A STUDY OF CHROMIUM INTERACTION WITH *N*-DONOR LIGANDS USING ELECTROSPRAY-IONIZATION MASS SPECTROMETRY

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A study of the interaction of chromium(III) with 2'2-bypiridine and two pesticides, atrazine and acetamiprid, as N-donor ligands was performed using electrospray-ionization mass spectrometry (ESI-MS) and ultraviolet/visible (UV/Vis) spectrophoto¬metry. Atrazine and acetamiprid, both widely used pesticides, were chosen for their approved toxic environmental effect, while 2,2'-bipyridine was selected for comparison, because of its powerful metal complexing ability. Chromium(III) is the toxic heavy metal ion that forms numerous stable complex compounds. The heterogeneity and complexity of natural matrices justify the usage of model systems such these, with the aim of evaluation of complex processes in the environment. The intensity of the interaction is correlated with the acid-base and electron-donor properties, geometric and steric characteristics, and the number and the positions of *N*-donor atoms in the investigated ligands. UV/Vis data describing chromium interaction with ligands is placed in correlation with ESI-MS data on the complexes. UV/Vis spectroscopy and ESI-MS quantification showed a large difference in the information they give in describing the interaction of chromium(III) with the ligands, the ESI-MS technique being superior. ESI-MS technique can be used for quantitative analysis of the system Cr(III)–ligand. ESI-MS ion current chromatograms of 20 µL *loop* injections of systems Cr(III)–ligand indicate a stabile peak and signal integrity.

Introduction

The world production trend for chromium is expected to reach 8 million tons/year of alloyed metal or its compounds in 2020, permanently increasing human and animal exposition to the dispersed toxic chromium species in the environment. Approximately 28.8 million tons of marketable chromite ore was produced in 2013, and converted into 7.5 Mt of ferrochromium [1]. On the other side, the estimated 1 to 2.5 million tons of active pesticide ingredients are used annually, mainly in agriculture [2,3]. A significant portion of pesticides (about 19%) find their way into the soil through spray drifts, or as wash-off from treated plants [4]. From the soil, these pesticide residues may get directly vaporized along with the evaporating soil moisture or are migrated into the ground or surface waters by leaching or run-off [5-8].

Agricultural soils may contain both classes of these compounds – heavy metals and pesticides – originating from various agricultural or industrial practices. Their state, form, mobility, sorption, bioavailability and mutual interaction depend on physical and chemical environmental conditions in which they are located, and on the nature of all chemical species present in the environment, which they can interact with. Thus, the migration of heavy metals and pesticides from soil into groundwater or plant tissue is strongly dependent on terrestrial conditions, such as

physical and chemical characteristics of soil, the clay mineral content and the presence of natural or artificial organic matter [9, 10].

Pesticide degradation is an important phenomenon that helps in remediating the contaminated soils and water bodies. It is shown to be assisted by the number of factors such as the moisture content of soil, the soil texture, and soil mineral contents [11]. The presence of metals in the soil has recently also been evidenced to affect the pesticide degradation pathways [12]. Metals may catalyze the photolysis/hydrolysis of pesticides or influence the microorganisms methabolomics [13]. On the contrary, complexation interactions between pesticides and metals may lead to a decrease in the rate of their degradation [14]. It was found that certain paramagnetic metal ions inhibit photodegradation of pesticides with humic acids [15], and the decreased imazapyr photolysis has been explained by coordinative binding of metal ions [16].

In natural environments such as soil, chromium primarily interacts with soil organic matter – humic substances [17-19] containing *O*- and *N*-donor groups, but strong complexation potential of Cr(III) ions is worth to be investigated in cases of pesticides coexistence, among them many exhibiting the behavior of typical *O-, N-, P-* or *S*-ligands. The

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present study focuses on tracing these correlation patterns, specifically on three *N*-only donor ligands – triazine herbicide atrazine, insecticide acetamprid and 2,2'-bipyridine. The last one had been chosen for comparison purposes, for its well-known electron donor ability. Since the discovery of 2,2'-bipyridine at the end of the nineteenth century, it was extensively used in the complexation of metal ions [20-22].

Electrospray-ionization mass spectrometry, ESI-MS is used for the detection and characterization of one or more ionic species in the solution, and is confirmed as very useful in the studies of various complex systems [23]. In the case of metal–ligand coordination interactions, it is often used for verification of the stoichiometry of a newly synthesized complexes [24] that do not participate in the liquid phase equilibrium, and after decomposition does not produce other molecular species (if it is still the case, the created equilibrium is usually ignored).

UV/Vis spectrophotometric characterization of chromium complexes with *N*-donor ligands was correlated with the ESI-MS characterization of the complexes, in both quantitative and qualitative terms.

The aim of this study was to closer define the processes that chromium ions can undergo in interaction with pesticides and other natural or artificial substances with *N*-donor atoms. This research contributes to the investigation of complex processes of chromium and pesticides translocation and transformation in the natural systems, after which the pollutants could be further transported and dispersed in biotic and abiotic systems.

Experimental ———————————————————

Chemical reagents, solutions, instrumentation

All reagents used were p.a. or of HPLC grade purity. Acetamiprid, atrazine and 2,2'-bipyridine were purchased from *Sigma-Aldrich* (USA). Methanol, purchased from Baker (Analyzed Reagent Bio) was of HPLC grade purity. As a source of chromium(III), crystalline chromium(III) chloride hexahydrate (Merck, Germany) was used. The employed glass and plastic laboratory ware was rinsed with 4.0 mol L^{-1} hydrochloric acid (*Baker* Analyzed), then with 0.6 mol L^{-1} nitric acid (Environmental Grade *Anachemia*) and finally with purified water (< 0.1 µScm⁻¹) to remove trace metals and other contaminants.

