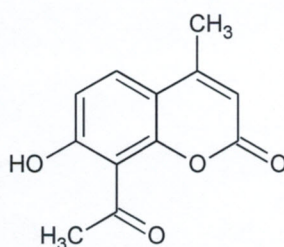
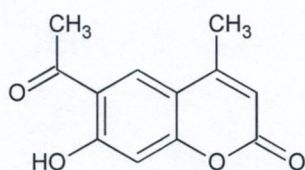
#### 4.6.2 Initial applications (papers H5 and H6)

Papers **H5** and **H6** are related to the synthesis and structural studies of copper (II) complexes with hydroxycoumarin derivatives. At the same time, these are the first research in which DFT calculations were included into the analytical and spectroscopic methods. It was done to optimize the geometry of the molecular models proposed during the structural analysis. Thus, the process of comparing the experimental and theoretical spectra of XANES was improved because it allowed to exclude non-energy-optimizing models or delivering results that are not reliable.

The tested compounds were not obtained in a crystalline form, that is why in the **H5** and **H6** there is a lack of support from diffraction methods. Both studied complexes were obtained by electrochemical method. The structure of the ligands is shown in the figure 9. The difference between the ligands is the position of the acetyl group in the coumarin ring. For HL1 this is the C6 position and for HL2 it is C8.

In addition, both the initial ligands as well as the obtained complexes were microbiologically and cytotoxically tested. Increased activity of complexes with respect to parent ligands was observed. Complex with HL2 ligand for selected fungal strains showed activity comparable to the commercially available drug - Fluconazole. In contrast, the complex with the HL1 ligand was found to be selective for prostate cancer cells (DU145).

To describe the structure of the obtained compounds, elemental analysis, IR spectroscopy and XAS were used. The whole was supported by the DFT calculations. In **H5**, structural studies were carried out for compounds in the form of microcrystalline powder while in **H6** compounds were tested after dissolving in common organic solvents. The studied complexes did not dissolve in water. The solvents used were: (i) dimethyl sulfoxide (DMSO), (ii) dimethylformamide (DMF). They are commonly used solvents in preparatory processes for microbial or cytotoxic activity tests.





## HL2

## HL1

Figure 9. Schemes of initial ligand structures: HL1 and HL2 coumarin derivatives (applies to **H5** and **H6**).

In the XAS analysis, the full potential of both regions was applied. XANES provided information on the oxidation state of copper in the complexes (+2), as well as confirmed the molecular structure of the complexes proposed on the basis of EXAFS analysis and DFT calculations. Experiments have shown that complexes are formed by two ligands coordinated bidently with copper atom by four oxygen atoms at an average distance of 1.94 Å. Oxygens came from an acetyl and hydroxy group. Coordination polyhedron is in the form of a flat square (see Figure 10c).

