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Interaction of cobalt(II), nickel(II) and zinc(II) with humic-like ligands studied by the ESI-MS and ion-exchange methods

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Abstract: The interactions of Co(II), Ni(II) and Zn(II) with humic acid and O-donor humic-like ligands were studied by the ion-exchange and electrospray ionization mass spectrometry (ESI-MS) methods. Interactions were confirmed by differences between the values of chromatogram peak areas for monocomponent (ligand) and binary systems (ligand with metal ion) by a newly developed ESI-MS 5 μL-loop injection technique. The Schubert ion exchange method was used for the determination of the stability constants of the formed complexes at pH 4.0 and $I = 0.01$ mol dm^{-3}. Comparing the values of log K for complexes formed by di-positive metal ions with humic, benzoic and salicylic acids, it could be concluded that the interaction strengths of d-metals followed the Irving–Williams order: Co(II) < Ni(II) < Cu(II) > Zn(II). The obtained values of log K indicated that Pb(II) ions affect the strongest interactions with all the investigated ligands. Complexation of humic acids macromolecules possessing O-donor binding sites as major binding sites could be predicted and modeled following the same order of interaction strength as the Irving–Williams order.

Keywords: humic acid; humic-like ligands; heavy metals; ion exchange; ESI-MS.

INTRODUCTION

Environmental pollution by heavy metals is a well-known problem that threatens nature as well as human health, due to their high toxicity even in small amounts. Heavy metals can be involved in a series of complex chemical and biological interactions in soil, water and sediments.[1,2] An important factor in determining toxicity, bioavailability and transport of heavy metals is the binding or complexation of metal ions by humic substances (HS).[3,4]

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Humic substances represent a major fraction of the natural organic compounds in the environment. One of characteristics of HS is their heterogeneity in terms of elemental composition, molecular size distribution and chemical functionality.[4,5] Bearing in mind that humic substances can derive from any organic material, including plant and animal debris, bio-wastes and meiofauna, their heterogeneity is obvious. The elemental composition of HS is 40–60 % carbon, 30–50 % oxygen, 1–3 % nitrogen and 0.1–2 % sulfur. HS contain many functional groups such as carboxylic, alcoholic, phenolic, amino, amide and sulfhydryl groups. Oxygen functional groups are the most abundant groups in HS macromolecules.[5,6] Due to the acidity of these groups, such as carboxyl and hydroxyl groups, the acid–base behavior of humic acids, an operational fraction of HS, play an important role in the acid–base balance of natural waters.[7] The study of the pH behavior of humic acid is very significant, because the complexation of metal cations and binding of different xenobiotics, such as polycyclic aromatic hydrocarbons, pesticides *etc.*, with humic acids is pH-dependent.[8]

Metal ions react with humic compounds and form organometal complexes with different stabilities and solubilities. The high affinity of humic substances for interactions with metal ions affects the distribution of the metal ions in terrestrial and aquatic systems. These organometal reactions facilitate the transport, mobilization or deposition of trace metals in soil and sediments, which influences their bioavailability in the natural environment. The obtained information of the complexing properties of humic substances with metal ions is important from the ecotoxicological viewpoint. For this reason, knowledge of the most reactive functionalities of humic materials is required for a full understanding of the role and function of these constituents in the environment.[9]

Despite the application of powerful techniques for structural investigation of humic substances, including nuclear magnetic resonance spectrometry, infrared spectroscopy with Fourier transform, mass spectrometry, spectroscopic analysis, *etc.*, the structural identification of HS at the molecular level has not been completely revealed.[10–13] Bearing in mind that the number and type of functional groups determine the reactivity of HS, it is important to know such characteristics and not the strict/completely defined chemical structure of HS in order to predict their complexation and acidic properties in the environment.

The major binding sites in the structure of HS, *i.e.*, carboxylic and phenolic groups, are ionizable groups, the dissociation of which gives rise to a net negative charge. These two functional groups allow two types of interactions with heavy metals: carboxylate–metal cation and phenolate–metal cation interactions. Metal ions could interact with humic acid through electrostatic interactions and/or formation of chelate structures with charged carboxylic and phenolic group.[14]

The general rules of metal interaction with HS ligands are based on their relative ionic and covalent bonding properties. Based on the Pearson rule, small

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metal cations prefer less polarizable sites (O-containing sites) in humic macromolecule, while the larger and more polarizable cations prefer humic functional groups with N- and S-donor atoms.[15]

As low-molecular organic substances (such as phenolic acids) were identified as structural elements of natural humic acids, they were used in this paper as humic-like models in the investigation of the correlation between humic functional composition and their acidic/complexation properties. In order to investigate heavy metal interaction with complex and structurally heterogeneous humic substances, the investigation was performed with stoichiometrically defined substances that resemble structurally the most important parts of the binding sites of humic macromolecule. Benzoic (BA) and salicylic (SA) acid have phenolic hydroxyl and/or carboxylic groups bonded on aromatic rings, and they are denoted as humic-like ligands.[16]

