Journal of the Serbian Chemical Society

JSCS-info@shd.org.rs • www.shd.org.rs/JSCS

J. Serb. Chem. Soc. 78 (1) 137–154 (2012)
JSCS–4403

UDC 546.763+539.196+547.992:
543.422.3:543.51
Original scientific paper

A study of chromium interaction with *O*-donor humic-like ligands using electrospray-ionization mass spectrometry

DARKO H. ANĐELKOVIĆ[1]*, RUŽICA S. NIKOLIĆ[1], DEJAN Z. MARKOVIĆ[2],
TATJANA D. ANĐELKOVIĆ[1], GORDANA M. KOCIĆ[3], ZORAN B. TODOROVIĆ[2]
and ALEKSANDAR LJ. BOJIĆ[1#]

[1]*Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia,*
[2]*Faculty of Technology, University of Niš, Bulevar oslobođenja 124, 16000 Leskovac, Serbia*
and [3]*Faculty of Medicine, University of Niš, Bulevar dr Zorana Đinđića 81, 18000 Niš, Serbia*

(Received 20 March, revised 3 July 2012)

Abstract: A study of the interaction of chromium with *O*-donor humic-like ligands war performed using electrospray-ionization mass spectrometry (ESI-MS) and ultraviolet/visible (UV/Vis) spectrophotometry. The heterogeneity of the functional groups justifies the use of model compounds of humic substances. For studying the interaction of chromium with humic substances, benzoic, salicylic, phthalic and citric acid, which include *O*-donor atoms also present in heterogeneous and polydispersed ligands, such as humic and fulvic acids, were used as model substances. 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 the *O*-donor atoms in the investigated ligands. UV/Vis data describing chromium interaction with humic-like ligands was placed in correlation with ESI-MS data of the complexes, both in quantitative and in qualitative terms. UV/Vis spectroscopy and ESI-MS quantification showed a large difference in the information they yield in describing the interaction of chromium(III) with the ligand, the ESI-MS technique being more informative. 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.

Keywords: chromium; interaction; humic; ligand; electrospray-ionization mass spectrometry.

INTRODUCTION

The presence of heavy metals in nature has a negative impact on human health and the environment. Their state, form, mobility, sorption and bioavail-

---

* Corresponding author. E-mail: darko.andjel@gmail.com
[#] Serbian Chemical Society member.
doi: 10.2298/JSC120320071A

ability depend on the physical and chemical environmental conditions in which they are located, and on the nature of all chemical species, present in that environment, with which they can interact. Thus, the migration of heavy metals from soil into groundwater is strongly dependent on the terrestrial conditions, such as physical and chemical characteristics of soil, clay mineral content and the presence of natural organic matter - humic substances.[1,2]

Humic substances are complex organic molecules formed by the decomposition of plant, animal and/or microbial material. They are ubiquitous and persistent in the biosphere, playing an important role in the mobilization of metal ions in soil and aquatic environments, and influencing the bioavailability and toxicity of these ions. By creating a water-soluble complex between HS and metal ions, an increase in the concentration of ions in the groundwater and other natural waters, far above their solubility, could be expected. In addition, the possibility that complexation of humic substances in solid phase could mobilize metals present in landfills, which leads to their penetration into the biosphere,[3–6] should be taken into consideration.

Chromium is an essential, yet toxic trace element depending on its valence state and the nature of the ligand to which it is bound. Chromium in the environment can be found in two oxidation states +3 and +6, which have different chemical, physicochemical and biochemical reactivity. Cr(VI) chemical species are more soluble, mobile and bioactive than Cr(III) chemical species.[7,8]

In natural environments, such as soil, chromium primarily interacts with soil organic matter – humic substances.[9–11] The heterogeneity of functional groups and their versatility justifies the use of model compounds of humic substances. “Hard” binding sites are the most common in the humic structure, with mainly oxygen in carboxylic and phenolic binding sites as the donor atom.[12–14] As model substances for studying the interaction of chromium with humic substances, benzoic, salicylic, phthalic and citric acid, which include all *O*-donor atoms of heterogeneous and polydispersed ligands, such humic and fulvic acids, were used. Therefore, an investigation of the interactions of chromium with each individual *O*-donor atom ligand, with a further investigation of the complex mixture of *O*--donor atoms in humic acid, present a good methodology for defining the interactions of metals with polyfunctional, polydispersed and polyelectrolytic ligands, such as humate macromolecules.[15–17]

Electrospray-ionization mass spectrometry, ESI-MS is used for the detection and characterization of one or more ionic species in solution, and has been confirmed as very useful in studies of various complex systems.[18] In the case of metal–ligand coordination interactions, it is often used for verification of the stoichiometry of a newly synthesized complex,[19] that do not participate in the liquid phase equilibrium, and after decomposition do not produce other molecular species (if it is still the case, the created equilibrium is usually ignored).

Studies of solution equilibrium, simultaneous detection, identification and quantification of numerous species, the distribution of which depends on pH, the stoichiometric concentration, *etc*. are less routine, although very interesting applications of ESI-MS.

Another convenient feature of ESI-MS is its low detection limit; thus, concentrations down to 10^{-6} mol L^{-1} can be detected.[20–22]

This paper deals with a study of the interaction of chromium(III) with *O*-donor humic-like ligands by ESI-MS and UV/Vis spectrophotometry. Application of ESI mass spectrometry is a novelty in the study of the interactions of chromium and model humic ligands.

UV/Vis spectrophotometric characterization of the chromium complexes with *O*-donor humic-like 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 processes that chromium ions in interaction with humic substances in the system soil/water can undergo. This research contributes to complex processes of chromium translocation in the groundwater systems, *i.e.*, leaching from the soil, after which the chromium could be further transported and dispersed in the biotic and abiotic systems of the natural environment.