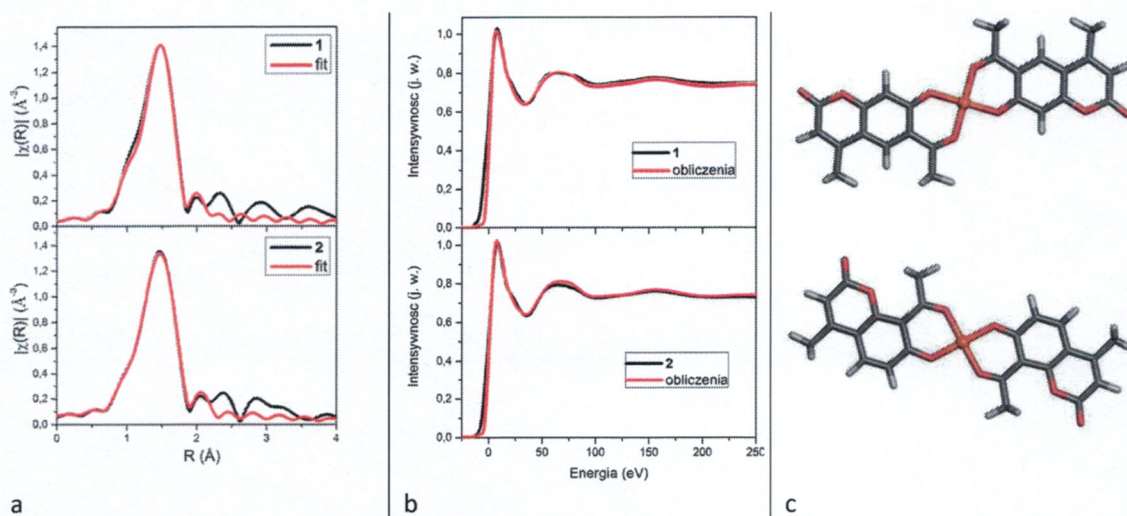


Figure 10. Fourier transforms of the EXAFS oscillations along with the result of the fit for the identified environment in the copper(II) complexes with hydroxycoumarin derivatives (a), comparison of the experimental and theoretical XANES spectra of the studied complexes (b), molecular models of the studied complexes proposed on the basis of XAS (c) analysis. The data in the top line refers to the complex with the HL1 ligand, in the bottom line to the complex with the HL2 ligand - both in the powder form.

The obtained results for the powder form were used in the analysis of complexes in the form of solutions in DMSO and DMF (**H6**). The work was aimed at investigating whether and, if so, to what extent the structure of copper complexes with hydroxycoumarin derivatives was modified after dissolving in an organic solvent. Also in these studies, the analysis of both the EXAFS and XANES regions was used, and the whole analysis was supported by theoretical calculations. The copper oxidation state remained +2, which was derived from the qualitative XANES analysis. From the comparison of the FT EXAFS oscillations for powder forms and dissolved in both solvents, it could be noted that significant differences in the local environment are not unambiguous. EXAFS analysis confirmed this assumption. It was observed that the DMSO environment modifies the structure of the complex with the HL1 ligand, whereas the DMF environment remains inert. For the complex with the HL2 ligand, there is an opposite relationship - DMSO remains inert, while DMF modifies the structure of the complex. To investigate the structural changes induced by interaction with solvent molecules, EXAFS analysis was performed up to a distance of approximately 3.3 Å from the absorbing atom. It was possible thanks to properly prepared and energetically optimized models, proposed on the basis of the preliminary EXAFS analysis (for the nearest environment) and XANES spectra calculations. Models ultimately optimized using DFT calculations allowed to use both single and multiple scattering paths in the analysis. The whole analysis



allowed to describe structural changes occurring under the influence of both solvents. For a complex with an HL1 ligand, two additional DMSO molecules through sulfur atoms to the copper atom were identified. In the case of complex with the HL2 ligand, the structure is modified by two molecules of DMF joining via oxygen atoms to the copper atom. In the figure 11 the obtained structural results are shown.

