

Article

Physico-Chemical Aspects of Metal–Fulvic Complexation

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Abstract: The interactions of metal ions with fulvic acids were investigated from the point of view of the thermodynamic aspects of complexation as well as the size and charge of the formed complexes. Thermodynamic aspects were studied by means of isothermal titration calorimetry. Particle size distribution was determined by the method of dynamic light scattering and charge by the measurement of zeta potential. Complexation resulted in changes in particle size and charge. The particle size distribution was trimodal for fulvic acids and bimodal for fulvic complexes with calcium and magnesium, while copper–fulvic complexes had only one size fraction. The compensation of the negative charge of carboxylic and phenolic functional groups by positively charged metal ions resulted in an increase in zeta potential which became closer to zero in the case of copper–fulvic complexes. However, all metal–humic complexes behaved as colloiddally unstable, which resulted in visually observable sedimentation. Calorimetric measurements provided positive values for changes in enthalpy, which indicated endothermic processes. In contrast, quantum chemical calculations as well as experiments with model compounds provided negative values indicating exothermic processes. Changes in Gibbs energy were determined as negative and changes in entropy as positive.

Keywords: fulvic acid; metal ion; interaction; thermodynamics; particle size; charge

1. Introduction

Fulvic acids as a soluble fraction of humic substances are highly beneficial molecules promoting several soil functions such as nutrient absorption, and the transport of metals, and improving soil structure. Due to their solubility at all pH values, they are more active in comparison with humic acids and can participate in metal speciation as well as metal complexation and aggregation [1–4]. Most studies on the migration of metal ions in natural systems are focused on free (hydrated) ions. However, metal ions are often dissolved as complex species such as with humic substances. Comparing the diffusion coefficients of metal complexes with fulvic acid and humic acid showed that the size of the ligand is of primary importance for the mobility of metal–humic complexes [5–7]. In contrast, the binding constants are usually higher for humic acids than for fulvic acids [4,8–11]. Some authors (e.g., Bertolli et al. [12]) calculated thermodynamic stability as a change in reaction enthalpy between fulvic anions and metal cations. Thermodynamic aspects of interactions between humic substances and metals can contribute to a detailed understanding of the cation-binding properties of humic substances and have particular importance for developing long-term solutions to the problems of waste storage and remediation as well as metal ion migration and bioavailability in natural systems [13]. Thermodynamic parameters of metal–fulvic interactions are usually based on experimental data obtained for adsorption [14–16] and theoretical calculations [12,13,17,18], which can include different model binding sites [13,15,18]. The calculation of changes in enthalpy, entropy and Gibbs energy was based on experimental adsorption data obtained for several temperatures. Values were calculated for an initial pH value of 5.95 ± 0.05 (adjusted by the addition of NaOH and HNO₃) and $I = 0.01 \text{ mol dm}^{-3}$ (NaNO₃) [14]. Another approach was based on the determination of an apparent equilibrium constant K for the interactions of copper(II)



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ions with carboxylic groups in peat and Equation (1). The change in Gibbs energy was calculated as the average value in a pH range between 3 and 4, which is typical for the specified environments. Calculations were performed for normal conditions (25 °C and 101,325 Pa) by the GIBBS algorithm and UNITHERM database of “HCh” software described in detail in [19]. Theoretical calculations were based on semi-empirical methods, e.g., PM6, which is parameterized for transition metals and applies some parameters obtained from experimental data [12], PM3 accomplished with a Digital 3000/700 AXP and a Silicon Graphics Indy workstation using spartan 4.0 and MOPAC [13], and the INDO method of molecular orbitals parameterized for compound of transition elements [18]. Several semi-empirical methods (AM/1, MNDO, PM3, PM5, PM6 and PM7) were used in [17] to compute the heat of formation, enthalpy, and entropy and compared. It was shown that the PM6 and PM7 methods in the MOPAC 2012, v.15.229W (Molecular Orbital Package) and MOPAC 2009, v.9.283W software package provided good compliance with experimental and calculated heats of formation for copper complexes and alkaline earth metal complexes with organic acids.