EXPERIMENTAL

Chemical reagents, solutions, instrumentations

All reagents used were *p.a.* or HPLC grade purity. Salicylic, benzoic, phthalic and citric acid were purchased from 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 were 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 μS cm^{-1}) to remove trace metals and other contaminants.

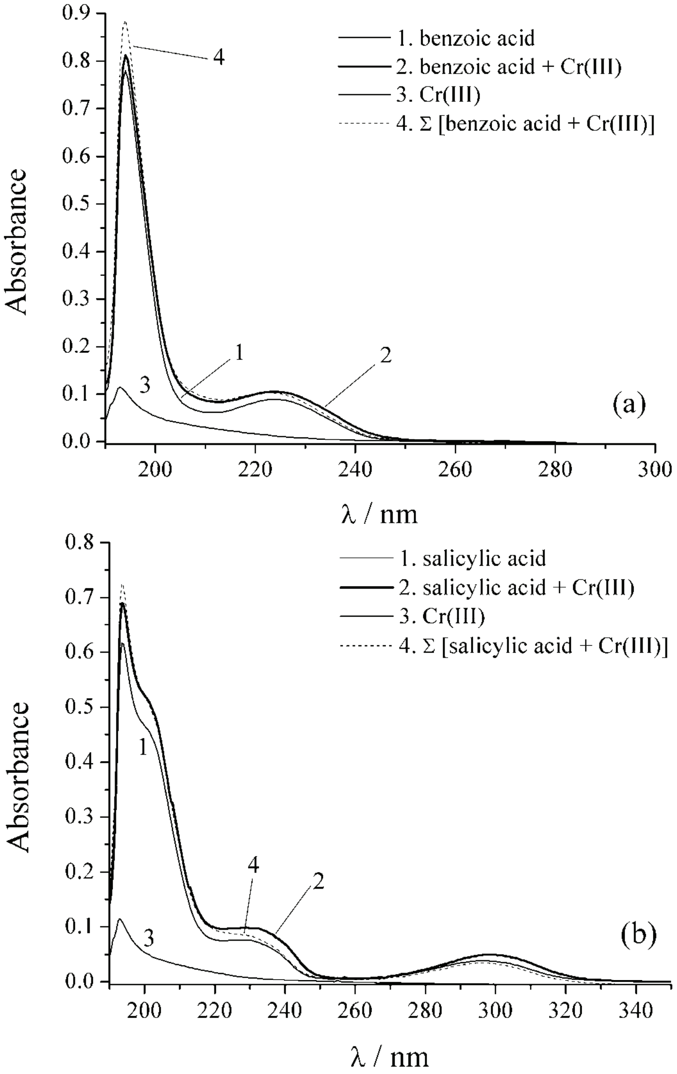
Standard stock solutions of the ligands were prepared by weighing the respective acid with an accuracy of ±0.0001g and dissolving in methanol. The solutions were kept in a refrigerator at 4 °C. Working solutions were made by dilution of the standard solutions in a methanol/water mixture (50/50, v/v). Working solutions of the ligand–chromium mixtures were prepared in polyethylene vials of 5, 2 and 1.5 cm^3 (Eppendorf, Germany) previously washed with 0.1 mol L^{-1} HCl, deionized water and methanol to remove any adsorbed surface impurities and reduce the 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 investigation was performed on a LCQ Deca Ion Trap Mass Spectrometer (Thermo Finnigan, USA) with auxiliary equipment.

Experimental procedure for the UV/Vis characterisation of the Cr–ligand interactions

A mixture of methanol and deionized water (< 0.1 μS cm^{-1}) in ratio 50/50 (v/v) was used as the blank. 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 solution, considering the relatively low concentration range of the components. In the 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.

For the obtained spectral curves (Fig. 1), the areas of the surface integrals for all spectral curves 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