Electrospray ionization mass spectrometry (ESI-MS) is used for the detection and characterization of ion species in solution and is often the method used for confirming the stoichiometry of newly synthesized complexes in metal–ligand systems. The soft conditions that provide a spectrum with sufficient sensitivity and low detection limit, at concentration values down to 10^{-6} mol L^{-1}, represent one of the main advantages of the ESI-MS method for analysis of metal–organic complexes. This allows the analysis of solutions at concentration levels, which are more close to those usually encountered in the environmental samples. In addition, many ionic species can be determined and simultaneously analyzed without prior sample preparation. ESI-MS was used to determine the stoichiometry of metal–ligand complexes in solution, as confirmation of the results obtained from other techniques.[17]

The stability constants of humic acid–M(II) complexes may be determined by different analytical techniques, such as ion selective, liquid membrane electrode (ISLME) techniques, equilibrium dialysis, potentiometric titration and dynamic light scattering techniques.[16,18–22] Among other popular methods for the determination of stability constants of metal ions and humic acid, the Schubert ion-exchange method, which is based on competition of metal to bind ligand and/or resin, is often used and thus applied in this study.[23,24]

This paper deals with an investigation of the interaction between M(II) ions with humic acid and O-donor humic-like ligands, *i.e.*, benzoic and salicylic acid, in order to closely define the interaction of M(II) ions with HS in the natural environment, by comparison of interaction data with stoichiometrically defined ligands using the ion-exchange method and electrospray ionization mass spectrometry (ESI-MS). The results of this study define the stoichiometry of the complexes, *i.e.*, whether they are mononuclear or polynuclear complexes. By comparing the results of the existence and strength of the interactions of metals with stoichiometrically defined ligands, it is possible to predict the complexing cap-

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acity of humic substances with the investigated metals, if their chemical characteristics, such as the presence of oxygen functional groups and the total acidity, are known. The obtained results have importance in the theory of coordination compounds of transition metals and the interaction with O-donor ligands, and practical importance in defining the distribution, migration and mobility of the d-metals Co, Ni and Zn in soil, sediments and aquatic systems.

EXPERIMENTAL

Chemical reagents, solutions, instrumentation

All the used chemicals were *p.a.* or HPLC (high-performance liquid chromatography) grade purity. Benzoic acid was purchased from Fluka (Switzerland); salicylic acid from Merck (USA); humic acid from Sigma–Aldrich (USA) and methanol from Baker (Analyzed Reagent Bio). Stock solutions of each metal were prepared from metal salts: $Zn(NO_3)_2\cdot 6H_2O$, $Co(NO_3)_2\cdot 6H_2O$, $NiSO_4\cdot 6H_2O$. All metal salts were purchased from Merck, Germany. Humic acid (Aldrich) was purified by the same procedure as recommended by The International Humic Substances Society (IHSS) for purification of humic acid isolated from soil, in order to remove ash content, residual fulvic acids and heavy metals.[25] An estimation of the molecular weight ($\overline{M}_w$) of the investigated humic acid was obtained from the measurement of the absorption coefficient at 280 nm (ε_{280}). The molecular weight was calculated using the following equation:[26]

$$\overline{M}_w = 3.99\varepsilon_{280} + 490 \tag{1}$$

The absorption coefficient, ε_{280}, was found to be 263, and the calculated molecular weight 1539 g mol^{-1}. According to these results, an appropriate amount of humic acid was dissolved and a working solution prepared. The spectrophotometric measurements were performed using a Shimadzu UV-1650PC spectrophotometer.

All standard stock solutions were prepared by dissolving the required amounts of the chemicals, measured with an accuracy of ±0.0001 g. The solutions of all chemicals were kept in a refrigerator at 4 °C.

ESI-MS analysis

Working solutions for the ESI-MS analyses were made by dilution of the standard solutions in a methanol/water mixture (80/20, *V*/*V*). The ESI-MS measurements were performed on a LCQ Advantage, Thermo Finnigan (USA) instrument, with auxiliary equipment, implementing the loop injection technique, rather than syringe flow injection, in order to obtain separate peaks of the analytes of interest. The calculated peak area was considered more reliable for concentration determination than the fluctuating abundance of the analyte ions usually obtained through flow injection. Solutions of ligands were analyzed by ESI-MS immediately after preparation. Mixed solutions of M(II) ions and benzoic and salicylic acid were stored at 25 °C and were analyzed 24 h after mixing. The concentrations of ligands and metal ions were 1, 3, 5, 7 and 9 µmol L^{-1}.

The Schubert ion exchange method

Three different concentrations of ligands were used (5×10^{-3}, 10×10^{-3} and 15×10^{-3} mol L^{-1} for benzoic and humic acids; 5×10^{-3}, 7.5×10^{-3} and 10×10^{-3} mol L^{-1} for salicylic acid). All solutions were prepared using double deionized water (conductivity less than 0.1 µS cm^{-1}). Concentrations of all metal ions were 5, 10, 15 and 20 mg L^{-1}.