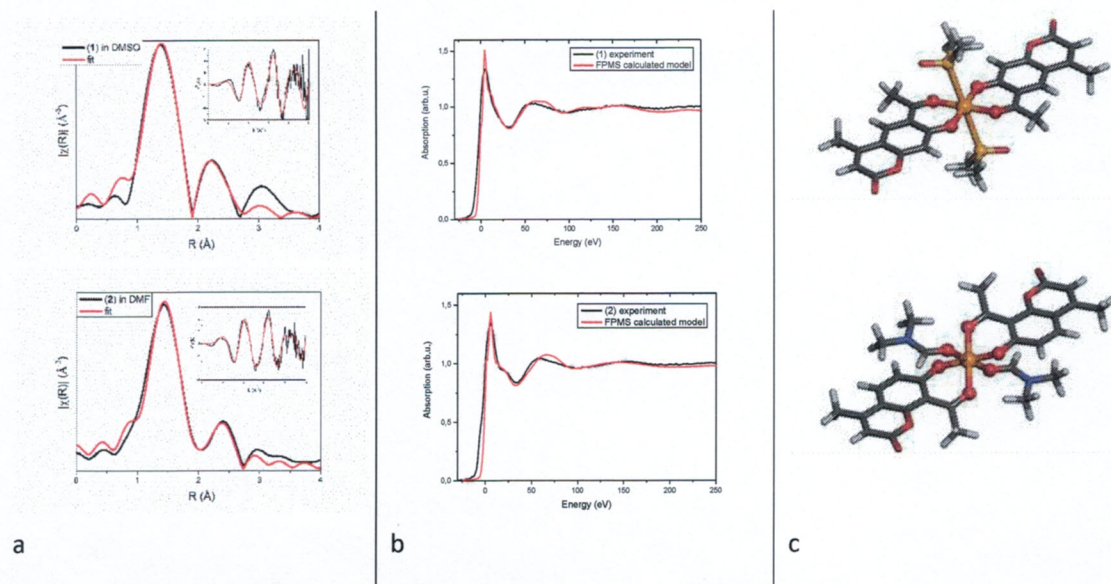


Figure 11. Fourier transforms of the EXAFS oscillations along with the result of the fit for the identified environment in the copper(II) complexes with hydroxycoumarin derivatives (a), comparison of the experimental and theoretical XANES spectra of the studied complexes (b), molecular models of the studied complexes proposed on the basis of XAS (c) analysis. At the top the data for the complex of ligand HL1 as a solution in DMSO. At the bottom of the data for the complex of ligand HL2 as a solution in DMF.

The coordination polyhedron in newly formed hydroxycoumarin derivative complexes with copper ions is the tetragonal bipyramid, where oxygen atoms derived from coumarin ligands are in one plane, while solvent molecules, DMSO or DMF, are axial ligands coordinated by sulfur and oxygen atoms respectively.

#### 4.6.3 Research using the methodology (papers H7 and H8)

The H7 and H8 represent the methodology applied in a complete form. All information obtained in previously described studies were used to lay the order of successive stages of the methodology, to organize it. The research concerns the structural studies of copper (II) complexes with coumarin acids derivatives (H7, figure 12) and hydroxycoumarins derivatives (H8, figure 13). In the latter case, they are the equivalents of the ligands shown in papers H5 and H6 with bromine atoms as substituents. The use of bromine substituents was intended to increase biological activity, but no measurable effect on the microbiological activity of the complexes was observed.



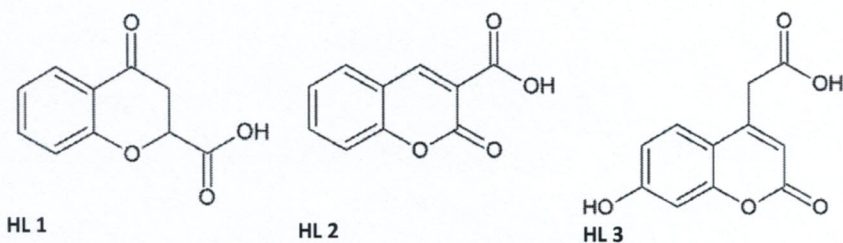


Figure 12. Schemes of initial ligand structures: coumarin acids derivatives (applies to paper H7).

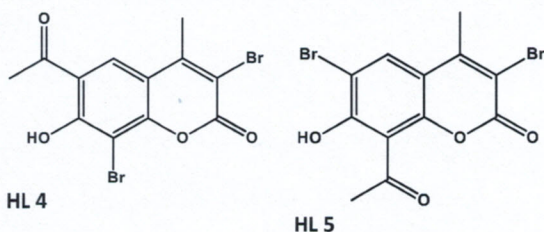


Figure 13. Schemes of initial ligand structures: hydroxycoumarin derivatives (applies to paper H8).

In H7 complexes obtained by two methods of synthesis were compared and their molecular structure was described. The used methods were classical synthesis in solution and electrochemical one. The comparison of FT EXAFS oscillations did not show structural differences between the complexes synthesized by both methods (see figure 14).

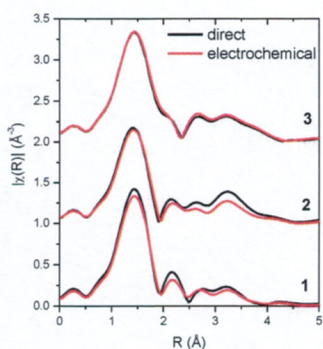


Figure 14. Fourier transforms of EXAFS oscillations for copper(II) complexes with coumarin acid derivatives obtained by two methods of synthesis. 1 complex with the ligand HL1, 2 with the ligand HL2, 3 with the ligand HL3 (regarding the paper H7).