Standard stock solutions of ligands were prepared by weighing the respective acid with an accuracy of ± 0.0001 g and dissolving in methanol. The solutions were kept in a refrigerator at 4 °C. Working solutions were made by dilution of standard solutions in the methanol/water mixture (50/50 v/v). Working solutions of the ligand–chromium mixtures were prepared in polyethylene plastic vials of 5, 2 and 1.5 cm³ (*Eppendorf*, Germany) previously washed with 0.1 mol L^{-1} HCl, deionized water and methanol in order to remove adsorbed surface impurities and reduce plastic additives in the working solutions.

Spectrophotometric characterization in the UV/VIS region was performed on a double beam UV/Vis spectrophotometer (*Shimadzu* UV-1650 PC). The ESI-MS in-

vestigation was performed on a LCQ Deca Ion Trap Mass Spectrometer (*Thermo Finnigan*, USA) with the auxiliary equipment.

Experimental procedure for UV/Vis characterisation of Cr-ligand interactions

A mixture of methanol and deionized water (< 0.1 µScm⁻¹) in ratio 50/50 (v/v) was used as a blank. The samples with the appropriate ligand and Cr(III) in the same solvent mixture were recorded 30 min after mixing the components to ensure the formation of the complex in the solution, considering the relatively low concentration range of the components. In preparation of the samples of ligand, and ligand–chromium(III) mixtures, any pH adjustment was avoided, so as not to disrupt the equilibrium in the reaction mixture. Otherwise, the occurrence of secondary chemical reactions and the formation of various coordination compounds with buffer components could not be excluded. Concentrations for ligands were in the range $c = 11 - 12$ µmol L^{-1}, while Cr(III) was kept at $c = 15.00$ µmol L^{-1}.

For the obtained spectral curves (Fig. 1.), the areas of surface integrals under each spectral curve were calculated and compared. The calculation was performed in order to obtain the relative area difference ($\Delta P_\%$) between the area of the binary ligand–Cr(III) system (P_2) and the area of the arithmetical summation of two spectra of monocomponent systems, *i.e.*, the solution of ligand and the solution of Cr(III) (P_4), according to the Eq. (1):

$$\Delta P_\% = \frac{P_2 - P_4}{P_2} \times 100 \dotfill (1)$$

The correlation coefficient (*r*) is calculated for the range of wavelengths in which the absorption occurred, from $\lambda_{min} = 190$ nm till $\lambda_{max} = 285 - 340$ nm, depending on the ligand.

The sum of absolute values of absorbance differences for every binary ligand-chromium system is calculated for the wavelength (λ_i), according to Eq. (2):

$$S_{\Delta A} = \sum_{\lambda_{min}}^{\lambda_{max}} \mathrm{abs}(x_i - y_i) \dotfill (2)$$

where the wavelength range was 190 to 285–340 nm, in which the absorption is observed. The parameter $S_{\Delta A}$ could be calculated in this way, as all the data were equidistant following the abscise (wavelength), because all spectra were recorded with an identical sampling interval of $\Delta\lambda = 0.5$ nm.

The sum of squares of absorbance differences ($S^2_{\Delta A}$) for the specific wavelength (λ_i), between two series of spectral curves data was calculated according to Eq. (3):

$$S^2_{\Delta A} = \sum_{\lambda_{min}}^{\lambda_{max}} (x_i - y_i)^2 \dotfill (3)$$

Experimental procedure for ESI-MS characterisation of Cr-ligand interactions

Monocomponent solutions of ligands were made in

pre-washed polyethylene Eppendorf tubes of 1.5, 2 and 5 cm³, with the solvent methanol/water (50/50, v/v). Binary systems of chromium(III)-chloride and the corresponding ligands were obtained in the same way, in the same solvent, by dilution of the concentrated stock solutions. All the obtained solutions were stored in a cold (4 °C) and dark place. The presence of 50% deionized water in the solvent was intended to facilitate the desolvation electrospray process and provide better ion yields.

Solutions of pure ligands were analyzed by ESI-MS immediately after preparation, while the two-component systems of chromium(III) and ligands were analyzed after maturation for at least 30 minutes after mixing of the components, to allow sufficient time for chemical interaction. Bearing in mind that compared to UV/Vis spectrophotometric analysis, ESI-MS is much more time consuming, it was ensured that the prepared working solutions are not older than a few hours, in order to avoid side-reaction products. Considering the chemical nature of all investigated ligands and their relative chemical stability at a given pH, it was assumed that the formation of side products due to photolysis or catalytic degradation did not occur to the extent that could jeopardize the relevance of the results.

By using ICIS® module of Thermo Excalibur™ 1.3 software, the areas of each ESI-MS ion current chromatogram of 20 μL *loop* injection for the defined mass range of ligand (P_1) and ligand–chromium solution (P_2) were calculated (Fig. 2). Five different ligand concentrations are investigated, within the range $1 – 12 \times 10^{-6}$ mol L⁻¹.

In order to quantitatively describe the interaction between ligand and chromium(III), the comparison of the obtained areas P_1 and P_2 in Fig. 2., *i.e.*, difference of areas ΔP(1–2)% was performed following Eq. (4):

$$\Delta P_{(1-2)\%} = \frac{P_1 - P_2}{P_1} \times 100 \ \% \ \dots\dots\dots\dots\dots(4)$$

The areas are presented in absolute non-dimensional (arbitrary) units of XcaliburTM software (count–second units) which have no physical meaning.