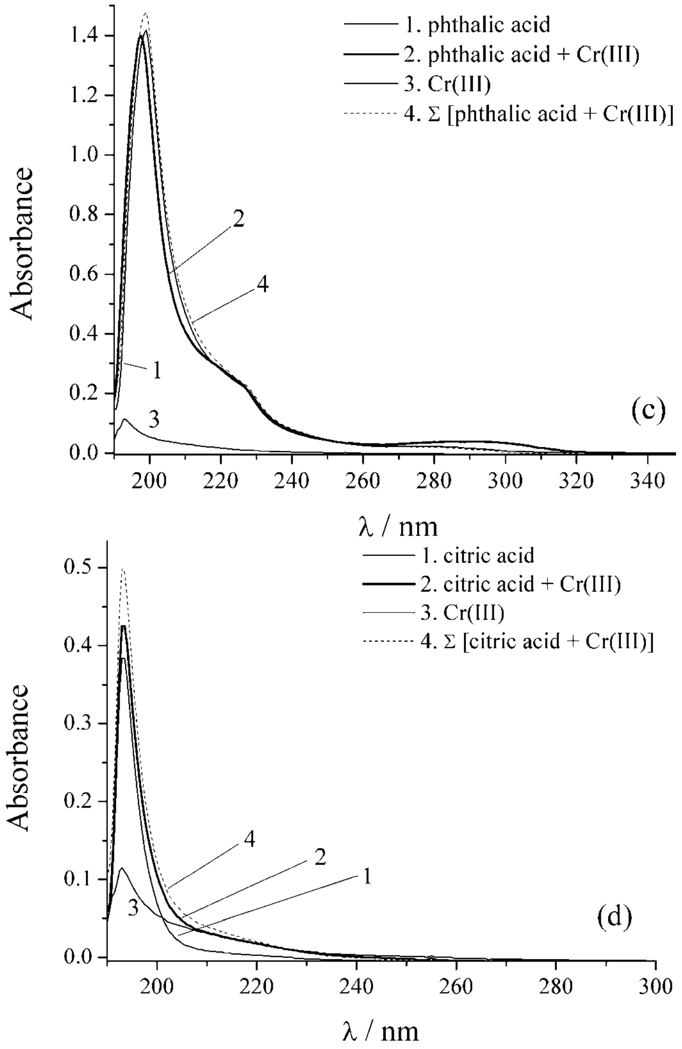


Fig. 1. UV/Vis Spectra of the ligands: benzoic (a), salicylic (b), phthalic (c) and citric acid (d). 1 – ligand solution, 2 – ligand + Cr(III) solution, 3 – Cr(III) solution and 4 – mathematical sum of ligand spectrum and chromium spectrum. *l* = 1 cm, solvent: CH_3OH/H_2O = 50/50.

the area of the arithmetical summation of the two spectra of the monocomponent systems, *i.e.*, the solution of ligand and the solution of Cr(III) (P_4), according to Eq. (1):

$$\Delta P_{\%} = \frac{P_2 - P_4}{P_2} \times 100 \tag{1}$$

The correlation coefficient (r) was calculated for range of wavelengths in which the absorption occurred, from $\lambda_{\text{min}} = 190$ nm to $\lambda_{\text{max}} = 245 – 330$ nm, depending on the ligand.

The sum of absolute values of absorbance differences for every binary ligand–chromium system was calculated for the wavelength (λ_{i}), according to Eq. (2):

$$S_{\Delta A} = \sum_{\lambda\text{min}}^{\lambda\text{min}} \text{abs}\left(x_i - y_i\right) \tag{2}$$

where the wavelength range was 190 to 245–330 nm, in which the absorption was 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 the absorbance differences ($S_{\Delta A}^2$) for the specific wavelength (λ_{i}), between two series of spectral curves data, was calculated according Eq. (3):

$$S_{\Delta A}^2 = \sum_{\lambda\text{min}}^{\lambda\text{max}} \left(x_i - y_i\right)^2 \tag{3}$$

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

Monocomponent solutions of ligands were made in pre-washed polyethylene Eppendorf tubes 1.5–5 cm^3, 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 the cold (4 °C) and dark. 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 min after mixing of the components, in order 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 were not older than a few hours, in order to avoid side-reaction products. Considering the chemical nature of all the 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 an extent that could jeopardize the relevance of the results.

By using the ICIS® module of Xcalibur™ 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).

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.*, the difference of areas $\Delta P_{(1–2)\%}$ was performed following Eq. (4):

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

The areas are presented in absolute non-dimensional (arbitrary) units of the Xcalibur™ software (count–second units) that have no physical meaning.

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

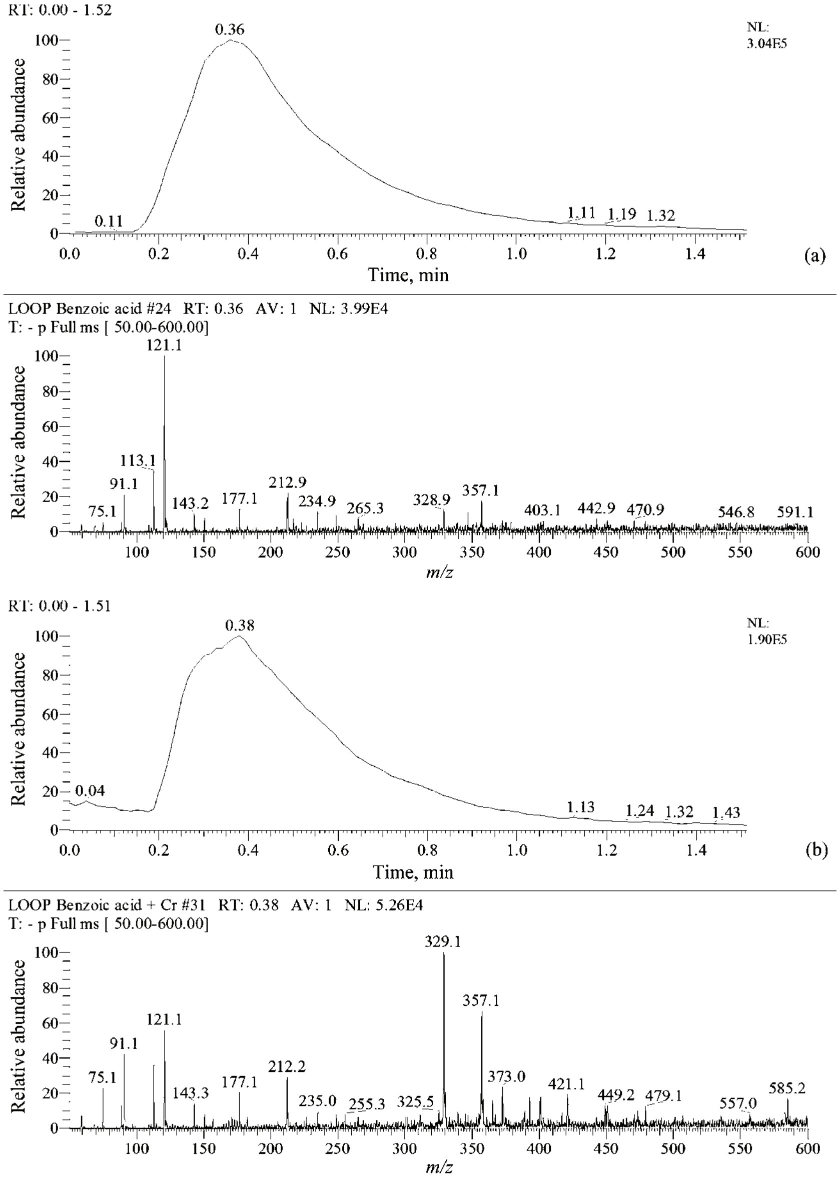


Fig. 2. a) ESI-MS Ion current chromatogram for mass range *m/z* 120–122 of the loop injection of ligand without chromium: benzoic acid, c = 7.00 μmol L^{-1}; b) ESI-MS ion current chromatogram for mass range *m/z* 120–122 of the loop injection of ligand with chromium: benzoic acid, c = 1.00 μmol L^{-1} and chromium, c = 9.0 μmol L^{-1}. Solvent: CH_3OH/H_2O = 50/50, negative ionization mode, flow rate = 100 μL min^{-1}.