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The pH measurements were made with a Hach sension 3 pH-meter (precision of 0.1 mV or 0.001 units of pH) using a Hach gel-filled combination glass electrode (51935-00). All experiments were performed at 25±1 °C. The prepared solutions were analyzed by flame atomic absorption spectroscopy (AAS) using a Perkin Elmer AAnalyst 300.

For each experiment, the cation exchange resin Dowex 50WX8, 100–200 mesh, Na-form was used. Approximately 50 g of the resin was stirred with deionized water and the fine particles were decanted off. Subsequently, the resin was transferred to a glass column and the resin was rinsed with 2 L of deionized water, followed by 2 L of 2 mol L^{-1} HCl, 2 L of 2 mol L^{-1} NaOH, and finally, with 2 L of deionized water. After this rinsing, the resin was passed into the Na-form using 2 L of 2 mol L^{-1} NaCl, followed by 2 L of deionized water. After converting into Na-form, the resin was air-dried for 24 h, and then stored in an airtight glass container.

Experimental procedure for establishing the metal/ion-exchange distribution coefficients (D_0 *and* D*)*

Time of equilibrium was defined by taking accurate amount of Na-saturated cation exchange resin and 50.0 mL of each metal ion solution, and these solutions were shaken. Period of attaining the equilibrium was determined by monitoring the metal concentration in supernatant by flame AAS. Equilibrium was achieved in 1 h.

For each metal ion (Co(II), Ni(II) and Zn(II)), the ion-exchange isotherm was measured at pH 4.0 at a constant ion strength $I = 0.01$ mol L^{-1}. Solutions of metal ions were prepared by adding different volumes of metal stock solutions to a 50.0 mL volumetric flask. The pH was adjusted with the addition of 0.1 mol L^{-1} NaOH and/or 0.1 mol L^{-1} HCl and the ion strength was adjusted with 0.01 mol L^{-1} NaCl. In each volumetric flask, 100.0 mg of cleaned, Na-saturated cation exchange resin was added, and all solutions were shaken at a constant temperature of 25 °C for 2 h.

Determination of distribution coefficients of metal ions in solutions with ligand (D) was performed with same procedure as for the determination of D_0, except that the solution contained ligand. Each solution contained metal ions in variable concentrations 5, 10, 15 and 20 mg L^{-1}, and ligands (benzoic, salicylic or humic acids) in concentrations from 5×10^{-3} to 15×10^{-3} mol L^{-1}. The pH value for all mixed solutions was adjusted to pH 4.0; the ion strength was adjusted at 0.01 with a solution of NaCl, and finally, 100.0 mg of cleaned Dowex 50WX8 (100–200 mesh) resin in the Na-form was added. All solutions were shaken for 2 h at 25 °C. The total concentration of free metal ion and complexed metal ion remaining in the equilibrium solution was determined in supernatant by flame AAS.

RESULTS AND DISCUSSION

ESI-MS analysis of the M(II) ion–humic-like ligand systems

Humic-like ligands with O-donor atoms in their structure, as –OH and –COOH groups, due to their tendency to deprotonate, were examined in the ESI negative mode. Monitoring ions $[M–H]^{–}$ were used in all cases, and their signal intensities were good.

ESI-MS total ion current chromatogram of 5 μL loop injections were obtained by analyzing series of solutions of ligands and binary systems – mixed solutions of metal–ligand. The peak areas of the selected mass range ion current chromatograms of the ligands and binary systems were used for the investigation.

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The areas of each ESI-MS total ion current chromatogram were calculated for the defined mass range, $[M–H]^{-}$ m/z±1, for all investigated ligands by using ICIS© modul of Xcalibur™ 1.4 software. The ESI-MS selected mass range ion current chromatograms of benzoic acid and of the binary system benzoic acid–Co(II) are shown in Fig. 1.