Bearing in mind that the chromium concentration was constant (9×10 μmol L⁻¹) in all binary systems, but ligand concentrations were different, the normalization of all $\Delta P_{(1-2)\%}$ values was performed, providing the corrected relative area differences $\Delta P_{ESI\%}$, expressed as in Eq. (5):

$$\Delta P_{ESI\%} = \Delta P_{(1-2)\%} \frac{c_{max}(\text{ligand})_m}{(1/n)\sum_{i=1}^{n}[c_{max}(\text{ligand})_i]} \ \%$$

$$\frac{1}{n}\sum_{i=1}^{n}[c_{max}(\text{ligand})_i] = 11.696 \ \mu mol \ L^{-1} \ \dots\dots\dots\dots(5)$$

where: $c_{max}(\text{ligand})_m$ is the maximum ligand concentration (μmol L⁻¹) in the ligand–chromium system, while the denominator presents the average maximal concentration of the investigated ligand, *i.e.*, 11.696 μmol L⁻¹.

The obtained $\Delta P_{ESI\%}$ values were used for the compari-

son of the interactions between chromium(III) and ligands. A higher $\Delta P_{ESI\%}$ value confirms a stronger interaction in the investigated binary systems.

Results and disscusion

UV/Vis analysis of Cr(III)–ligand system

The results of UV/Vis spectrophotometric studies of chromium(III) interaction with 2,2'-bipyridine, acetamiprid and atrazine are shown in Fig. 1.
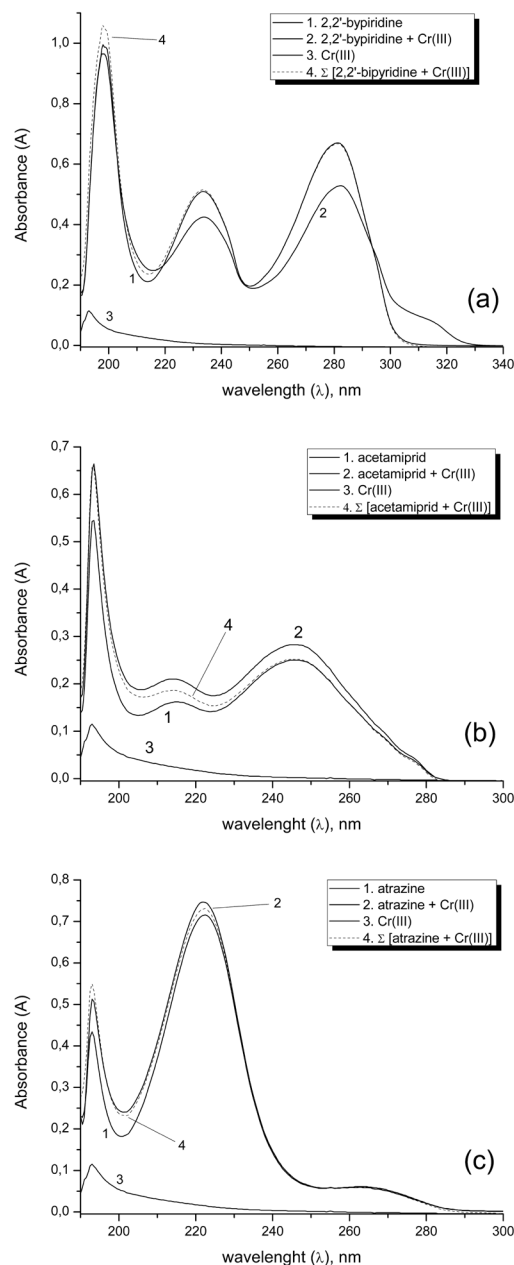

Figure 1. UV/Vis spectra of ligands: (a) 2,2'-bipyridine c = 11.43 μmol L⁻¹ , (b) acetamiprid c = 11.68 μmol L⁻¹ and (c) atrazine c = 11,89. μmol L⁻¹; chromium(III) c = 15.00 μmol L⁻¹ in all systems. Labels: 1 – ligand solution, 2 – ligand + Cr(III) solution, 3 – Cr(III) solution and 4 – mathematical sum of ligand spectrum and chromium spectrum. *l* = 1 cm, solvent CH₃OH/H₂O = 50/50.

The hypochromic effect in the binary system Cr(III) and 2,2'-bipyridine (Fig. 1a-2) is noticed on λ_{max} = 199 nm, within the band which is slightly broader and shifted to higher wavelengths in comparison to other two ligands (Fig. 1b-2, 1c-2). At λ_{max} = 233 and 282 nm where the hypochromic effect is even more pronounced, while the bathochromic effect is very small. A significant difference of intensity is noticed in 300 – 330 nm region due to Cr(III) and 2,2'-bipyridine reaction, giving the absorption with not defined maximum ("shoulder").

For the binary system Cr(III)–acetamiprid (Fig. 1b-2), the spectrum at λ = 200 – 275 nm shows a relatively high absorbance gain, related to pure ligand and simulated summarized spectra of separate components (Fig. 1b-1 and 4, respectively), as well. At λ < 200 nm difference is neglectable.

The spectrum of the atrazine–chromium binary system (Fig. 1c-2), related to the simulated summation of the components spectra (Fig. 1c-4) is quite similar, both in terms of shape and intensity of absorption. A small absorption difference can be observed at λ_{max} = 193 nm where the band has lesser intensity in comparison to the sum of spectra from individual Cr(III) and atrazine mono-component systems (negative difference). On the contrary, at λ_{max} = 222 nm a positive difference occurs. For λ > 230 nm the related spectra are practically overlapped, and the peak shift ($\Delta\lambda$) is also neglectable.

The differences in the spectra can indicate the existence of interaction and/or coordination in the binary system of ligand–Cr(III), because there is no basis for assuming that some other causes could lead to the measurable hypochromic, hypsochromic, bathochromic or hyperchromic shifts under the given conditions of the measurement. The hypothesis that the presence of Cr(III) could lead to the catalytic degradation of the ligand or redox reaction with the ligand is unlikely because of the short preparation and maturation time of the system (about 30 min), a relatively low redox potential (positive or negative) of the ligands and chromium(III), low concentrations and mild pH of solutions. In addition, in the case of structural degradation of the ligand molecule in the presence of chromium(III) chloride, it would be expected that the spectra of resulting products would be significantly different in shape and intensity and would not, for example, show the hyperchromic effect while maintaining approximately the same shape of the spectrum.