Direct calorimetric measurements are relatively scarce. Bryan et al. [20] used microcalorimetry in determining the thermodynamic characteristics of interactions between humic and fulvic substances and with a range of metal ions, including Cu^{2+} , Cd^{2+} , La^{3+} , and Al^{3+} . Tris(hydroxymethyl)aminoethane (Tris) was used to dissolve metal salts to make a pH of 7. Humic and fulvic acids were dissolved in Tris buffer solution (pH = 9.61) and then the pH value was adjusted to 7 by HNO_3 . Enthalpy measurements were made at 25 °C. Antonelli et al. [21,22] used calorimetry to determine the binding capacity of fractionated humic acids and fulvic acids towards Cu^{2+} ions and the heat of reaction in the complexation of copper ions with small molecules containing functional groups similar to fulvic acid. A copper(II) solution was adjusted to pH = 5, and humic and fulvic acids were adjusted to different pH values (between 5 and 9) by NaOH and HNO_3 ; solutions of small molecules had a pH equal to 5 and 7. Measurements were carried out at 25 °C [21,22]. Qi et al. [23] determined negative values for the change in Gibbs free energy and positive values for enthalpy change and entropy change (revealing that copper sorption was spontaneous) for interactions of Cu^{2+} ions with organic matter from the decomposition of rice straw. Their experiments were realized at 25 °C and pH = 5 adjusted by NaOH and HNO_3 . Sheng et al. [24] employed isothermal titration calorimetry and determined similar thermodynamic characteristics of the binding between aqueous metals and extracellular polymeric substances of activated sludge. Measurements were realized at 25 °C in Tris buffer solutions adjusted to pH = 6. Taraba [25] measured the adsorption enthalpy of Pb^{2+} ions on natural coal at 30 °C and compared it with the calculated value, showing that the calculated values of the adsorption enthalpy can differ considerably from these measured ones. The pH value was not controlled (or measured). Du et al. [26] coupled isothermal titration calorimetry with batch adsorption experiments and determined changes in Gibbs energy, adsorption enthalpy, and entropy for copper and humic acid (and goethite and cells of *Pseudomonas putida*). While the changes in Gibbs energy and adsorption enthalpy were negative, the change in entropy was determined as positive, similarly as in the case of Qi et al. [23]. Both studies [23,26] were carried out at 25 °C and pH = 5. In contrast to [23], Du et al. [26] used KOH instead of NaOH. Kimuro et al. [27] combined potentiometry and calorimetry to determine the thermodynamic values of complexation for typical humic acid and groundwater humic acid with copper and uranyl ions. Experiments were realized at 25 °C, and pH was adjusted by NaOH and HCl or HClO_4 . They revealed a unique complexation mechanism for groundwater (Horonobe) humic acid based on a comparison of the complexation enthalpies of groundwater humic acid with those of typical humic acid and homogeneous polyacrylic acid. Their results are important for the migration of metal cations in the deep underground environment.

Interactions of humic substances with metal ions can result in their coagulation or aggregation [28–34] which affects their mobility. The size, shape, and charge of dissolved humic and fulvic particles and their complexes as well as their secondary structure in a

water environment can be very dynamic. Coagulation by hydrolysing metal salts is strongly affected by solution chemistry and the nature of the interacting colloids. The presence of specific cations and anions may affect the metal ion hydrolysis reactions and precipitation with humic substances. These processes are strongly affected by environmental conditions such as concentration, pH, and ionic strength (in which case the character of ions can be important). They have a strong propensity to aggregate which controls their interactions with other components, their mobility, and their functioning in the environment [31–41]. Liu et al. [40] reported that the hydrophobic fraction was selectively decreased after interaction with added Ca^{2+} , Al^{3+} , or Fe^{3+} , which was possibly due to the formation of their insoluble complexes with dissolved organic matter. The type of metal ions is an important factor that influences the chemical fractionation of dissolved organic matter (and humic substances) in cation-induced coagulation due to various binding affinities of metal ions for their special compounds or functional groups [10,42–44]. In general, carboxylate and phenolic groups are considered predominant metal coordination sites in humic and fulvic acids [13,29,30,45–47]. Other functional groups can be possible ligands, although they are less prevalent. It is known that dissolved humic substances such as fulvic acids can easily bind metal ions due to their higher content of ionizable functional groups (supporting their solubility) and more accessible binding sites in a water environment [21,42,47–53]. On the other hand, metal ions are also able to bind with non-ionizable active centres on the surface of solid humic particles as was shown in [54] and specific metal ions can interact with humic substances in different ways. It was reported that Ca^{2+} did not show any preference for distinct molecular fractions of humic substances, while Fe^{3+} and Al^{3+} preferentially interact with the most oxidized compounds in dissolved organic matter [40,55]. Calculations based on the NICA-Donnan model revealed that Ca^{2+} mainly binds to carboxylic-like functional groups, while Fe^{3+} and Al^{3+} were also bound to phenolic-like functional groups [40,56].