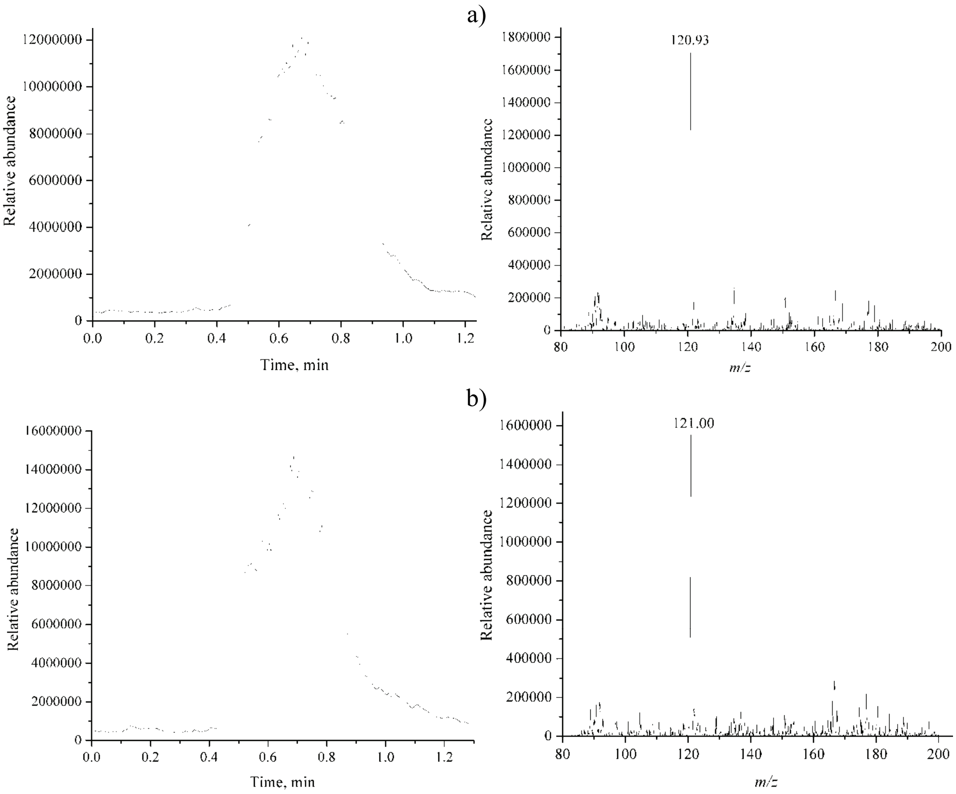

Fig. 1. a) ESI-MS selected mass range total ion current chromatogram and mass spectrum of the loop injection of benzoic acid without M(II) ions, $c = 7.00\times10^{-6}$ mol L^{-1}; b) ESI-MS selected mass range total ion current chromatogram and mass spectrum of the loop injection of the binary system benzoic acid, $c = 7.00\times10^{-6}$ mol L^{-1} and cobalt(II), $c = 3.0\times10^{-6}$ mol L^{-1}; solvent: CH_3OH/H_2O = 80/20, negative ionization mode, flow rate = 20 µL min^{-1}.

The values of the peak areas obtained for the selected mass range ion current chromatogram were plotted *versus* the ligand concentrations (Fig. 2). In order to describe the interaction between the investigated metal ions and ligands, comparison of the obtained integrals under the set of experimental points for monocomponent (P_L) and binary systems (P_{ML}) were compared (Fig. 2). The percentage difference between these areas ($\Delta P_{\%}$) was calculated from the following equation:

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$$\Delta P_{\%} = 100\frac{P_{\mathrm{L}} - P_{\mathrm{ML}}}{P_{\mathrm{L}}} \tag{2}$$

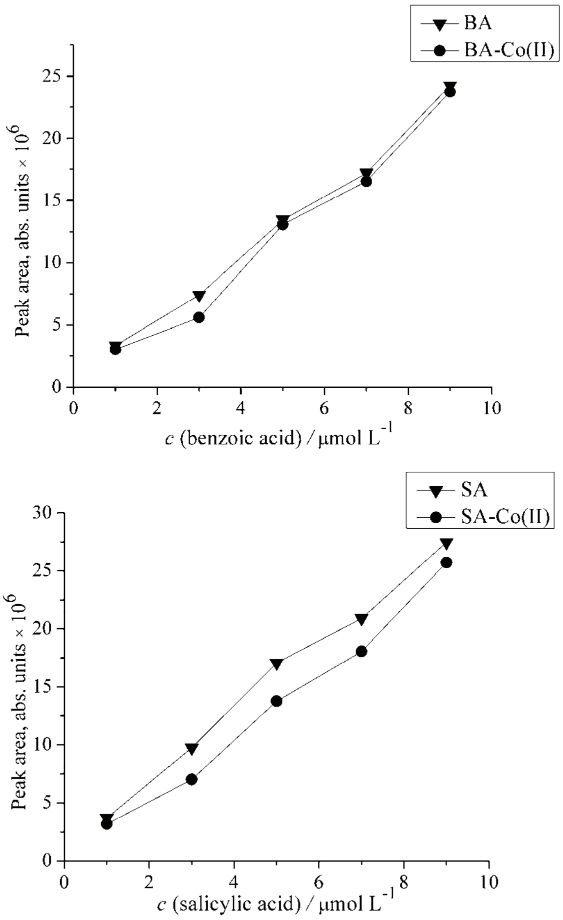

Fig. 2. a) Plot of the peak areas of the ESI-MS selected mass range ion current chromatograms of the loop injection of benzoic acid (▼) and mixed solutions benzoic acid–Co(II) (●) for the mass range *m*/*z* 120–122 *vs.* ligand concentration; b) plot of the peak areas of the ESI-MS ion current chromatograms of the loop injection of salicylic acid (▼) and mixed solutions salicylic acid–Co(II) (●) for the mass range *m*/*z* 136–138 *vs.* ligand concentration; solvent: CH_3OH/H_2O 80/20 mixture, negative ionization mode.