The calculated parameters, a relative difference between areas ($\Delta P\%$), the linear correlation coefficient (r), the sum of the absolute values of absorbance difference ($S_{\Delta A}$) and the sum of squares of the absorbance difference ($S^2_{\Delta A}$), according to Eqs. (1) – (3), are presented in Table 1. These parameters can only be considered as qualitative or, at most, as semi-quantitative indicators of the chromium(III)–ligand interaction, bearing in mind that the differences in the spectra, *i.e.*, the intensities and shifts of the positions of λ_{max} and A depend on the nature of the ligands, the characteristics of molecular orbitals, changes in bond energies and the electronic configuration of the complex in relation to the individual types of ligands and metal ions, the stability constants of complexes in the circumstances, etc.

Table 1. Comparative overview of the differences in the numerical quantificators of UV/Vis spectra between the monocomponent system (ligand solution) and the binary system (ligand-Cr(III) solution)

Ligand	$\Delta P/\%$	r	$S_{\Delta A}$	$S^2_{\Delta A}$	Range λ / nm
2,2'-Bipyridine	−6.342%	0.97937	18.187	1.64790	190 – 330
Acetamiprid	11.94%	0.99581	3.7523	0.08411	190 – 285
Atrazine	3.471%	0.99836	1.4191	0.03912	190 – 295

Generally, larger $\Delta P\%$, lower r, higher $S_{\Delta A}$ and larger $S^2_{\Delta A}$ reveal the significant interaction between the ligand and chromium(III), to the extent to which the physico-chemical nature of the process observed in the binary system can be reflected in the UV/Vis absorption characteristics.

Overall, UV/Vis spectral characterization in terms of the observation of the interaction of different systems can have only qualitative or semi-quantitative character because, despite the statistical analysis of systems, it could not provide the correct conclusions about the strength of the interaction of chromium(III) and the observed series of ligands.

Even if the difference in the spectrum of the ligand and analog spectrum of Cr(III)–ligand is small or negligible, a coordinating interaction cannot be excluded with certainty because the formed UV/Vis absorption systems, with their own new electronic configuration may be similar in absorption of the unbound ligand, and therefore the differences in UV/Vis spectrometric analysis are difficult to distinguish.

Based on the previous discussion, it could be stated that UV/Vis spectroscopy could be employed for a fast, easy to perform and preliminary study of interactions due to the simplicity of the preparation of solutions, a low-cost instrument and the easiness of the interpretation of the experimental results.

ESI-MS analysis of system Cr(III) – ligand system

A preliminary ESI-MS investigation of ligand solutions by the flow injection analysis showed that the selected polarity ESI source coincides with ionization characteristics of molecules, which are expected based on their structure. Thus, ligands with *N*-donor atoms with a tendency to be protonated were examined in the ESI positive polarity mode.

Quantification was performed by calculating the area of ESI-MS ion current chromatograms of loop injection for the appropriate mass range (*m/z* molecular ion ±1.0) obtained by analyzing series of mono-component (ligand without chromium) and two-component systems (ligand with chromium). The recorded MS spectra of mono- and two-component systems in the case of atrazine–Cr(III) are shown in the Fig. 2. The areas of ESI-MS selected mass range ion current chromatograms of the loop injection that are considered in the calculation are shown in Fig. 2. The same procedure was repeated for the other

two ligands: acetamiprid and 2,2'-bipyridine, the spectra of which are not presented in the paper.

The values of ion current chromatogram peak areas are plotted vs. ligand concentrations, and a two-variable correlation was described by linear fitting (Fig. 3).