As described above, interactions of humic substances with metal ions can result in heat effects and changes in the size and charge of particles. Thermodynamic parameters such as enthalpy, entropy, and Gibbs energy (or rather their changes) can characterize such interactions from the point of view of the ability of metal to bind with humic substances, the stability of the formed complexes, and the spontaneity of the interactions. They can affect the mobility of metal ions through their interactions with humic substances which can suppress or enhance their migration in natural systems. The size and charge of formed complexes can affect the mobility of metal ions directly because of the dependence of the diffusion coefficient on the size of diffusing particles and their colloidal stability and conformational changes.

The aim of this study was to investigate the interactions of copper, calcium, and magnesium ions with fulvic acids from the point of view of thermodynamics and the size and charge of the resulting metal–fulvic complexes. Enthalpy changes were measured directly by isothermal titration calorimetry. Fulvic acids were chosen due to their solubility resulting from their higher content of dissociable functional groups and smaller size in comparison with humic acids [1,2,43–45], which classes them as the most active fraction of humic substances. Copper was used as a metal ion with high affinity to humic substances [4,8,9,12,14,15,18,23,26,46,47,51,52,54]. Calcium and magnesium are known as natural components of soil which have a much lower affinity to humic substances in comparison with copper [57–59] but can affect the binding of copper and suppress its complexation by humic substances [60–63]. Interactions between metal ions and fulvic acids can thus affect the mobility of metal ions by a decrease in the content of free metal ions in the water and soil solution as well as an increase in the size of metal particles caused by the formation of metal–fulvic complexes.

2. Materials and Methods

2.1. Chemicals

Copper(II) nitrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium(II) nitrate and magnesium(II) nitrate were purchased from Lach-Ner s.r.o. (Neratovice,

Czech Republic). Aqueous solutions of the salts were prepared in the concentration range of $0.1\text{--}1\text{ mol dm}^{-3}$. Pyrocatechol, salicylic acid, phenol, phthalic acid, and benzoic acids used as model compounds structurally similar to binding sites in fulvic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of p.a. grade.

Suwannee River Fulvic Acids (2S101F) were purchased from the International Humic Substances Society, which provides their characteristics such as the elemental composition, contents of functional groups, IR and NMR spectra, etc. (details can be found at <https://humic-substances.org/#products>, accessed on 29 February 2024 standard sample of Cat. No. 2S101F). This fulvic standard was also studied from the point of view of its content and strength of acidic functional groups, its interactions with metal ions, and conformational changes, e.g., [2,5,35,52,64,65]. The fulvic acid was purchased in the form of powder. The powder was dissolved in deionized water and used in the form of an aqueous solution with concentration of 1 g dm^{-3} . This means that 1 g of the sample contained 5.02 mmol of carboxylic groups and 1.22 mmol of phenolic groups [65]. The total content of acidic functional groups thus was 6.24 mmol dm^{-3} . In experiments, the solutions were kept at constant temperature ($25.0 \pm 0.1\text{ }^{\circ}\text{C}$) in an air-conditioned laboratory.

2.2. Determination of the Size, Charge, and Colloidal Stability of the Formed Complexes

The aqueous solution of fulvic acid (10 cm^3) was titrated by metal ion solutions (approximately 0.25 cm^3). The total addition was 15 cm^3 . Conductivity and pH values (Mettler Toledo SevenGo Duo multimeter, Mettler Toledo, Küssnacht, Switzerland) and zeta potential and particle size distributions (Zetasizer Nano ZS with backscattering detection, Malvern Panalytical, Malvern, UK) were measured after each addition. For homogeneous mixing in the cell, the stirrer speed was kept constant at 90 rpm. Measurements were triplicated. Measurements were realized at $25.0 \pm 0.1\text{ }^{\circ}\text{C}$ in an air-conditioned laboratory.