The molecular structure of copper (II) complexes with coumarin acid derivatives was determined for compounds obtained by electrochemical synthesis. On the basis of elemental analysis, the ratio of metal to ligand 1:2 was determined. Analysis of the FT-IR spectra allowed to determine that the active groups in the binding process to copper ions are deprotonated carboxylate groups. The XANES analysis allowed to estimate the oxidation state of copper ions: +2, while the EXAFS analysis determined that oxygen atoms are in their immediate vicinity. Their number is 6 at a distance of 1.95  $\text{\AA}$  for the complex with HL1 (1) and 5 for the complex with HL2 (2) and HL3 (3) at average distances of 1.94 and 1.97  $\text{\AA}$ , respectively. This information was used to propose initial structural models. EXAFS analysis of the further environment showed the presence of two carbon atoms at a distance of 2.72  $\text{\AA}$  for 1 and four carbon atoms at 2.99 and 2.84  $\text{\AA}$  for 2 and 3 respectively. In addition, 3 showed the presence of another Cu at 2.64  $\text{\AA}$ . This allowed to state that this complex is dinuclear. Finally, the



proposed models were verified by XANES analysis after prior optimization using DFT calculations. The results of the analysis are presented in the figure 15.

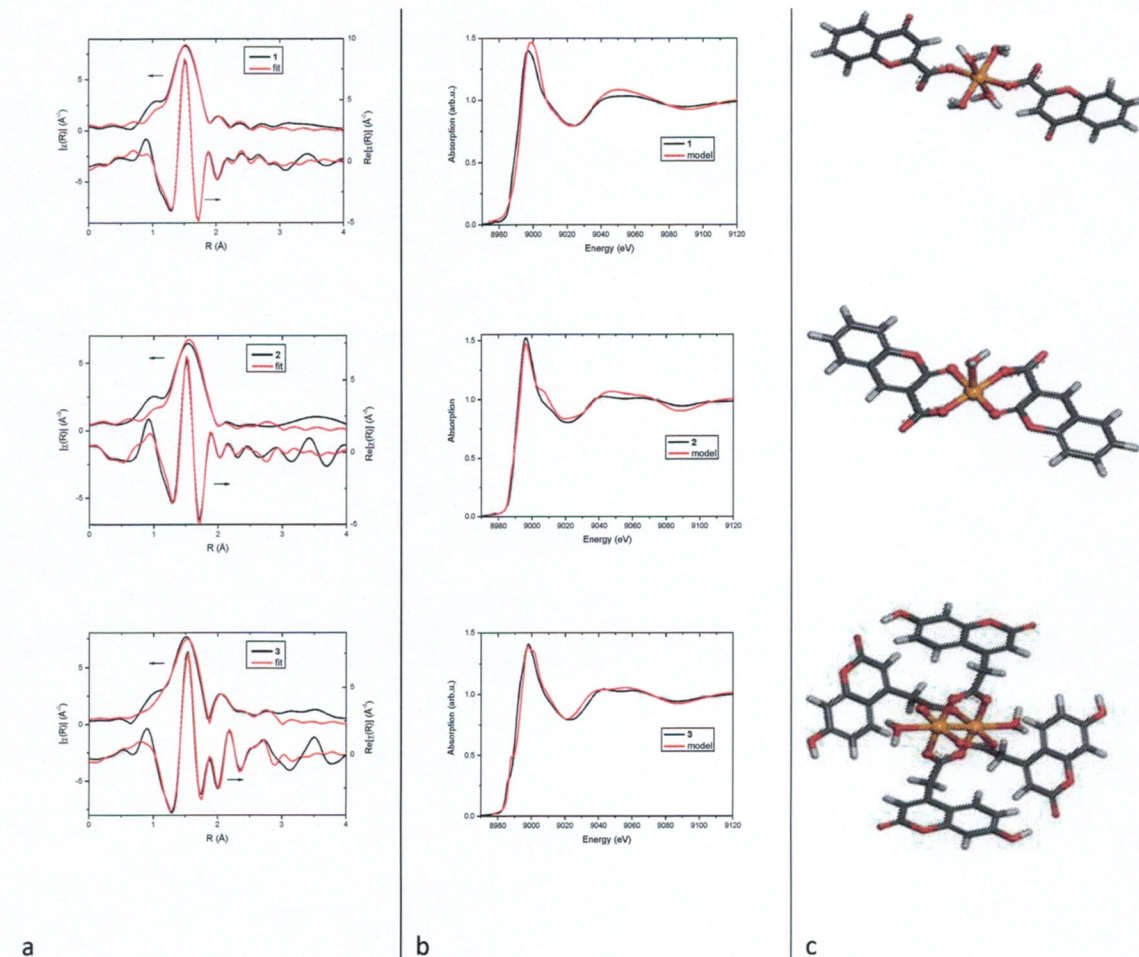


Figure 15. Fourier transforms of the EXAFS oscillations along with the result of the fit for the identified environment in the coumarin acid derivatives complexes (a), comparison of the experimental and theoretical XANES spectra of the studied complexes (b), molecular models of the studied complexes proposed on the basis of XAS (c) analysis. On the top complex with the HL1 ligand, in the middle with the HL2 ligand, at the bottom with the HL3 ligand (regarding the H7 paper)