The quantification is based on the fact that all areas of the chromatograms are in direct function of the peak intensities obtained by observing the signal of ions in the required mass range m/z. In binary systems where there is an interaction with the ligand and chromium(III), the intensity of the observed characteristic ion is reduced, because the coordination bonding or other interactions of chromium(III) and ligand change the molecular and ionic composition of the sample. The creation of new ionic species with chromium also results in a decrease of the concentration of monitored ligand ions because of the mass balance preservation. This leads to the reduction in the number of ions generated in a unit of time, in the case of the analysis of binary systems with sufficient interactions. Simultaneously, by a similar but not quite identical dependence, there is a reduction in the concentrations of other ionic species that have a ligand molecule incorporated as a component (adducts and/or dimers), since all the species present are in dynamic equilibrium in the injected solution and are desolvated in the ESI source.
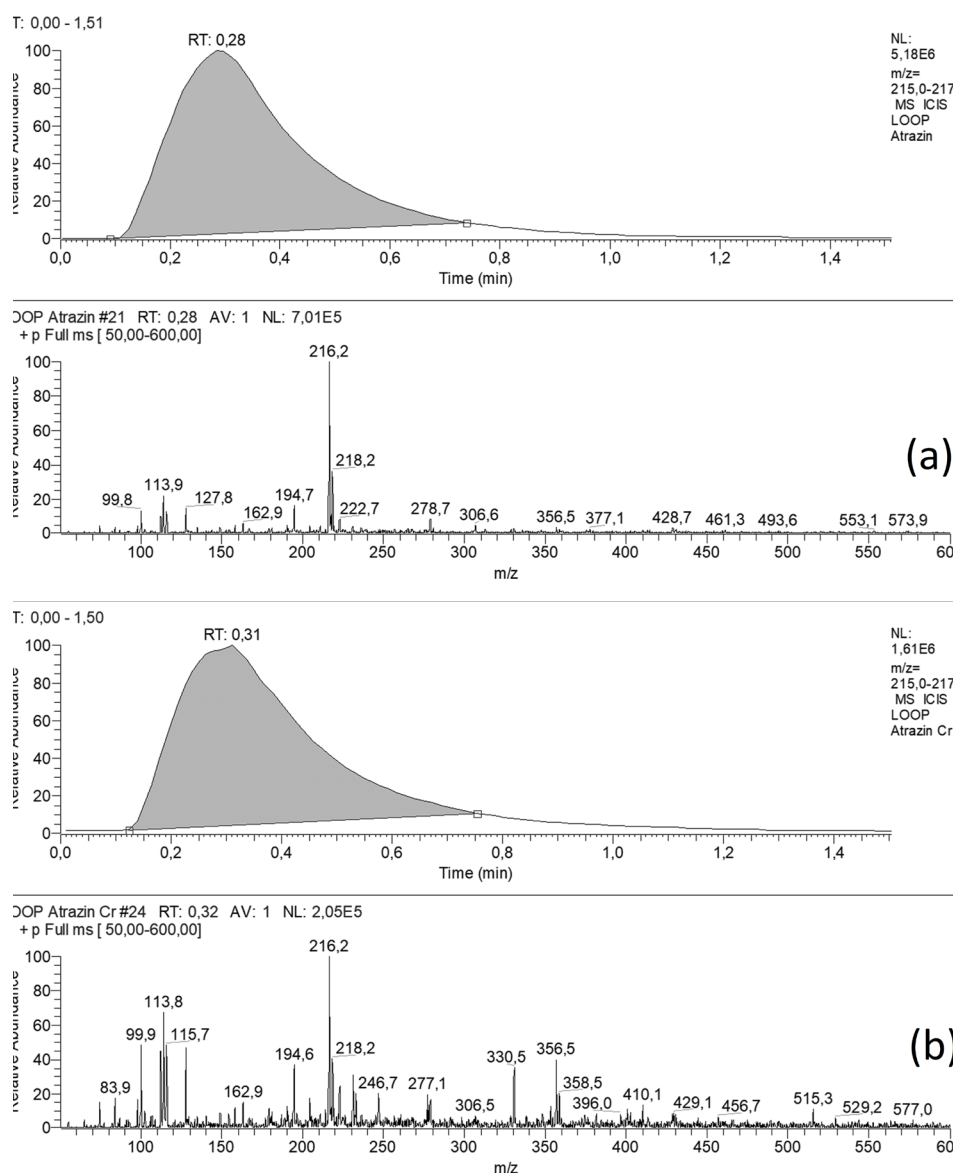

Figure 2. a) ESI-MS ion current chromatogram for mass range m/z 215 – 217 of the loop injection of ligand without chromium: atrazine, c = 5.00 µmol L^{-1}; b) ESI-MS ion current chromatogram for mass range m/z 215 – 217 of the loop injection of ligand with chromium: atrazine, c = 5.00 µmol L^{-1} and chromium, c = 9.0 µmol L^{-1}. Solvent CH_3OH/H_2O = 50/50, positive ionization mode, flow rate = 100 µL min^{-1}
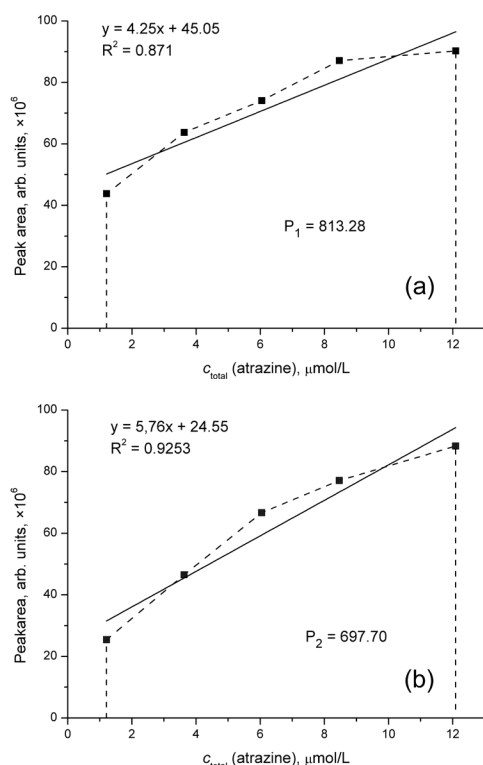
Figure 3. (a) Plot of the peak areas of ESI-MS ion current chromatograms of the loop injection of atrazine (without chromium) for the mass range m/z 215 – 217 vs. ligand concentrations; (b) plot of the peak areas of ESI-MS ion current chromatograms of the loop injection of the atrazine–chromium system (with chromium) for the mass range m/z 215 – 217 vs. ligand concentrations. Solvent CH_3OH/H_2O = 50/50, positive ionization mode.

This proportion is eventually reflected in the lower signal intensity of the selected ions by the MS detector, because the ESI source is sensitive to the concentration and not to the total amount of injected analyte. In this way, in binary systems of ligands with chromium(III), for the identical ligand concentration, a proportionally smaller area of the chromatographic peak is obtained, and a relative decrease in the area can be related to the strength of the interaction of chromium and the observed ligand because of the decrease in the monitoring ion concentration in the inlet capillary of the ESI source.

It should be noted that, for a variety of fundamental and methodological reasons, the changes in the area of chromatogram peaks of the loop injection can be treated as valid only in a relative comparison, in the analysis of the system of ligand–Cr(III) with an identical ESI source, ion optic and MS analyzer parameters and the same conditions of pH, ionic strength, solvent, etc. The comparison of the absolute values of the area of chromatogram peaks between different compounds (ligands) is not valid without a special study and knowledge of the complete stoichiometry of the investigated solutions, the equilibrium relationship between species, the evaluation of their reactivity and stability in desolvation and ionization processes, determination of specific response factors, etc.