2.3. Isothermal Titration Calorimetry

A TAM III microcalorimeter (TA Instruments, New Castle, DE, USA) was used for calorimetric measurements. An aqueous solution of fulvic acid (0.75 cm^3) was titrated by ion solutions (0.005 cm^3). The total addition was 0.25 cm^3 . The titrant was injected at 10 min intervals. Each injection lasted 10 s. The temperature was set at $25.0 \pm 0.1\text{ }^{\circ}\text{C}$. Calorimetric experiments were controlled by TAM assistant v 3.0.4 software (including temperature). For homogeneous mixing in the cell, the stirrer speed was kept constant at 90 rpm. The TAM III microcalorimeter was also used for the calorimetric measurement of pyrocatechol and salicylic acid used as model compounds structurally similar to binding sites in fulvic acids. The data were processed with TAM assistant v. 3.0.4 software and NanoAnalyze v 3.5 software, both from TA instruments. Measurements were triplicated. Results are stated as average values).

3. Results and Discussion

In initial experiments, conductivity and pH were measured during titrations (Figure 1). The measured curves had expected shapes. The conductivity increased gradually as metal ions were added. The strongest increase was observed for calcium and the lowest for magnesium, which corresponds with limiting molar conductivities of individual metal ions [66]. The conductivity dependence obtained for magnesium deviated much more strongly in comparison with calcium and copper. Minima on the pH curves can be observed. This phenomenon was described in our previous works [47,51]. In the beginning, the pH values decreased as a result of the liberation of H^+ ions; thereafter, they increased. The minimum was lowest for copper, which has the strongest affinity to fulvic acids.

The question is what caused the increase in pH after the achievement of the minimum and why conductivity increased during the whole titration experiment. The increase in pH value means that the concentration of hydrogen ions decreased. In contrast, the increase in conductivity means that the concentration of ions increased. The shape of dependence on the concentration of hydrogen ions is typical for a reaction intermediate in consecutive

reactions. It means that hydrogen ions are produced in the initial reaction and consumed in the following reaction step. We suppose that the mechanism of interactions between metal ions and fulvic acids is more complex than the simple exchange of metal and H^+ ions (in acidic functional groups) or their association with dissociated acidic groups. One possible mechanism could be that metal ions (in the beginning) can occupy a majority of functional groups which results in the liberation of a high amount of hydrogen ions and a decrease in pH. An indication could be that the minimum pH value corresponds approximately with the total concentration of hydrogen ions in fulvic acidic functional groups in the case of Cu^{2+} ions. Then, a re-arrangement in complex structures can follow. Because all bivalent ions need two functional groups, it is necessary to find them not far from each other to form metal–fulvic complexes. The occupied functional groups, which cannot be connected with metal ions, are able to re-connect with hydrogen ions. It can result in an increase in pH. The conductivity is influenced by all ions in the system; therefore, its values can be affected by the liberation of hydrogen ions in the beginning and metal ions in the re-arrangement. However, this is only our hypothesis. Other studied ions (calcium, magnesium) had weaker effects, probably due to their lower affinity to fulvic acids. The used metal ions undergo hydrolysis because of the weak character of their hydroxides. The hydrolysis produces hydrogen ions. On the other hand, if the addition of metal ions results in their complexation with fulvic acids, their amount decreases, which shifts the equilibrium of hydrolysis in the direction of the reactants and the concentration of hydrogen ions decreases. The conductivity increases because of the continuing titration and the further addition of metal ions and corresponding anions.