In summary, no structural differences in **H7** for complexes obtained by various chemical methods were observed. The methodology used, based on XAS, allowed to describe the molecular structures of the studied complexes. It was found that they differ structurally and demonstrate monodent (**1**) and bident coordination around the metal cation (**2** and **3**). Complexes **1** and **2** are mononuclear with octahedral and tetragonal pyramidal geometry respectively. However, complex **3** is a dinuclear one in the form of a tetragonal pyramidal.

The **H8** paper presents the application of methodology to describe the molecular structure of complexes with hydroxycoumarin derivatives, shown in the figure 13. The application of the methodology has made it possible to describe the molecular structures of these complexes. Elemental analysis allowed to estimate the metal to ligand ratio (1:2), FTIR spectroscopy confirmed the formation of complexes and allowed to indicate that the active groups in the ligand structure are carbonyl and acetyl one. EXAFS analysis showed that for both complexes there are 4 oxygen atoms in the immediate vicinity. However, a difference was observed in the number of carbon atoms in the farther distance



four carbon atoms were found in the case of complexes with HL1 (**1**) and two atoms in the case of complex with HL2 (**2**). This suggested the existence of two types of coordination - monodent and bident (see figure 16). In the **H8** UV-VIS spectroscopy was included into the methodology. This was to support and confirm the monodent and bident coordination mode. The final models were proposed using XANES calculations and DFT optimizations and confirmed in the full analysis of EXAFS region.

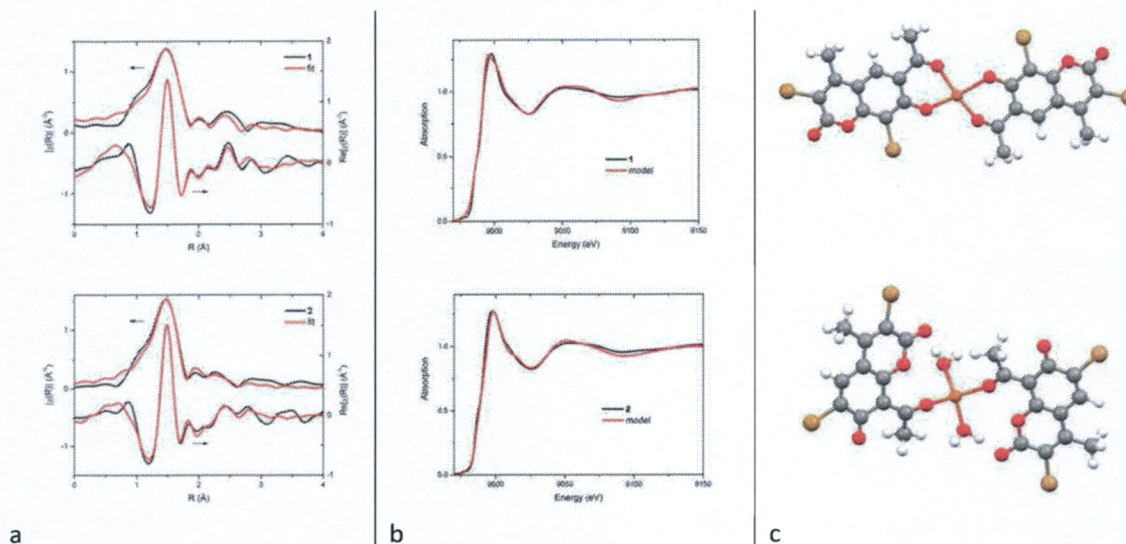


Figure 16. Fourier transforms of the EXAFS oscillations together with the result of the fit for the identified environment in the copper(II) complexes with hydroxycoumarin derivatives (a), comparison of the experimental and theoretical XANES spectra of the studied complexes (b), structural models of the studied complexes proposed on the basis of XAS (c) analysis.