$$\Delta P_{\mathrm{ESI}\%} = \Delta P_{(1-2)\%} \frac{c_{\max}\left(\mathrm{ligand}\right)_m}{(1/n)\sum_{i=1}^{n}\left[c_{\max}\left(\mathrm{ligand}\right)_i\right]}\,\%,\quad \frac{1}{n}\sum_{i=1}^{n}\left[c_{\max}\left(\mathrm{ligand}\right)_i\right] = 11.696\,\mu\mathrm{mol\ L}^{-1} \quad (5)$$

where $c_{\max}(\mathrm{ligand})_{\mathrm{m}}$ is maximum ligand concentration (μmol L^{-1}) in the ligand–chromium system, while the denominator presents the average maximal concentration of the investigated ligands, *i.e.*, 11.696 μmol L^{-1}.

The obtained $\Delta P_{\mathrm{ESI}\%}$ values were used for comparison of the interactions between chromium(III) and the ligands. A higher $\Delta P_{\mathrm{ESI}\%}$ value confirms stronger interaction in the investigated binary system.

RESULTS AND DISCUSSION

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

The results of UV/Vis spectrophotometric studies of chromium(III) interaction with benzoic, salicylic, phthalic and citric acids are shown in Fig. 1. The obtained UV/Vis spectra of the investigated ligands correspond well with literature data.[23]

Spectra of benzoic acid and binary system chromium(III)–benzoic acid (Fig. 1a) have very similar absorption profiles, with a small positive ($\lambda > 225$ nm) or negative difference (λ, 204–225 and 193–202 nm) between spectrum of the binary system and mathematical sum of the spectra of the separate components. Neither shifts of the absorption maximum and minimum nor do significant absorbance (A) changes exist, which indicates small coordination or interaction between the ligand and metal ion, or a similarity in the electronic configuration of the free and bound ligand, in terms of orbital energy and the probability of electronic transitions.

In contrast to benzoic acid, salicylic acid showed difference in spectra of free salicylic acid and binary system of chromium(III)–salicylic acid, expressed in bands, 220–250 nm and 275–325 nm, in terms of increasing the absorption (A) (Fig. 1b). A bathochromic shift of $\Delta\lambda = 2$ nm could be seen in the spectrum of complexed salicylic acid at $\lambda = 298$ nm, while the other bands did not change their positions.

The binary system Cr(III)–phthalic acid showed suppression of the absorbance, except at 266–317 nm, where the absorbance increased with the appearance of a poorly defined peak at $\lambda_{\max} = 293$ nm (Fig. 1c). The most prominent peak below 200 nm is, in comparison to the other investigated ligands, bathochromically shifted by approximately $\Delta\lambda = 6$ nm, and also the molar absorption coefficient (ε) shows the highest value compared to the other ligands.

The spectra of citric acid and citric acid complexed with chromium(III) showed a similarity of the spectral curves, as well as the lack of a bathochromic effect; only a pronounced peak was located at $\lambda_{\max} = 193$ nm (Fig. 1d). The slightly lower absorption intensity of the complex in solution compared to the mathematical sum of the spectra of the individual components (hypochromic effect) can be noted, indicating a lower probability of excitation of electrons in the resulting complex, while the absorption at $\lambda > 240$ nm decreased due to the absence of π-conjugated electrons and low energy excitation chromophores.

The differences in the spectra can indicate the existence of interaction and/or coordination in the binary systems 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 measurements. The hypothesis that the presence of Cr(III) could lead to the catalytic degradation of ligand or redox reaction with the ligand is unlikely because to the short preparation and maturation time of the system (about 30 min), relatively low redox potential (positive or negative) of the ligands and chromium(III), low concentrations and mild pH of the solutions. In addition, in the case of structural degradation of the ligand molecules in the presence of chromium(III) chloride, it would be expected that the spectra of the resulting products would be of significantly different in shape and intensity and would not show, for example, the hyperchromic effect while maintaining approximately the same shape of the spectrum.

The calculated parameters, the relative difference between the areas ($\Delta P_{\%}$), the linear correlation coefficient (r), the sum of the absolute values of the absorbance difference ($S_{\Delta A}$) and the sum of the squares of the absorbance difference ($S_{\Delta A}^2$), according Eqs. (1)–(4), are presented in Table I. These parameters can be considered only as qualitative or, at most, as semi-quantitative indicators of the chromium(III)–ligand interaction, bearing in mind the differences in the spectra, *i.e.*, the intensities and shifts of the positions of $\lambda_{\max}$ and A depend on the nature of the ligands, the characteristics of the molecular orbitals, changes in bond energies and 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 I. Comparative overview of the differences in the numerical quantifiers of UV/Vis spectra between the monocomponent system (ligand solution) and the binary system (ligand––Cr(III) solution)

Ligand	ΔP / %	r	$S_{\Delta A}$	$S_{\Delta A}^2$	Range λ / nm
Benzoic acid	3.629	0.99756	1.5490	0.06964	190–255
Salicylic acid	8.542	0.99808	2.9655	0.05298	190–330
Phthalic acid	–3.056	0.98782	9.0534	1.01692	190–320
Citric acid	–6.011	0.99790	1.3350	0.05400	190–245

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

Overall, the UV/Vis spectral characterization in terms of the observation of the interaction in the 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 the analog spectrum of Cr(III)–ligand is small or negligible, a coordination interaction can not be excluded with certainty because the formed UV/Vis absorption systems with their own new electronic configuration may be similar to the absorption of the unbound ligand and therefore differences in the 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 the solutions, a low-cost instrument and the easiness of the interpretation of the experimental results.