The values $\Delta P_{(1-2)\%}$ and $\Delta P_{ESI\%}$ calculated according to Eqs. (4) and (5) for the series of ligands are presented in Table 2. In the group of studied *N*-donor ligands, the strength of ligand–Cr(III) interaction follows the decreasing order: 2,2'-bipyridine > acetamprid > atrazine.

Table 2. Values of $\Delta P_{(1-2)}$ and ΔP_{ESI} obtained by ESI-MS investigation

Ligand	m/z [M+H]$^+$	$\Delta P_{(1-2)}$ / %	ΔP_{ESI} / %
2,2'-Bipyridine	157	67.44%	60.95%
Acetamprid	223	31.92%	31.88%
Atrazine	216	16.55%	17.11%

Among the studied N-donor ligands, the strongest interaction of Cr(III) is with 2,2'-bipyridine, and it is quantitatively expressed with value of $\Delta P_{ESI\%}$ = 60.95%. Bidentate and chelate effects of its *cis*-conformation are well related with the observed strong interaction and the formation of series of Cr(L-L)$_3$ complexes, from [Cr(bipy)$_3$]$^{3+}$ to [Cr(bipy)$_3$]$^{3-}$ (Fig. 4a), as well as Cr(L-L)$_2$ and Cr(L-L), where central Cr-ion may be coordinated with other species in the investigated solution (OH–, CH_3OH, H_2O). According to *Pearson's principle* of Hard and Soft Acids and Bases (HSAB), Cr(III) ion – with its radius/charge ratio – belongs to the group of hard acids, like other ions of the first series of d-elements in the higher oxidation state. On the other hand, nitrogen with high *absolute hardness* (η) value is pronounced hard base, just slightly weaker than oxygen is. These characteristics favor strong coordination binding, again in accordance with high $\Delta P_{ESI\%}$ value.

Acetamiprid, with remarkable Brønsted–Lowry acidity (pK_a = 0,7 due to –*I* effect of –CN group), with $\Delta P_{ESI\%}$ = 31.88% shows a notable but weaker interaction with Cr(III) then 2,2'-bipyridine, despite the presence of several potential binding locations (4×N, 1×Cl, 3×C). However, the most perspective position with nitrogen in –CN group cannot donate electrons simultaneously with neighboring groups due to the distance and steric hindrances. This implies that acetamiprid cannot act as a chelate agent and the formation of bi- or poly-dentate complexes is not probable. The basic character of pyridine N-atom is decreased by strong –*I* effect of Cl-atom, thus partly losing its electron donor ability (Fig. 4b). Some C-atoms with the partial negative charge (δ$^-$) are not potent coordination centers.

Atrazine evidenced the lowest value $\Delta P_{ESI\%}$ = 17.11% despite the presence of five N-atoms, again due to its disability to make bidentate complexes with metal. Triazine ring cannot coordinate Cr(III) with more than one N-atom at the time, while composing of 4-atom ring via N-8 and N-3 with Cr-ion is not likely due to the steric repulsion (Fig. 4b). In addition, negative charge at N-3 atom is decreased due to –*I* influence of two other triazine N-atoms and Cl-atom. The coordination of metal most likely occurs via N-8 with greater electronic density (+R effect from two –CH$_3$ groups, instead of one –CH$_2$– on N-13) (Fig. 4c).
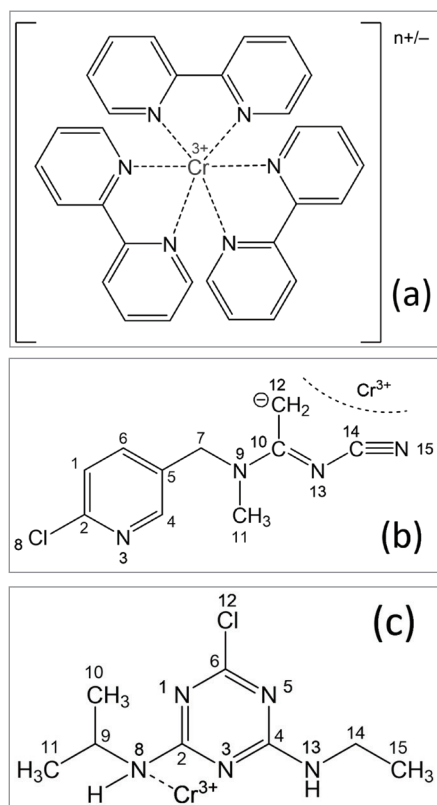
Figure 4. Possible structure and binding sites of complexes of chromium(III) with (a) 2,2'-bipyridine (b) acetamiprid and (c) atrazine ligands.

Comparing a series of the studied systems of chromium(III) with ligands, the values of $\Delta P_{ESI\%}$ are in good correlation with the acid–base and electron-donor properties, geometric and steric characteristics and molecular structures of the investigated compounds.

The general evaluation of the application of the ESI-MS technique for the qualitative and quantitative analysis of metal-ligand systems, based on the presented results, is that it can be considered that idealized descriptions and theoretical considerations and advantages of ESI-MS techniques do not often match with the real conditions, which may affect the evaluation of the results obtained by the ESI-MS technique for defining metal–ligand interactions. During the ESI soft ionization process, the composition of the solution can be changed in relation to the initial equilibrium conditions: a) volume changes due to spray evaporation, which increases the concentration of species and the modification of ionic strength of the solution, b) there is a variation of the temperature in the droplet spray due to the high temperature that exists in the ESI source and the constant evaporation from the droplet surface, c) changing the pH, as influenced by changes in the temperature and the concentration in the spray and as a result of redox reactions in the solution in the capillary ion source, especially in systems with redox-active ligands and metal ions, while redox reactions may also occur in other parts of the ES chamber,

not only in the capillaries.