In summary, the use of XAS methodology allowed to describe the molecular structures of two new compounds. In both complexes, the coordination polyhedron proved to be a flat square, and the coordination of the metal ions occurs through the oxygen atoms. Ligands in **1** coordinate bidently with the copper atom, while in case **2** it is the monodent coordination of two coumarin ligands and two water molecules.

#### 4.7 Summary

The use of XAS in the described successive studies allowed to develop the methodology by which a mechanism of metals binding in non-crystalline metal-organic ligand systems can be described. The chronology of development of the methodology and how the XAS analysis was extended for the needs of the research was shown in examples in the papers **H1-H8**. This methodology allows to effectively analyze the molecular structure of virtually any compounds. Its use is of particular interest for the description of metal-organic ligand systems in non-crystalline forms as well as solutions for which the structure is often modified. The contribution to the development of the scientific field is the increase of the application of spectroscopic methods, in this case X-ray absorption spectroscopy, in non-crystalline metal-organic ligand systems. Research on such systems is a challenge both from the



coordination chemistry and physics point of view. My work in this direction has shown that using the full analytical potential of XAS, based on the EXAFS analysis including geometrically sensitive multiple scattering paths, allows to describe the mechanism of metal bonding in such systems. The key to success is of course a construction of a realistic structural model to use in the XAS. XAS is practically the only technique that allows obtaining direct structural information for non-crystalline systems.

The latest unpublished research on the mechanism of silver binding using the methodology, in similar systems, allowed to determine what is the structural architecture of the system. The studied complexes exhibited the characteristics of both polymers and layered systems.

It is the development of the methodology based on XAS that I consider the most important achievement of my scientific work. The methodology without the use of XAS spectroscopy would not be able to determine the molecular structure of the non-crystalline compound.

The currently conducted research on the mechanism of metal binding in metal-organic ligand systems with potential pharmacological application has been extended and will be performed mainly for solutions, simulating both the environment of living cells and the environment in which broadly understood biological research is conducted. Organic solvents, water and physiological saline will be used as solvents. Well-developed cooperation with domestic and foreign scientific centers provides more and more new projects and challenges. What's more, the presentations on the methodology and its application meet the interest of another research groups, and the publications on it are very popular.

## 5. Discussion of other scientific and research achievements

### 5.1 Description of scientific achievements unrelated to the topic of habilitation

During last few years, most of my scientific work has been concentrated on the use of X-ray absorption spectroscopy for structural studies of metal-organic ligand systems. However, there are other activities and scientific topics in which I have participated. Among them, structural studies of various semiconductor materials can be distinguished. The used technique was also X-ray absorption spectroscopy. In my opinion, the most interesting scientific papers in this subject are:

- A. Wolska, K. Lawniczak-Jablonska, M. Klepka, M.S. Walczak, A. Misiuk, 2007, "Local structure around Mn atoms in Si crystals implanted with Mn+ studied using x-ray absorption spectroscopy techniques", *Physical Review B* 75 113201;
- A. Wolska, K. Lawniczak-Jablonska, S. Kret, P. Dłuzewski, A. Szczepanska, M. Klepka, M.S. Walczak, Y. Lefrais, M.J. Hytch, A. Misiuk, 2008, "Atomic order in magnetic Mn inclusions in Si crystals: XAS and TEM studies", *Journal of Non-Crystalline Solids* 354 4189-4192;
- K. Lawniczak-Jablonska, J. Libera, A. Wolska, M.T. Klepka, R. Jakiela, J. Sadowski, 2009, "The ratio of interstitial to substitutional site occupation by Mn atoms in GaAs estimated by EXAFS", *Radiation Physics and Chemistry* 78 80-85;
- A. Wolska, K. Lawniczak-Jablonska, M.T. Klepka, A. Barcz, A. Hallen, D. Arvanitis, 2010, "Study of the Local Environment of Mn Ions Implanted in GaSb", *Acta Physica Polonica A* 117 286-292;
- W. Paszkowicz, A. Wolska, M.T. Klepka, S. abd el All, F.M. Ezz-Eldin, 2010, "Combined X-Ray Diffraction and Absorption Study of Crystalline Vanadium-Doped Lithium Disilicate", *Acta Physica Polonica A* 117 315-318;
- K. Lawniczak-Jablonska, A. Wolska, M.T. Klepka, S. Kret, J. Gosk, A. Twardowski, D. Wasik, A. Kwiatkowski, B. Kurowska, B.J. Kowalski, J. Sadowski, 2011, "Magnetic properties of MnSb