ESI-MS Analysis of the Cr(III)–ligand system

A preliminary ESI-MS investigation of ligand solutions by flow injection analysis showed that the selected polarity ESI source coincides with the ionization characteristics of the molecules, which are expected based on their structure. Thus, ligands with *O*-donor atoms with a tendency to deprotonize because of the presence of OH and COOH groups were examined in the ESI negative polarity mode.

Quantification was performed by calculating the area of ESI-MS ion current chromatograms of loop injections for an 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 the mono- and two-component systems in the case of benzoic acid––Cr(III) are shown in the Fig. 2. The areas of the 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 three ligands: salicylic, phthalic and citric acid, the spectra of which are not presented in the paper.

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

The quantification was 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 in which there is interaction of the ligand with chromium(III), the intensity of the observed characteris-

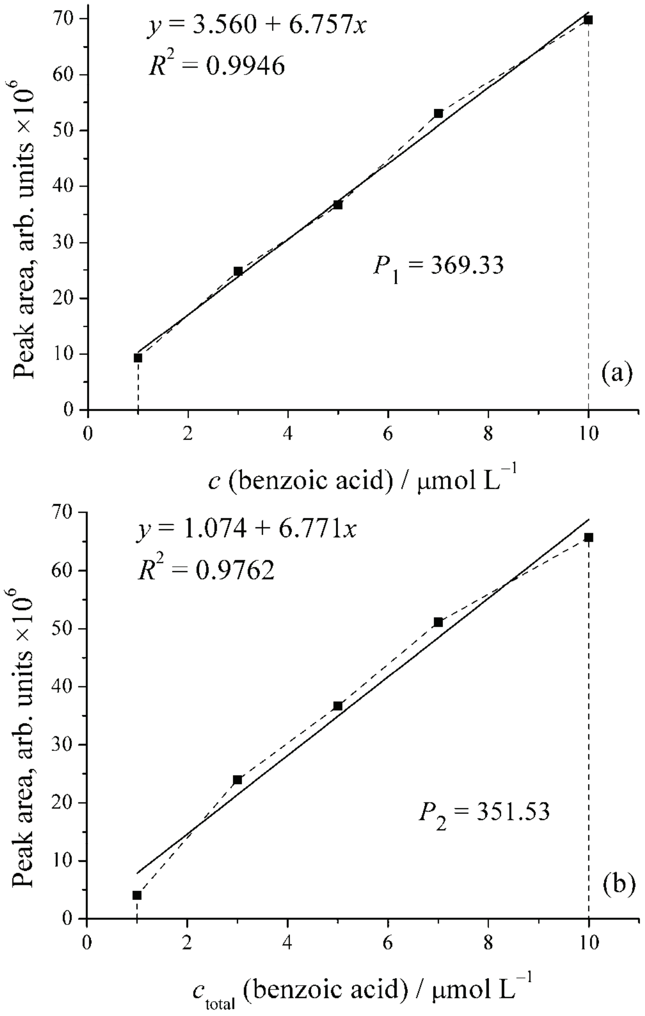


Fig. 3. a) Plot of the peak areas of the ESI-MS ion current chromatograms of the loop injection of benzoic acid (without chromium) for the mass range *m/z* 120–122 *vs*. ligand concentration; b) plot of the peak areas of the ESI-MS ion current chromatograms of the loop injection of the benzoic acid–chromium system (with chromium) for the mass range *m/z* 120––122 *vs*. the ligand. concentration. Solvent: CH_3OH/H_2O = 50/50, negative ionization mode.

tic 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 concentration of the monitored ligand ions because of mass balance preservation. This leads to a 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 by 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. This proportion is eventually reflected in lower signal intensities of the selected ions registered by the MS detector, because the ESI source is sensitive to concentration and not to the total amount of injected analyte. In this way, in binary systems of the ligands with chromium(III), for identical ligand concentrations, proportionally smaller area of the chromatographic peak are obtained, and the relative decrease in area can be related to the strength of the interaction of chromium and the observed ligand because of a 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, changes in area of chromatogram peaks of loop injections 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 optics, MS analyzer parameters and the same conditions of pH, ionic strength, solvent, *etc*. Comparison of the absolute values of the area of chromatogram peaks between the 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, evaluation of their reactivity and stability in the desolvation and ionization processes, determination of specific response factors, *etc*.

The values $\Delta P_{(1–2)\%}$ and $\Delta P_{\mathrm{ESI}\%}$, calculated according to Eqs. (4) and (5), for the series of ligands are presented in Table II. In the group of studied *O*-donor ligands, the strength of ligand–Cr(III) interaction follows the decreasing order: citric acid > phthalic acid > salicylic acid > benzoic acid.