The areas have no physical meaning because they are presented in absolute non-dimensional units of the Xcalibur^{TM} software (count–second units). The obtained $\Delta P_{\%}$ values were used for comparison of the interactions between M(II) ions and ligands. A higher value of $\Delta P_{\%}$ confirms stronger interaction between metal ions and ligands in the investigated solutions.

The values of the peak areas of the selected mass range ion current chromatogram for different concentrations of benzoic acid and for mixed Co(II)–benzoic acid solutions are presented in Table I. Each measurement was performed three times, and the mean values were calculated. The same procedure was repeated for salicylic acid.

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TABLE I. Values of the peak areas of the total ion current chromatogram (abs. units) for benzoic acid and the binary systems M(II)–benzoic acid

Parameter	System				
	I	II	III	IV	V
c_{BA} / 10^{-6} mol L^{-1}	1	3	5	7	9
(Peak area ± *SD*)×10^6	3.36±0.27	7.41±0.32	13.45±0.49	17.22±0.73	24.26±0.19
c_{BA} / 10^{-6} mol L^{-1} + $c_{Co(II)}$ / 10^{-6} mol L^{-1}	1+9	3+7	5+5	7+3	9+1
(Peak area ± *SD*)×10^6	3.03±0.06	5.60±0.21	13.08±0.58	16.56±0.50	23.75±0.19

The confirmation of interaction was based on the area of the recorded chromatogram being in direct relation with the peak intensities, regarding the signal of ions in the required *m*/*z* mass range. In the binary systems, interaction of investigated M(II) ion and ligand led to changes in the molecular and ionic species in the sample and thus, the intensity of the observed characteristic ion decreased. Proportionally smaller areas of the chromatographic peak were obtained in the binary systems for identical ligand concentrations. Relative decrease in area could be related to the strength of the interaction of the M(II) ions and the ligand.

Since many properties of a solution and the recording conditions affect the obtained result, such as the equilibrium relationship between species and stoichiometry of the investigated solutions, changes in the area of chromatogram peaks can be treated as valid only in a relative comparison.[27]

The values of the integrals under the set of points in Fig. 2 for the ligands and binary systems of ligands with metals (P_{ML}), and the $\Delta P_{\%}$ values are presented in Table II. In the group of studied O-donor ligands (PL), the strength of M(II)–ligand interaction follows the decreasing order: salicylic acid > benzoic acid.

The Schubert ion exchange method

Schubert's method was used for determination of stability constants of complexes formed between M(II) ions and organic ligands. The principle of ion exchange equilibrium was applied to investigate the complex composition. This method is based on measuring the distribution coefficients of a metal ion between a cation exchange resin and the solution phase, in the presence and absence of a ligand (as complexing agent).

The equilibrium reactions between M(II) ions and the ion exchange resin (R), and M(II) ions and the ligands (L) can be given by the following equations:

$$r\mathrm{M} + \mathrm{R} \rightleftarrows \mathrm{M}_r\mathrm{R} \tag{3}$$

$$m\mathrm{M} + n\mathrm{L} \rightleftarrows \mathrm{M}_m\mathrm{L}_n \tag{4}$$

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TABLE II. Integrals under the set of experimental points for monocomponent and binary systems. Integrals under the set of experimental points obtained for ligands (P_L), binary systems of ligands with metals (P_{ML}), and the $\Delta P_{\%}$ values

Parameter	Ligand without metal	Co	Ni	Zn
	Benzoic acid (BA)			
P_L	105.12	–	–	–
P_{ML}		99.25	98.51	98.88
P_L–P_{ML}		5.87	6.61	6.24
$\Delta P_{\%}$		5.58	6.29	5.94
	Salicylic acid (SA)			
P_L	126.30	–	–	–
P_{ML}		108.46	102.90	99.23
P_L–P_{ML}		17.84	23.40	27.07
$\Delta P_{\%}$		14.12	18.53	21.43

The distribution coefficient for a metal ion, M(II), between the resin and solution phase in absence of an organic ligand is defined by the equilibrium ratio given by Eq. (5):

$$D_0 = \frac{[M_R]}{[M_f]} \tag{5}$$

where $[M_R]$ is the concentration of metal ions on the resin and $[M_f]$ is the concentration of free metal ions in solution.

The distribution coefficient for metal ions in the presence of an organic ligand is determined by the following equation:

$$D = \frac{[M_R]}{[M_f]+[M_c]} \tag{6}$$

where $[M_c]$ is the concentration of complexed metal ions.

In these distribution coefficients equations, $[M_R]$ presents the amount of metal per g of resin while $[M_f]$ and $[M_c]$ present the amount of metal per volume unit of solution, and the distribution coefficients have unit of cm^3 g^{-1}.