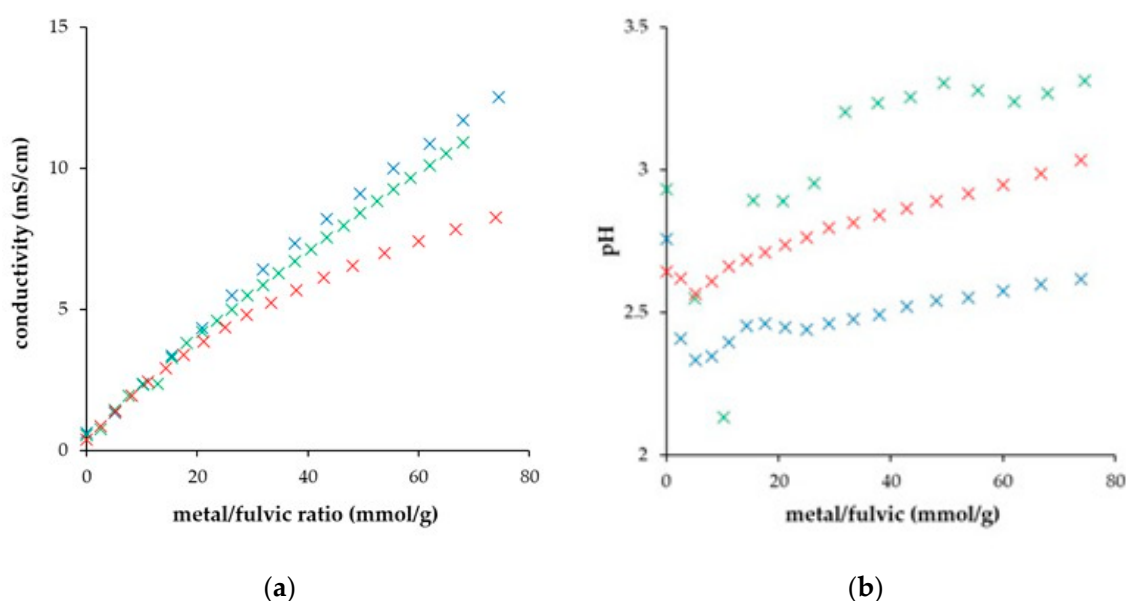


Figure 1. Dependencies of conductivity (a) and pH (b) on the ratio between metal ions and fulvic acid in the titration: Ca (blue), Cu (green), Mg (red).

The addition of metal ions to fulvic acids caused a gradual increase in the average diameter of metal–fulvic particles as well as the zeta potential. A higher increase in the average diameter of metal–humic complexes was observed for copper, which is known as a metal with a very high affinity to humic substances. The sizes of the complexes with calcium and magnesium were very similar. The average size of copper–fulvic complexes increased to about 30 nmol per gram of fulvic acid and then remained constant. The increase in the average size of fulvic complexes with calcium and magnesium was gradual, with stabilization above 40 nmol per gram of fulvic acids. The final average diameter was slightly below 4000 nm for copper and slightly above 1000 nm for calcium and magnesium. The charge of metal–fulvic particles increased and became closer to zero in the case of copper–fulvic complexes. The final zeta potential of fulvic complexes with calcium and

magnesium was about -10 mV. This means that fulvic particles became less stable through their interaction with metal ions and their colloidal stability was lower in the case of complexes with copper. It is necessary to explain that particles with zeta potentials below -30 mV and above $+30$ mV can be considered stable ones [35,37,43]. Particles with a zeta potential in the range between these two boundary values can be considered colloidally unstable and can easily undergo aggregation and sedimentation [34,41,45]. The interaction of fulvic acids with metal ions resulted in the compensation of the negative charge of carboxylic and phenolic groups; therefore, its value increased. In the case of calcium and magnesium, some of the functional groups probably remained charged due to their lower affinity to fulvic acids; therefore, the final charge was not close to zero.

An interesting phenomenon observed in Figure 2a is the local decrease in the average particle size at the beginning of the titration which was more perceptible for calcium and magnesium. In order to analyse this phenomenon, the particle size distribution was measured gradually in the titration.