TABLE II. Values of $\Delta P_{(1–2)\%}$ and $\Delta P_{\mathrm{ESI}\%}$ obtained by ESI-MS

Ligand	$\Delta P_{(1–2)}$ / %	ΔP_{ESI} / %
Benzoic acid	4.8	4.1
Salicylic acid	54.5	54.8
Phthalic acid	60.8	63.7
Citric acid	63.0	66.5

The minimum value of $\Delta P_{\mathrm{ESI}\%}$ of 4.1 % among the studied *O*-donor ligands, can be explained by the fact that ionized benzoic acid with one carboxyl group in the molecule has no ability to build a high stability complex with chromium(III). The first reason is that, compared to all the examined *O*-donor ligands, it is the weakest acid (pK_a = 4.21),[24] which means that in the methanol/water matrix it has the greatest tendency to bind H_3O^+ to the conjugate anion (benzoate), which possess ligand properties. As ligands act as bases, acids and metal ions in water-containing solutions, this may lead to competition between the protons and chromium(III) ions for the ligand. If the interaction between ligand and protons is strong, protonation of ligand occurs, leading to a decrease in the coordination interaction with chromium(III).

The other investigated *O*-donor ligands have, in comparison to benzoic acid, an order of magnitude larger acidity constants (citric acid, $pK_{a1} = 3.09$; phthalic acid, $pK_{a1} = 2.98$ and salicylic acid, $pK_{a1} = 2.97$)[23] allowing them stronger interactions with the chromium cations in the studied binary systems. These acidity constants are valid for aqueous solutions of these compounds, while in a mixture of methanol/water 50/50 (v/v), the corresponding constants are about 6–10 times smaller because of the lower basicity of methanol compared to water, although this does not interfere substantially in the above-described relative relations. Groups in the *ortho*-positions, such as –OH (salicylic acid), by its $+R$ effect, lower the acidity of the neighboring –COOH groups, but also by its strong $-I$ effect, lower the electronic density of the carboxyl groups, which favor deprotonation. In the case of citric, salicylic and phthalic acid anions, stabilization is enhanced by the presence of intramolecular hydrogen bonds.

In addition to the above-discussed acid–base characteristics, ligand dentate is another important factor in the formation of chromium complexes. Benzoic acid, or benzoate, can be considered as a bidentate ligand because of the presence of carboxylate anions. However, due to the slightly higher effective ionic radius of Cr(III) ions (61.5 pm), four-atom cyclic structures are not stabile and benzoic acid can be considered as a monodentate ligand. On the contrary, salicylate and phthalate ($HPht^-$, Pht^{2-}) are typical bidentate ligands with the possibility of creating five- or six-membered ring structures with Cr(III), which is more suitable for ligand field stabilization of the octahedral coordination sphere of chromium ions.

Salicylic acid with an OH group in the *ortho*-position possesses two binding sites with three *O*-donor atoms (ambidentate ligand), which significantly enhances its interaction with chromium compared to that of benzoate. It should be noted that further deprotonation of the OH groups in the salicylate is present to a very small extent ($pK_{a2} \approx 13.80$); hence, this structure can be excluded from further consideration. The deprotonized carboxyl group and the *ortho*-OH group of salicylate may, with low steric hindrance, form a stable bidentate complex with chromium, which is confirmed by the higher value of $\Delta P_{ESI\%} = 54.78$ %.

Phthalic acid with two COOH groups in the 1,2-position is subject to two-step deprotonation and can appear as a monodentate or bidentate ligand at the pH values of the investigated solutions, providing for complexes of different stoichiometric composition and type. The forms of the neutral ligand, phthalate and hydrogenphthalate (H_2Pht, Pht^{2-} and $HPht^-$) are more convenient for coordination of Cr(III) than salicylate, as evidenced by the higher value $\Delta P_{ESI\%} = 63.72$ %.

As a consequence of intermolecular hydrogen bonds, there is a tendency of dimerization of hydroxycarboxylic and dicarboxylic acids, especially in non-polar solvents, which therefore have the possibility of forming open or cyclic dimeric forms, (H_4L_2) in neutral or ionized form ($H_3L_2^-$, $H_2L_2^{2-}$, *etc*.). This variety of forms with electron-donor ability favors the formation of different interactions with the chromium in the observed binary systems, which has the consequence of significantly reducing the concentration of the monitored analyte ions in solution and the relatively high values of $\Delta P_{\mathrm{ESI\%}}$ for the *O*-donor ligands, except for benzoic acid.

Citric acid as a natural three-carbon acid with four acidic hydrogen atoms (H_4Cit) plays an important role in the transport processes of chromium(III) in soil systems and may represent a good model system for studying the properties of natural organic matter. Despite its aliphatic character, it possesses substantial acidity due to the strong –*I* effect, because of which the adjacent –COOH groups act on one another. The effect is strong enough to disable the partial dissociation of the carboxylic H-atom from the $-CH_2$ group. In addition to the polar effect, statistically there is greater probability that one of the three equivalent carboxyl groups will dissociate and give protons. However, the citrate forms, in contrast to other *O*-donors, and most of the other studied ligands, form typical chelate complexes, which further favor coordination with chromium. In addition, there is the possibility of forming a homoleptic complex series of the type $[\mathrm{CrCit_2}]^{n+/-}$, because the steric hindrances are relatively small and the flexible conformation of the σ-bonds allows favorable orientation for the formation of chromium coordination octahedron with oxygen. For these reasons, citric acid shows the highest value $\Delta P_{\mathrm{ESI\%}} = 66.46\%$ among the studied *O*-donors, indicating it has the strongest interaction with Cr(III) in the series of studied ligands.