The distribution coefficient, D_0, between the resin and solution phase for metal ion in the absence of ligand and the distribution coefficient, D, between resin and solution phase for metal ion in the presence of ligand was calculated using the following equation:

$$D_0 \text{ or } D = \frac{\alpha_0 V}{(100-\alpha_0)m_R} \tag{7}$$

where α_0 is percentage of total metal bound to the exchange resin; $(100–\alpha_0)$ is percentage of total metal remaining in solution; V is the volume of the solution (cm^3) and m_R is the weight of the exchange resin (g).

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From Eq. (4), the stability constant *K* could be determined using the equation:

$$K=\frac{[\mathrm{M}_m\mathrm{L}_n]}{[\mathrm{M}]^m[\mathrm{L}]^n} \tag{8}$$

Combining equations for the determination of the distribution coefficients and the equation for the calculation of the stability constant gives the following equation:

$$\frac{D_0}{D}-1=\frac{[\mathrm{M_c}]}{[\mathrm{M_f}]}=\frac{m[\mathrm{M}_m\mathrm{L}_n]}{[\mathrm{M_f}]}=\frac{mK[\mathrm{M}]^m[\mathrm{L}]^n}{[\mathrm{M}]}=mK[\mathrm{M}]^{m-1}[\mathrm{L}]^n \tag{9}$$

Taking the logarithm of both sides, the following equation is obtained:

$$\log\left(\frac{D_0}{D}-1\right)=\log m+\log K+(m-1)\log[\mathrm{M}]+n\log[\mathrm{L}] \tag{10}$$

This equation presents the full form of the Schubert method, which can be applied for polynuclear complexes. For mononuclear complexes, Eq. (11) is obtained since $m = 1$:

$$\log\left(\frac{D_0}{D}-1\right)=\log K+n\log[\mathrm{L}] \tag{11}$$

Equation (11) presents the basic equation for the determination of the stability constant of mononuclear complexes. When the complex is mononuclear, the slope of the plotted data from Eq. (10) has an integer value, and the intercept is equal to the value of the stability constant. The slope, *n*, shows the metal:ligand ratio of the formed complex, while the intercept, log *K*, gives the stability constant for mononuclear complexes.

This method could be applied if the following conditions are met: pH and ion strength must be constant; the equilibrium temperature of the investigated system must be constant; the experiment must be performed within the linear range of the ion exchange isotherm; there is no absorption on the resin of the ligand or formed complex and the total concentration of the organic ligand must be higher than the total concentration of the metal ion. The experiment was performed at pH 4.0 to avoid hydrolysis of metal ions and the formation of carbonate or hydroxide.

The percentage of the total metal bound to the exchange resin, α_0, the distribution coefficients, D_0 and D, the values of the slope, *n*, and the stability constants, log *K*, obtained for the investigated interaction of M(II) ions with benzoic acid (for concentrations of M(II) and ligand of 5 mg $\mathrm{L^{-1}}$ and 0.005 mol $\mathrm{L^{-1}}$, respectively) at pH 4.0 are summarized in Table III. The same parameters for the

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investigated systems of M(II) ions with salicylic and humic acid are presented in Tables IV and V, respectively.

TABLE III. Data obtained by the Schubert method for the systems M(II)–benzoic acid. Experimentally determined percentages of the total metal bound to the cation exchange resin, α_0, distribution coefficients D_0 and D, values of slope, n, and values of the logarithm of the stability constants, log K, for complexes of Co(II), Ni(II) and Zn(II) (5 mg L^{-1}) with benzoic acid, at pH 4.0 and ionic strength I = 0.01 mol L^{-1}

Metal ion	$c_{BA}\times10^3$ / mol L^{-1}	α_0	D_0 (D)	log (D_0/D–1)	n	log K	log $\overline{K}$
Co(II)	0	61.14	786.67	–	1.00	–	0.55
	5	60.72	772.91	–1.75		0.55	
	10	60.28	758.81	–1.44		0.56	
	20	59.90	746.88	–1.27		0.55	
Ni(II)	0	12.48	71.30	–	0.53	–	0.97
	5	8.00	43.48	–0.19		0.96	
	10	6.96	37.40	–0.04		0.96	
	20	6.02	32.03	0.09		1.00	
Zn(II)	0	31.04	225.06	–	1.01	–	1.03
	5	29.96	213.88	–1.28		1.02	
	10	28.88	203.04	–0.96		1.04	
	20	27.98	194.25	–0.80		1.02	

TABLE IV. Data obtained by the Schubert method for the systems M(II)–salicylic acid. Experimentally determined percentages of the total metal bound to the cation exchange resin, α_0, distribution coefficients (D_0 and D), values of the slope, n, and the values of logarithm of the stability constants, log K, for complexes of Co(II), Ni(II) and Zn(II) (5 mg L^{-1}) with salicylic acid, at pH 4.0 and ionic strength I = 0.01 mol dm^{-3}