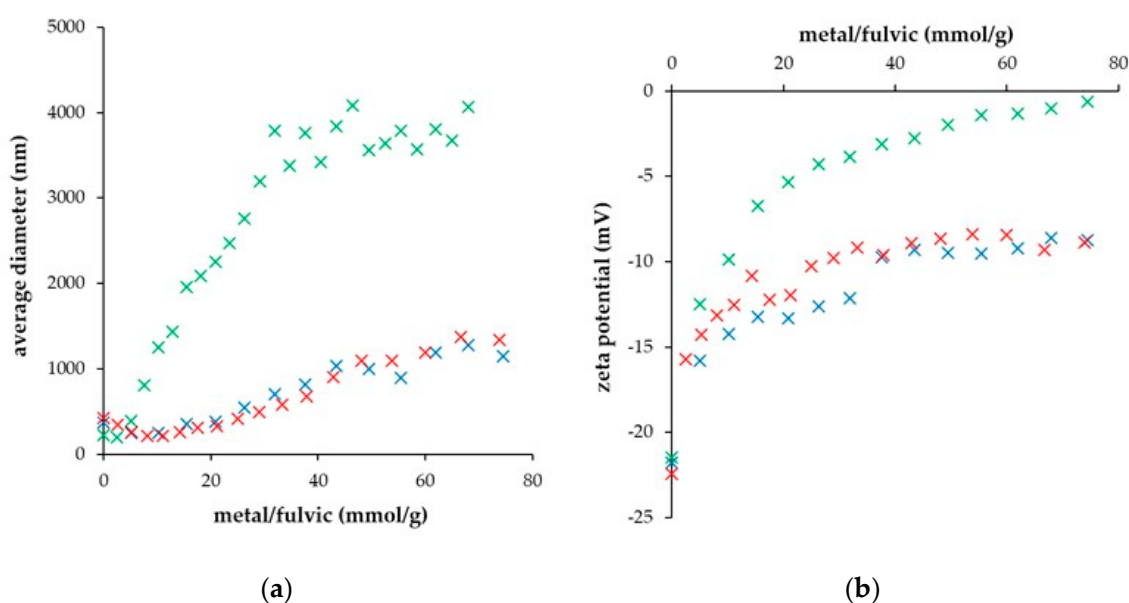


Figure 2. Dependencies of average diameter (a) and zeta potential (b) on the ratio between metal ions and fulvic acid in the titration: Ca (blue), Cu (green), Mg (red).

In Figure 3, a comparison of the particle size distribution of final metal–fulvic complexes with that of pure fulvic acids as well as the development of the distribution for the formation of the magnesium–fulvic complex in the titration are shown. We can see that pure fulvic acids had three size populations, with maxima at 28, 530, and 6440 nm. In the titration, the first population gradually disappeared, which was accompanied by an increase in the population with the biggest diameter. Temporarily, the intermediate population decreased and then re-increased. The final content of the fraction with the biggest diameter was lower than that in the course of the titration. The final magnesium–fulvic and calcium–fulvic complexes thus had a bimodal particle size distribution with maxima around 530 nm (Ca), 615 nm (Mg), and 5600 nm (both). Comparing individual metal–fulvic complexes, we can state that the character of the interactions between copper and fulvic acids differed from the character of interactions involving the other two metal ions. The system had a particle size distribution with only one maximum at 1100 nm, which was smaller in comparison with the bigger fraction of fulvic complexes with calcium and magnesium, and it was closer to the intermediate fraction of fulvic acids and the smaller fraction of calcium and magnesium complexes. In contrast to the other two metal ions, the particle size distribution of copper–fulvic complexes was relatively narrow and all particles had similar sizes. The initial decrease in average size was thus caused by the change from

a trimodal to a bimodal character of particle size distribution and the change in particle contents in the individual populations.

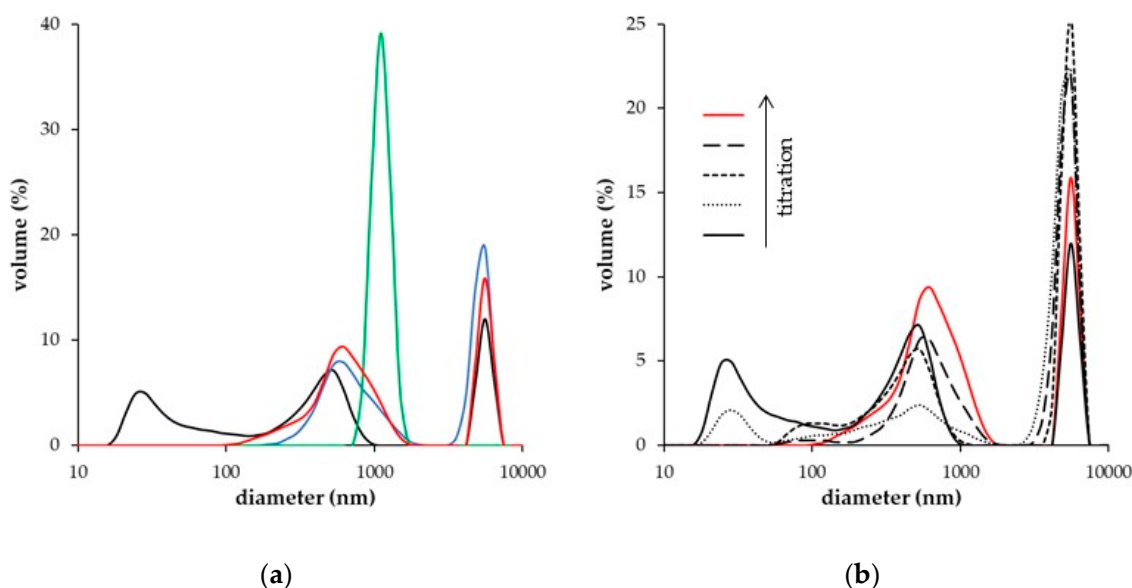


Figure 3. Particle size distributions for fulvic acids (black) and their complexes with Ca (blue), Cu (green), Mg (red) after the end of titration addition (a). The development of size distribution in the titration from pure fulvic acids (black) to final magnesium–fulvic complex (red) (b).