inclusions formed in GaSb matrix directly during molecular beam epitaxial growth", *Journal of Applied Physics* 109 074308;

- M. Sawicki, E. Guziewicz, M.I. Lukasiewicz, O. Proselkov, I.A. Kowalik, W. Lisowski, P. Dłuzewski, A. Wittlin, M. Jaworski, A. Wolska, W. Paszkowicz, R. Jakiela, B.S. Witkowski, L. Wachnicki, M.T. Klepka, F.J. Luque, D. Arvanitis, J.W. Sobczak, M. Krawczyk, A. Jablonski, W. Stefanowicz, D. Sztienkiel, M. Godlewski, T. Dietl, 2013, "Homogeneous and heterogeneous magnetism in (Zn,Co)O: From a random antiferromagnet to a dipolar superferromagnet by changing the growth temperature", *Physical Review B* 88 085204;
- J. Wojnarowicz, S. Kusnieruk, T. Chudoba, S. Gierlotka, W. Lojkowski, W. Knoff, M.I. Lukasiewicz, B.S. Witkowski, A. Wolska, M.T. Klepka, T. Story, M. Godlewski, 2015, "Paramagnetism of cobalt-doped ZnO nanoparticles obtained by microwave solvothermal synthesis", *Beilstein Journal of Nanotechnology* 6 1957-1969;
- A. Kulka, A. Braun, T.W. Huang, A. Wolska, M.T. Klepka, A. Szewczyk, D. Baster, W. Zajac, K. Swierczek, J. Molenda, 2015, "Evidence for Al doping in lithium sublattice of LiFePO<sub>4</sub>", *Solid State Ionics* 270 33-38.

I'm also involved in material research using a DualBeam microscope. It is a device that allows imaging using scanning electron microscopy (SEM) and focus ion beam (FIB). In addition, it allows for testing chemical composition using the energy dispersive spectroscopy (EDS) technique. I'm using mainly FIB technique to prepare samples that are later investigated using transmission electron microscopy (TEM). Selected scientific papers on this subject are:

- F. Ivaldi, N.A.K. Kaufmann, S. Kret, A. Dussaigne, B. Kurowska, M. Klepka, J. Dabrowski, P. Dłuzewski, N. Grandjean, 2011, "Effects of the annealing temperature on the structural and electronic properties of MBE grown InGaN/GaN quantum wells", *Journal of Physics Conference Series* 326 012012;
- S. Kret, A. Wolska, M.T. Klepka, A. Letrouit, F. Ivaldi, A. Szczepanska, J.F. Carlin, N.A.K. Kaufmann, N. Grandjean, 2011, "TEM and XANES study of MOVPE grown InAlN layers with different indium content", *Journal of Physics Conference Series* 326 012013;
- K. Sobczak, P. Dłuzewski, M.T. Klepka, B. Kurowska, E. Czerwosz, 2012, "Transmission electron microscopy studies of the Pd-C films obtained by physical and chemical vapor deposition", *International Journal of Hydrogen Energy* 37 18556-18562;
- G. Kamler, J. Smalc-Koziorowska, G. Nowak, I. Grzegory, M.T. Klepka, 2013, "Influence of substrate planar defects on MOVPE GaN layer growth", *Physica Status Solidi A-Applications and Materials Science* 210 503-506;
- A. Aquila, R. Sobierajski, C. Ozkan, V. Hajkova, T. Burian, J. Chalupsky, L. Juha, M. Stormer, S. Bajt, M.T. Klepka, P. Dłuzewski, K. Morawiec, H. Ohashi, T. Koyama, K. Tono, Y. Inubushi, M. Yabashi, H. Sinn, T. Tschentscher, A.P. Mancuso, J. Gaudin, 2015, "Fluence thresholds for grazing incidence hard x-ray mirrors", *Applied Physics Letters* 106 241905;
- R. Sobierajski, I. Jacyna, P. Dłuzewski, M.T. Klepka, D. Klinger, J.B. Pelka, T. Burian, V. Hajkova, L. Juha, K. Saksl, V. Vozda, I. Makhotkin, E. Louis, B. Faatz, K. Tiedtke, S. Toleikis, H. Enkisch, M. Hermann, S. Strobel, R.A. Loch, J. Chalupsky, 2016, "Role of heat accumulation in the multi-shot damage of silicon irradiated with femtosecond XUV pulses at a 1 MHz repetition rate", *Optics Express* 24 15468-15477.