Comparing a series of studied systems of chromium(III) with ligands, the values of $\Delta P_{\mathrm{ESI\%}}$ 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 ESI-MS technique for the quailtative and quantitative analysis of metal–ligand systems, based on the presented results, is that it can be considered that the idealized descriptions and theoretical considerations and advantages of ESI-MS techniques often do not match with the real conditions, which may affect the evaluation of the results obtained by 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) the volume changes due to spray evaporation, which increases the concentration of species and the modification of ionic strength of 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 temperature and 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 the other parts of the ES chamber, not only in the capillaries. The ions produced in the ESI source may be subject to a variety of reactions in the gas phase, before they reach the mass analyzer/detector. Among the factors that can complicate the ESI-MS analysis are the facts, which were also confirmed in this study, that solvent molecules can be added to (or taken away from) the central metal ion, the ligand or during the process of 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 of the investigated studied systems. The formation of pseudo molecular or adduct ions (*e.g.*, with Na^+) is very common in ESI-MS analyses. They do not compromise the analysis but make the recognition the origin of some peaks difficult. Despite the soft ionization characteristic of ESI-MS, fragmentation or polymerization phenomena can still play role (*e.g.*, with salicylic acid and other systems). In addition, the number of acidic protons in the metal–ligand complex cannot be determined. ESI-MS Spectra are sensitive to instrumental parameters for source and ion optics (recording conditions), such as spray voltage, capillary temperature, capillary voltage, ion optics potentials (tube lens offset) and the cone voltage. Change in these parameters can have an impact on the results for a given system. Different ions can show different response factors. The composition of solutions that can be subjected to the analysis is conditioned by significant limitations: no application of high ionic strengths, even relatively low concentrations of non-volatile components can be disturbing (*e.g.*, Na^+, Ca^{2+} and Mg^{2+}); the method requires the addition of an organic solvent to the aqueous solution before analysis, which can be somewhat disruptive to the equilibrium state in solution.

Despite these interfering factors, the ESI-MS technique was used in studies of metal–ligand systems, as evidenced by literature references. ESI-MS was applied not only to the speciation of particular elements,[24,25] 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 phase of determining the research methodology, it is necessary to define whether ESI-MS can provide qualitative determinations, giving reliable data on the number and stoichiometry of metal–ligand species in solution. It is necessary to know whether the ESI process introduces interference in equilibria, and whether gas-phase reactions occur and what are their products. Equilibrium misbalance can occur only if the metal–ligand system is kinetically unstable in the time range of ESI process, *i.e.*, about 0.01 sec. As for gas-phase reactions, they can occur under a variety of specific conditions and hence a simple conclusion on this issue cannot be given.

In addition, for quantification purposes, it is important to determine whether the ESI-MS technique can be successfully applied for the quantification, *i.e.*, if it provides a proper determination of the concentrations of the species in solution. For this application, besides the above-mentioned requirements (kinetic inertia and absence of gas phase reactions), it is essential to know the response factors of the individual ions. However, the different approaches proposed for their calculations are still not reliable enough, and in most papers, equivalence of the response factors is assumed, which is a relatively debatable assumption, except in cases of similar types of identical charge (for example, host–guest systems). Finally, an acceptable method for assessing the usefulness of ESI-MS results is comparison of the ESI-MS results with those from available conventional techniques, such as potentiometry and other methods.

In terms of qualitative results, this comparison often gives good matches. Thus, it would seem that in most cases 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. Therefore, the ESI-MS technique may be considered as a relevant and reliable technique for qualitative analysis of metal–ligand system.

With regard to the quantitative application ESI-MS, rigorous comparison with other techniques can be realized only with the correction of activity coefficients, due to the different composition of the solution ionic strength, while currently there is no generally applicable method for overcoming the problem of response factors. In summary, the ESI-MS technique, for now, cannot be considered as a completely reliable application for the quantitative determination of metal–ligand system (this of course is not true for quantitative analytical applications in other fields, *e.g.* with HPLC and other separation techniques), but due to some unique features, in combination with other techniques, ESI-MS is still a valuable source of data for a better understanding of the equilibrium of metal–ligand systems. In this paper, quantification by the employed techniques, UV/Vis spectroscopy and ESI-MS showed a large difference in the informativness of the experimental results, in favor of the ESI-MS technique, which is related to the fundamental limitations of the UV/Vis method. A comparative view of UV/VIS and ESI-MS method for the study of interactions in metal–ligand systems is given in Table III.

TABLE III. Comparison of the suitability of the UV/Vis and ESI-MS techniques for investigations of metal–ligand systems

Technique	Species number determination	Stoichiometry determination	Equilibrium constant determination	Detection limit mol L^{-1}
UV/Vis	Not ideal	Acceptable	Good	10^{-5}
ESI-MS	Good	Excellent	Problematic	10^{-6}

CONCLUSIONS

Chromium(III) interacted with the investigated *O*-donor humic-like ligands. The intensity of the interaction of chromium(III) with the *O*-donor humic-like ligands was correlated with the acid–base and electron-donor properties, geometric and steric characteristics, and the number and position of the *O*-donor atoms in the investigated ligands. The intensity of the interaction of chromium with the *O*-donor ligands followed the order: citric acid > phthalic acid > salicylic acid > benzoic acid, which correlates with the number of *O*-donor atoms in the structure. The study of the possibility of chromium interaction with humic model substances, benzoic, salicylic, phthalic and citric acid is a good method for defining the interaction with polyfunctional, polydispersed, polyelectrolyte ligand, such as humic macromolecules. 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 quantitative analysis of the system Cr(III)–ligand under certain circumstances. 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. This study was supported by the Ministry of Education, Science and Technological Development of the Republic Serbia and was performed as a part of the Project TR 31060.