In this study, it was confirmed that solvent molecules can be added to (or taken away from) the central metal ion or ligand during the process of ESI soft ionization. Ion adducts that do not normally occur in the solution can be formed in the process of ionization, as observed in the present study for acetamprid and atrazine systems. The formation of pseudo molecular or adduct ions (e.g., with Na^+) is very common in ESI-MS analyses, but here the analysis is not compromised with these effects since the metal-ligand equilibrium was investigated via monitor-ion only, not through the concentration of all ligand species. For quantification purposes, it is important to examine whether the ESI-MS can provide a proper determination of concentrations of the species in the solution. For this application, it is essential to know the response factors of the individual ions. However, different approaches proposed for their calculations are still not reliable enough and, in most papers, equivalence of response factors is assumed, which is a relatively debatable assumption except in the cases of similar types of the identical charge (e.g., *host-guest* systems). A general problem that different ions may show different response factors did not affect this investigation, because the same monitor-ion was selected both in Cr(III)-ligand binary system and a ligand only system.

Despite various interfering factors, the ESI-MS technique was widely used in the studies of the metal–ligand system, as evidenced by literature references. ESI-MS was applied not only to the speciation of particular elements [25] which can be defined by phenomenon of coordination interactions, but also in the domain of studying the non-covalent interactions between biomacromolecules [26,27].

In terms of qualitative results, this comparison often gives good matches. Thus, it would seem that in most cases the equilibrium misbalance and gas-phase reactions do not occur to a significant extent or, if they occur at all, then they do not modify the stoichiometry of the species present in the initial solution, but change only their quantitative relations. Equilibrium misbalance can only occur if the metal–ligand system is kinetically unstable in the time range of the ESI process, *i.e.*, about 0.01 sec which is enough time for most analyses. Therefore, the ESI-MS may be considered as a relevant and reliable technique for the qualitative analysis of the metal–ligand system.

In summary, so far the ESI-MS cannot be considered as a fully reliable application for quantitative determination of the metal–ligand system, but due to some unique features, in combination with other techniques, ESI-MS is still a valuable source of data for better understanding of the equilibrium of metal–ligand systems. In this paper, the quantification by the employed techniques, UV/Vis spectroscopy and ESI-MS showed a large difference in the usefulness and amount of information based on experimental results, in favor of the ESI-MS technique, which is related to the fundamental limitations of the UV/Vis method.

Conclusion

Chromium(III) interacted notably with all three investigated N-donor ligands. The intensity of the interaction of chromium with investigated compounds followed the order: 2,2'-bipyridene > acetamiprid > atrazine, which correlates with the acid–base and electron-donor properties, geometric and steric characteristics, and the number and position of *N*-donor atoms within the molecular structure.

The study of the possibility of chromium interaction with pesticides and toxic substances in model systems is a good method which may contribute to defining mutual interactions among toxic inorganic and organic agents in the natural environment. UV/Vis spectroscopy and ESI-MS quantification showed a large difference in their ability to define the interaction of chromium(III)–ligand, the ESI-MS technique being superior. The ESI-MS technique with loop injection can be used for the quantitative analysis of the system Cr(III)–ligand. The ESI-MS ion current chromatograms of loop injection indicated a stabile peak and signal integrity for both the total ion current and the range of *m/z* values.

Acknowledgement

References

[1] John F. Papp, "Mineral Yearbook 2015: Chromium". United States Geological Survey. Retrieved 2015-06-03.

[2] Food and Agriculture Organization of the United Nations (FAO), FAOSTAT, 2012 (http://www.fao.org/faostat/en/#home).

[3] U.S. Environmental Protection Agency (U.S. EPA), "Pesticides Industry – Sales and Usage" (Washington, DC, 2011).

[4] R. Rial Otero, B. Cancho Grande, M. Arias Estevez, E. Lopez Periago, J. Simal Gandara, Procedure for the measurement of soil inputs of plant-protection agents washed off through vineyard canopy by rainfall, Journal of Agricultural and Food Chemistry, 51 (17) (2003) 5041-5046.

[5] H. M. G. van der Werf, Assessing the impact of pesticides on the environment, Agriculture, Ecosystems and Environment, 60 (2-3) (1996) 81-96.

[6] G. W. Van Loonand, S. J. Duffy, Environmental Chemistry: A Global Perspective, Oxford University Press, Oxford, UK, 2nd edition, 2005.

[7] P. K. Goel, Water Pollution (Causes, Effects and Control), New Age International (P), New Delhi, India, 2nd edition, 2006.

[8] M. Arias-Estevez, E. Lopez-Periago, E. Martinez-Carballo, J. Simal-Gandara, J.-C. Mejuto, L. Garcia-Rio, The mobility and degradation of pesticides in soils and the pollution of groundwater resources, Agriculture, Ecosystems & Environment, 123 (4) (2008) 247-260.

[9] H. E. Allen, C. P. Huang, G. W. Bailey, A. R. Bowers, Metal Speciation and Contamination of Soil, Lewis Publishers, Ann Arbor, Michigan, 1995, p. 7

[10] M. Linde, Trace Metals in Urban Soils – Stockholm as a Case Study, PhD thesis, Swedish University of Agricultural Sciences, Uppsala, 2005

[11] P. J. Shea, T. A. Machacek, S. D. Comfort, Accelerated remediation of pesticide-contaminated soil with zerovalent iron, Environmental Pollution, 132 (2) (2004) 183-188.

[12] T. R. Sandrin, R. M. Maier, Impact of metals on the biodegradation of organic pollutants, Environmental Health Perspectives, 111 (8) (2003) 1093-1101.

[13] Y. P. Wang, J. Y. Shi, H. Wang, Q. Lin, X. C. Chen, X. Chen, The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter, Ecotoxicology & Environmental Safety, 67 (1) (2007) 75-81.