Metal ion	$c_{SA}\times10^{-3}$ / mol L^{-1}	α_0	D_0 (D)	log (D_0/D–1)	n	log K	log $\overline{K}$
Co(II)	0	61.14	786.67		3.00		6.23
	5	57.06	664.42	–0.74		6.17	
	10	44.30	397.67	–0.01		6.37	
	20	39.50	326.45	0.15		6.15	
Ni(II)	0	12.48	71.30		0.57		5.91
	5	0.50	2.51	1.44		6.07	
	10	0.42	2.11	1.52		5.90	
	20	0.34	1.71	1.61		5.77	
Zn(II)	0	31.04	225.06		2.67		6.22
	5	1.34	6.79	1.51		6.11	
	10	0.44	2.21	2.00		6.25	
	20	0.22	1.10	2.31		6.31	

Comparison of the results obtained by the analysis of the investigated systems showed that the percentage of metal bound to the ion-exchange resin decreased with increasing concentration of the ligand in solution. The value of this parameter depended on the metal type and ligand structure. The difference

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between the amount of total metal bound to the ion exchange resins in the absence of ligand and amounts in the presence of the ligands showed that humic and salicylic acid caused larger differences than benzoic acid, which was expected due to the higher number of O-donor binding sites in their structure. Smaller differences were observed with benzoic and then with salicylic acid. Moreover, the type of the metal influenced the amount of resin bound metal.

TABLE V. Data obtained by the Schubert method for the systems M(II)–humic acid. Experimentally determined percentages of the total metal bound to the cation exchange resin, α_0, distribution coefficients (D_0 and D), values of the slope, n, and the values of the logarithms of the stability constants, log K, for complexes of Co(II), Ni(II) and Zn(II) (5 mg L^{-1}) with humic acid, at pH 4.0 and ionic strength $I = 0.01$ mol L^{-1}

Metal ion	$c_{HA}\times10^3$ / mol L^{-1}	α_0	D_0 (D)	log (D_0/D–1)	n	log K	log $\bar{K}$
Co(II)	0	61.14	786.67	–	0.8	–	1.95
	5	50.36	507.25	–0.26		2.04	
	10	46.46	433.88	–0.09		1.91	
	20	41.78	358.81	0.08		1.90	
Ni(II)	0	12.48	71.30	–	0.96	–	2.24
	5	6.94	37.29	–0.04		2.26	
	10	4.84	25.43	0.26		2.26	
	20	4.10	21.38	0.37		2.19	
Zn(II)	0	31.04	225.06	–	0.83	–	2.25
	5	18.74	115.31	–0.02		2.28	
	10	14.46	84.52	0.22		2.22	
	20	11.02	61.92	0.42		2.24	

Since the values of n should be an integer, values of $n \approx 1$ indicate that Co(II) ions with benzoic and humic acid form 1:1 complex. In addition, it was noticed that for Co(II)–salicylate n is 3.0, and the stoichiometry 1:3 is assigned in this case. Co(II) and Zn(II) ions form mononuclear complexes, with metal:ligand ratio 1:1, with benzoic acid. Ni(II) ion forms mononuclear complex, but with 1:0.5 stoichiometry of complex. The stability constants, log K, and metal–ligand ratio of obtained M(II) complexes with all investigated ligands are summarized in Table VI.

In a previous study[28] of the metal ions Cu(II) and Pb(II) with humic acid and benzoic and salicylic acid as O-donor humic-like ligands, it was found that the most stable complex of the investigated M(II) ions with benzoic acid was the Pb(II)–benzoate complex with a log K value of 1.98, which corresponded to literature data.[29] In addition, the most stable complex with humic acid was the Pb(II)–humate complex. Furthermore, Cu(II) ions formed more stable complexes with all the investigated ligands than the complexes formed with Co(II), Ni(II) and Zn(II) ion.

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TABLE VI. Data obtained by Schubert's method for humic acid and humic-like ligands. Metal:ligand ratio (m:n) and log K_{mn} for humic acid and humic-like ligands with Co(II), Ni(II) and Zn(II) ions

Metal ion	m:n			$\log \bar{K}$		
	BA	SA	HA	BA	SA	HA
Co(II)	1:1	1:3	1:1	0.55	5.80	1.93
Ni(II)	1:0.5	2:1	1:1	0.92	5.57	2.19
Zn(II)	1:1	1:2	1:1	1.03	6.39	2.26

Comparing the values of log K in the systems M(II) ions with humic, benzoic and salicylic acids, it could be concluded that the strength of the interaction between the metal of the transition series of elements was in the order:

$$\text{Co(II)} < \text{Ni(II)} < \text{Cu(II)} > \text{Zn(II)}$$

This trend correlates with the trend in the Irving–Williams series for the stability of complexes of d-metals with ligands having N-donor binding sites.

The obtained values of log K indicate that Pb(II) ions present the strongest interactions with all the investigated ligands. Considering the relation between log K and the ligand structure, the weakest interactions were observed for M(II) ions with benzoic acid. The strongest interactions were established between M(II) ions and salicylic acid due to the presence of both carboxylic and hydroxyl groups and thus, a higher possibility for interaction.