Calorimetric titrations were performed to directly measure the changes in reaction enthalpy in the interaction of fulvic acids with metal ions and subsequently to calculate other thermodynamic parameters such as changes in Gibbs energy (ΔG°) and entropy (ΔS°). A simple mathematical model, adapted from previous works [26,27], was used for the calculation of ΔG° and ΔS° . Examples of raw data are shown in Figure 4. Similar records were obtained for all titrations and all metal ions. As can be seen, the measured heat flow is in positive values, which indicates an endothermic process. It is relatively surprising because of the model calculations which gave negative values for reaction enthalpies [18,19]. Some authors also presented negative values for measured enthalpies [22,24,26,27]. In contrast, other authors measured positive enthalpies for interactions of humic substances with metal ions [22,23]. Antonelli et al. [21] stated that the thermic character of interactions is affected by the pH value and changes from exothermic (lower pH values) to endothermic (higher pH values).

We performed additional experiments with model compounds of binding sites in the fulvic structure such as pyrocatechol and salicylic acid (Figure 5). We can see that the shapes of the obtained curves are totally different from those of fulvic acids. Negative values of enthalpy indicated exothermic processes as predicted by quantum chemical calculations [17,18]. A similar discrepancy was described by Taraba for the immobilization of lead(II) in natural coal. His measurement provided positive a value of enthalpy, indicating an endothermic process, while the calculated value was positive, meaning an exothermic process. Calorimetric data obtained for pyrocatechol had a sigmoidal character, which is typical for this type of calorimetric experiment [22–24,26,27]. A similar shape of the titration curve was also obtained for phenol. In contrast, the data obtained for salicylic acids had a distinct minimum and then the enthalpy returned gradually to its initial values. This behaviour was also observed for benzoic and phthalic acids. It seems that the minimum appeared for model compounds containing carboxylic functional groups, while models containing only hydroxylic functional groups provided standard sigmoidal curves. If we compare changes in enthalpy determined on the basis of Figure 5 with the results of quantum chemical calculations published in [18], we obtain a value around -4000 J/mol for pyrocatechol and -270 J/mol for salicylic acid from calorimetric titrations,

in contrast to -265 J/mol (acidic pH) and -1848 J/mol (alkaline pH) for pyrocatechol and -353 J/mol (acidic pH) and -2150 J/mol (alkaline pH) for salicylic acid. This phenomenon should be investigated in detail in future work. The measured values differed in magnitude which can be a result of the different pH values. While the character of salicylic acid was strongly acidic, the character of pyrocatechol approached neutrality. The character of fulvic acids was also acidic; the measured enthalpies were thus in absolute values of the same magnitude as those obtained for salicylic acid. It is well known that the bond strength of metal ions with carboxylic functional groups is stronger in comparison with phenolic ones. Interactions of metal ions with different functional groups can have different mechanisms. Observed differences between model compounds and fulvic acids can be caused by the simple structure of model compounds in comparison to fulvic acids. Fulvic molecules have much more possibilities in the conformational arrangement of their molecules as well as formed complexes with metal ions. Further investigation is needed as mentioned.