ИЗВОД

ИНТЕРАКЦИЈА ХРОМА СА О-ДОНОР ХУМАТНИМ МОДЕЛ ЛИГАНДИМА ЕЛЕКТРОСПРЕЈ МАСЕНОМ СПЕКТРОМЕТРИЈОМ

ДАРКО Х. АНЂЕЛКОВИЋ[1], РУЖИЦА С. НИКОЛИЋ[1], ДЕЈАН З. МАРКОВИЋ[2], ТАТЈАНА Д. АНЂЕЛКОВИЋ[1],
ГОРДАНА М. КОЦИЋ[3], ЗОРАН Б. ТОДОРОВИЋ[2] и АЛЕКСАНДАР Љ. БОЈИЋ[1]

[1]*Природно–математички факултет,Универзитет у Нишу,Вишеградска 33, 18000 Ниш,*
[2]*Технолошки факултет,Универзитет у Нишу, Булевар ослобођења 124, 16000 Лесковац и*
[3]*Медицински факултет, Универзитет у Нишу, Булевар др Зорана Ђинђића 81, 18000 Ниш*

Проучавање интеракције хрома са *О*-донор хуматним модел лигандима вршено је коришћењем електроспреј-јонизационе масене спектрометрије (ESI-MS) и ултраљубичасте/видљиве (UV/Vis) спектрофотометрије. Хетерогеност функционалних група оправдава примену модел једињења хуминских супстанци. Као модел супстанце за проучавање интеракције хрома са хуминским супстанцама коришћене су бензоева, салицилна, фтална и лимунска киселина, које садрже *О*-донор атоме, а који се налазе и у хетерогеном и полидисперзном лиганду, каква је хуминска или фулво киселина. Интензитет интеракције је у корелацији са ацидо–базним и електрон-донорским особинама, геометријским и стерним карактеристикама, као и бројем и положајем *О*-донор атома у испитиваним лигандима. UV/Vis карактеризација интеракције хрома са хуматним модел лигандима је доведена у корелацију са ESI-MS карактеризацијом комплекса, у квалитативном и квантитативном смислу. UV/Vis спектроскопија и ESI-MS квантификација су показале информативну разлику у погледу дефинисања интеракција хром–лиганд, и то у корист ESI-MS технике. ESI-MS техника се може користити за квантитативну ана-

лизу система Cr(III)–лиганд. ESI-MS хроматограми јонске струје добијени ињектирањем 20 µL система Cr(III)–лиганд, показују стабилне пикове и интензитете сигнала.

(Примљено 20. марта, ревидирано 3. јула 2012)

REFERENCES

1. 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
2. M. Linde, *PhD Thesis*, Swedish University of Agricultural Sciences, Uppsala, 2005
3. E. Tipping, C. Woof, *J. Soil. Sci.* **42** (1991) 437
4. E. Tipping, M. A. Hurley, *Geochim. Cosmochim. Acta* **56** (1992) 3627
5. R. A. Griffin, A. K. Au, R. R. Frost, *J. Environ. Sci. Health* **12** (1977) 431
6. D. G. Kinniburgh, W. H. V. Riemsdijk, L. K. Koopal, M. Borkovec, M. H. Benedetti, M. J. Avena, *Colloids Surf., A* **151** (1999) 147
7. B. R. James, R. J. Bartlett, in *Chromium in Natural and Human Environments*, J. O. Nriagu, E. Nieboer, Eds., Wiley Interscience, New York, 1988, p. 265
8. R. J. Bartlett, *Environ. Health Perspect.* **92** (1991) 17
9. J. Kotas, Z. Stasicka *Environ. Pollut.* **107** (2000) 263
10. R. J. Bartlett, J. M. Kimble, *J. Environ. Qual.* **5** (1976) 379
11. R. J. Bartlett, J. M. Kimble, *J. Environ. Qual.* **5** (1976) 383
12. T. Andjelkovic, J. Perovic, M. Purenovic, S. Blagojevic, R. Nikolic, D. Andjelkovic, A. Bojic, *Anal. Sci.* **22** (2006) 1553
13. T. Andjelkovic, R. Nikolic, A. Bojic, D. Andjelkovic, G. Nikolic, *Maced. J. Chem. Chem. Eng.* **29** (2010) 215
14. T. Arunachalam, R. Bhakyaraj, A. K. Sasi, *E-J. Chem.* **6** (2009) 743
15. E. Nakayama, T. Kuwamoto, S. Tsurubo, H. Tokoro, T. Fujinaga, *Anal. Chim. Acta* **130** (1981) 289
16. L. Bois, A. Ribes, M. Petit-Ramel, M. F. Grenier-Loustalot, *Chem. Ecol.* **19** (2003) 263
17. T. D. Burns, T. G. Spence, M. A. Mooney, L. A. Posey, *Chem. Phys. Lett.* **258** (1996) 669
18. P. Kebarle, L. Tang, *Anal. Chem.* **65** (1993) 972A
19. W. Henderson, M. J. Taylor, *Inorg. Chim. Acta* **277** (1998) 26
20. Y. Bai, F. R. Song, M. L. Chen, J. P. Xing, Z. Q. Liu, S. Y. Liu, *Anal. Sci.* **20** (2004) 1147
21. D. Baron, J. G. Hering, *J. Environ. Qual.* **27** (1998) 844
22. Z. L. Cheng, K. W. M. Siu, R. Guevremont, S. S. Berman, *J. Am. Soc. Mass Spectrom.* **3** (1992) 281
23. *NIST database*, National Institute for Standards and Technology, Gaithersburg, MD, USA
24. J. A. Dean, *Lange's Handbook of Chemistry*, McGraw Hill, New York, 1992
25. I. I. Stewart, *Spectrochim. Acta, B* **54** (1999) 1649
26. H. Chassaigne, V. Vacchina, R. Lobinski, *TrAC, Trends Anal. Chem*. **19** (2000) 300
27. J. L. Beck, M. L. Colgrave, S. F. Ralph, M. M. Sheil, *Mass Spectrom. Rev.* **20** (2001) 61
28. A. J. R. Heck, R. H. H. Van den Heuvel, *Mass Spectrom. Rev.* **23** (2004) 368.