A full list of scientific papers from all my previous activity is included in Annex 3.



## 5.2 Conferences activity

I had 5 invited presentations:

1. M.T. Klepka, K. Lawniczak-Jablonska, A. Wolska, 2011, "Iron Location In Fe-Chitosan Complexes Determined By X-ray Absorption Spectroscopy" 1st Annual Congress of Marine Biotechnology (WCMB-2011) April 25-30, Dalian, China.
2. M.T. Klepka, A. Wolska, A. Drzewiecka, K. Lawniczak-Jablonska, 2013, "X-ray absorption spectroscopy - tool to resolve structure" Krajowe Spotkanie Użytkowników promieniowania Synchrotronowego (KSUPS 2013) Stalowa Wola.
3. M.T. Klepka, A. Wolska, A. Drzewiecka-Antonik, P. Rejmak, 2014, "XAFS technique used to obtain structural information for complexes of coumarin derivatives" 12th Int. Symposium and School on Synchrotron Radiation in Natural Science (ISSRNS-12) Warszawa.
4. M.T. Klepka, A. Drzewiecka-Antonik, A. Wolska, 2016, "Structural studies of bioactive metal-organic ligand complexes using XAFS" 13th Int. Symposium and School on Synchrotron Radiation in Natural Science (ISSRNS-13), Ustroń.
5. M.T. Klepka, D. Kalinowska, A. Wolska, A. Drzewiecka-Antonik, C. Barboza, G. Aquilanti, M. Struga, 2018, XAFS studies of non-crystalline metal-organic ligand complexes, 17th International Conference on X-ray Absorption Fine Structure, Krakow, Polska.

Additionally, I had 9 presentations and 24 posters which were presented during international and national conferences. Twice my research was highlighted as the most important achievement of the Laboratory of X-Ray And Electron Microscopy Research of the Institute of Physics of the Polish Academy of Sciences. I have twice presented the results of my achievements during the Institute's reporting session.

## 5.3 Teaching and popularizing activity

Since 2015 till 2018 I was a member of the Scientific Council of the Institute of Physics of the Polish Academy of Sciences.

Since 2017 I am a member of the Board of the Polish Synchrotron Radiation Society.

Since 2015 I am one of the PhD student's scientific supervisors at the Institute of Physics of the Polish Academy of Sciences.

I was responsible for two interns one from the Adam Mickiewicz University in Poznan and the other from Warsaw University of Technology.

I was an external expert in the Inowator Mazowska competition.

## 5.4 Implementation of scientific projects

Project leader in:

- "Synthesis and characterization of copper and silver complexes with coumarin derivatives". National Science Center (UMO-2012/07/D/ST5/02251), 2013-2017.

I was the vice leader of the innovation capacity task in the project:



- „European Action towards Leading Centre for Innovative Materials (EAgLE)”, seventh framework program of EU (REGPOT-2012-2013), 2013-2016.

I am the main contractor in the Baltic TRAM project, which aims to strengthen cooperation between the world of science and industry, by enabling research using, among others, scientific infrastructure. The initiative concerns the region of the Baltic Sea countries. My main task included substantive evaluation of projects and suggesting appropriate research methods and techniques. I also in person carried out research for entrepreneurs.

- Baltic TRAM - Transnational Research Access in MacroRegion, project co-financed by the European Union.

I was also a contractor for a number of projects granted as part of Ministry of Science and Higher Education. In addition, I was the manager and contractor of 13 projects implemented on synchrotron sources. A detailed description can be found in Annex 3.

*Marcin Klepka*