When considering the stability constants of M(II) ions with humic acid, it must be taken into account that the reaction between metal ions and humic acids occurs in two ways. The main or dominant mode is when the carboxylic and phenolic groups participate simultaneously in the complexation. The secondary mode is when only the carboxylic groups participate in the complexation. This was justified by the good correlation between the complexing capacity of humic acid and its carboxylic acidity.[30]

The concept of hard/soft acids and bases (HSAB) could explain the established order of the interaction strength between the investigated M(II) ions and humic acids, as well as the investigated humic-like ligands. O-Donor groups, due to the considerable hard properties, generate strong relationships with hard metal acids. The investigated ions belong to the d-metals group, or boundary group between typical hard and typical soft metals.

Comparing the obtained values of log K, it was registered that all investigated M(II) ions form the most stable complexes with salicylic acid and the lowest values of log K were obtained for the interactions with benzoic acid. In addition, it could be seen that the strongest interaction between the M(II) ions and salicylic acid could be interpreted as this acid providing a higher possibility of interaction due to the presence of carboxylic and hydroxyl groups.

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The results of interaction strength for all investigated systems using the ion exchange method are in accordance with literature data and results obtained by the other methods. Investigation of the stability constants of complexes formed between following metal ions Co(II), Ni(II), Zn(II), Cu(II) and Pb(II) with benzoic acid in other investigations showed the same order of strength as was found in the present study.[29] Results found using the Schubert method showed that Co(II) ions and humic acid form complexes with a 1:1 stoichiometric ratio between the metal and the ligand.[31] Moreover, investigations of the stability constant for Ni(II), Zn(II), Cu(II) and Pb(II) ions with humic acid showed that the strongest interaction with humic acid was achieved by Pb(II), slightly weaker by Cu(II), and Ni(II) and Zn(II).[9,14,32–37]

CONCLUSIONS

Phenolic acids comprise a significant part of the chemical structure of humic substances showing an important role in complexation properties of HA. Benzoic and salicylic acid as humic-like ligands can be used for setting the range of stability constants of humic complexes with the investigated M(II) ions. The established trend of the stabilities of metal–humic-like ligand complexes could be used for predicting the strength of interaction between the O-donor atoms in humics and metal ions, as well as for predicting their competition in binding. Of the five divalent metal ions that were examined in the present and previous studies, it was found that Pb(II) ions, as the only non-d-metal ions, form the most stable complexes with O-donor humic-like ligands and humic acid. Comparing the obtained values of log K_{mn}, it can be concluded that the strength of interaction between the metal of transitional series of elements is in the order: Co(II) < < Ni(II) << Cu(II) > Zn(II), which correlates with the trend in the Irving–Williams series for the stability of complexes between d-metals and N-donor binding site ligands. Competition between Pb(II) ions and other investigated M(II) ions in the binding for O-donor binding sites of humic ligands in the environment could be expected. In the natural environment, under conditions of increased concentrations of Pb(II) ions, Pb(II) ions may displace the other investigated M(II) ions, and would cause their increasing mobility and bioavailability.

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ИЗВОД

ИСПИТИВАЊЕ ИНТЕРАКЦИЈЕ КОБАЛТ(II)-, НИКЛ(II)- И ЦИНК(II)-ЈОНА СА МОДЕЛИМА ХУМИНСКОГ ЛИГАНДА МЕТОДАМА ESI-MS И ЈОНСКЕ ИЗМЕНЕ

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У овом раду вршено је испитивање интеракције кобалт(II)-, никл(II)- и цинк(II)-јона са хуминском киселином и О-донорским моделима хуминског лиганда методом јонске измене и методом масене спектрометрије са електроспреј јонизацијом (ESI-MS). Постојање интеракције је потврђено разликом између вредности површина пика хроматограма монокомпонентних система (лиганда) и бинарних система (лиганда са металним јоном) помоћу ESI-MS технике убризгавања петљом од 5 μL. Шубертова јоноизмењивачка метода је коришћена за одређивање константи стабилности формираних комплекса на pH 4,0 и при јонској јачини од 0,01 mol L^{-1}. Упоређујући вредности log *K* за комплексе формиране између двовалентних металних јона и хуминске, бензоеве и салицилне киселине, може се закључити да јачина интеракције метала прелазне серије елемената прати Ирвинг–Вилијамсов редослед: Co(II) < Ni(II) < Cu(II) > Zn(II). Добијене вредности log *K* указују на то да Pb(II) јон остварује најјачу интеракцију са свим испитиваним лигандима у односу на остале M(II) јоне. Комплексирање макромолекула хуминских киселина, који садрже О-донорска везивна места као главна места везивања, може се предвидети и моделирати на основу редоследа јачине интеракције у Ирвинг–Вилијамсовој серији.

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