[14] A. A. Helal, D. M. Imam, S. M. Khalifa, H. F. Aly, Interaction of pesticides with humic compounds and their metal complexes, Radiochemistry, 48 (4) (2006) 419-425.

[15] M. Kamiya and K. Kameyama, Effects of selected metal ions on photodegradation of organophosphorus pesticides sensitized by humic acids, Chemosphere, 45 (3) (2001) 231-235.

[16] E. Quivet, R. Faure, J. Georges, J.-O. Paisse, P. Lanteri, Influence of metal salts on the photodegradation of imazapyr, an imidazolinone pesticide, Pest Management Science, 62 (5) (2006) 407-413.

[17] T. Andjelkovic, J. Perovic, M. Purenovic, S. Blagojevic, R. Nikolic, D. Andelkovic, A. Bojic, Spectroscopic and Potentiometric Studies on Derivatized Natural Humic Acid, Analytical Sciences, 22 (2006)1553-1558.

[18] T. D. Andjelkovic, J. M. Perovic, R. S. Nikolic, S. D. Blagojevic, D. H. Andelkovic, Binding of lead to humic acid related to its carboxyl and phenol groups contents, 23rd International Meeting on Organic Geochemistry – "IMOG" Torquay, United Kingdom, 2007, Book of Abstracts 775-776.

[19] T. Andjelkovic, R. Nikolic, A. Bojic, D. Andelkovic, G. Nikolic, Binding of cadmium to soil humic acid as a function of carboxyl group content, Macedonian Journal of Chemistry and Chemical Engineering, 29 (2) 215-224.

[20] B. P. Sullivan, D. J. Salmon, T. J. Meyer, Mixed phosphine 2,2'-bipyridine complexes of ruthenium, Inorganic Chemistry, 17 (12) (1978) 3334-3341.

[21] C. C. Scarborough, K. Wieghardt, Electronic structure of 2,2'-bipyridine organotransition-metal complexes. Establishing the ligand oxidation level by density functional theoretical calculations, Inorganic Chemistry, 50 (20) (2011) 9773-93.

[22] T. Yamamoto, T. Maruyama, Z.-H. Zhou, T. Ito, T. Fukuda, Y. Yoneda, F. Begum, T. Ikeda, S. Sasaki, Pi-Conjugated Poly(pyridine-2,5-diyl), Poly(2,2'-bipyridine-5,5'-diyl), and Their Alkyl Derivatives. Preparation, Linear Structure, Function as a Ligand to Form Their Transition Metal Complexes, Catalytic Reactions, n-Type Electrically Conducting Properties, Optical Properties, and Alignment on Substrates, Journal of the American Chemical Society, 116(11) (1994) 4832-4845.

[23] P. Kebarle, L. Tang, From ions in solution to ions in the gas phase, Analytical Chemistry 65(22) (1993) 972A-986A.

[24] W. Henderson, M. J. Taylor, An electrospray mass spectrometric investigation of gallium trihalide and indium trihalide solutions, Inorganica Chimica Acta, 277 (1) (1998) 26-30.

[25] I. I. Stewart, Electrospray mass spectrometry: a tool for elemental speciation, Review Article Spectrochimica Acta (Part B) Atomic Spectroscopy 54 (12) (1999) 1649-1695.

[26] J. L. Beck, M. L. Colgrave, S. F. Ralph, M. M. Sheil, Electrospray ionization mass spectrometry of oligonucleotide complexes with drugs, metals, and proteins, Mass Spectrometry Review 20 (2) (2001) 61-87

[27] A. J. R. Heck, R. H. H. Van den Heuvel, Investigation of intact protein complexes by mass spectrometry, Mass Spectrometry Review 23 (5) (2004) 368-389.

Izvod —————

ANALIZA INTERAKCIJE HROMA SA *N*-DONOR LIGANDIMA PUTEM ELEKTROSPREJ-JONIZACIONE MASENE SPEKTROMETRIJE

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Izvršena je analiza interakcije hroma(III) sa 2,2'-bipiridinom i dva pesticida, atrazinom i acetamipridom, kao *N*-donor ligandima, putem elektrosprej-jonizacione masene spektrometrije (ESI-MS) i UV/Vis spektrofotometrije. Atrazin i acetamiprid, pesticidi u širokoj upotrebi, su odabrani za ispitivanje zbog njihovog dokazanog toksičnog efekta u životnoj sredini, dok je 2,2'-bipiridin odabran za poređenje, usled svoje izražene sposobnosti da gradi komplekse sa metalima. Hrom(III) kao jon toksičnog teškog metala, formira brojna stabilna kompleksna jedinjenja. Heterogenost i kompleksnost prirodnih matriksa opravdavaju upotrebu model sistema kao ovde ispitivanih, u cilju evaluacije kompleksnih procesa u životnoj sredini. Intenzitet interakcija je doveden u korelaciju sa kiselo-baznim i elektron-donor osobinama, kao i brojem i pozicijom *N*-donor atoma u ispitivanim ligandima. Rezultati dobijeni UV/Vis spektrofotometrijom su upoređeni sa rezultatima dobijenih ESI-MS metodom. UV/Vis spektrofotometrija i ESI-MS kvantifikacija su pokazale značajne razlike po informacijama koje pružaju u opisivanju interakcija hroma(III) sa ligandima, pri čemu se ESI-MS metoda pokazala superiornom. ESI-MS tehnika može biti uzeta za kvantitativne analize sistema Cr(III)–ligand. ESI-MS hromatogrami jonske struje pri radu sa 20 µL *loop* injektiranjem sistema Cr(III)-ligand pokazuju stabilne pikove i integritet signala.

Ključne reči: hrom; pesticidi; ligandi; elektrosprej-jonizaciona masena spektrometrija