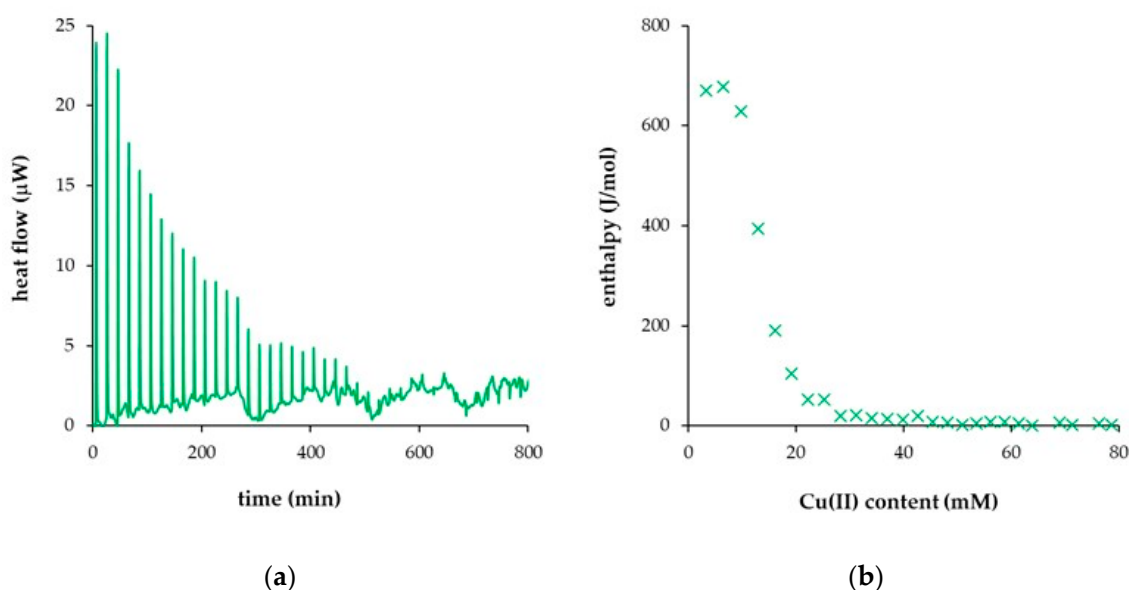


Figure 4. Raw calorimetric data for the titration of copper(II) ions into fulvic acids (a) and experimental data after determination (integration) of peak areas (b).

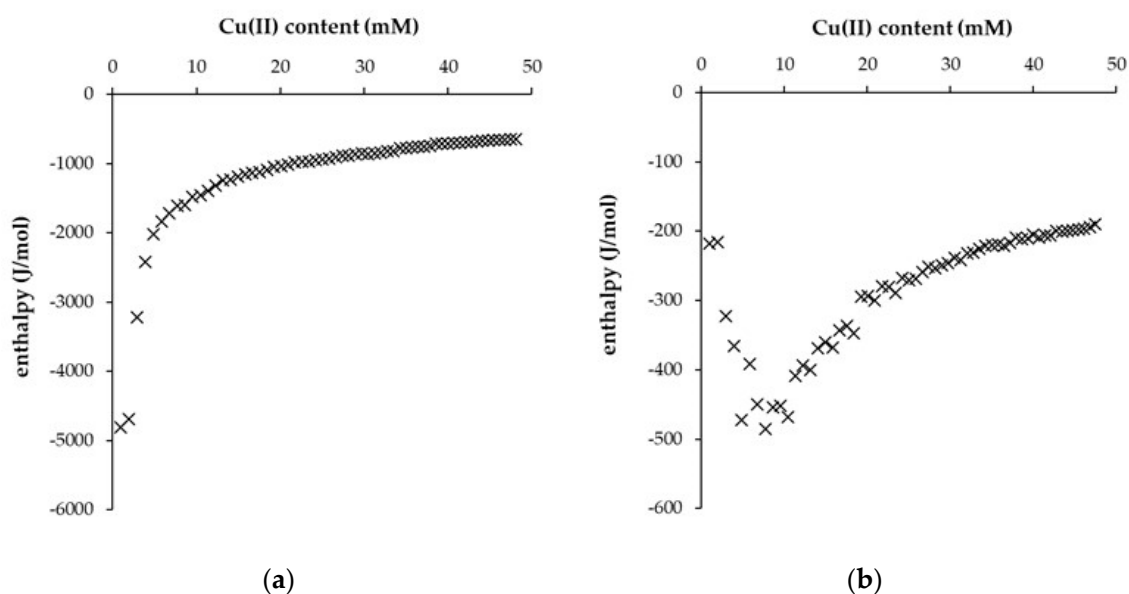


Figure 5. Calorimetric data for the titration of copper(II) ions into pyrocatechol (a) and salicylic acid (b) as model compound.

We adopted the simple mathematical model presented by Du et al. [26] and Kimuro et al. [27] where the change in Gibbs energy ΔG° can be expressed as

$$\Delta G^\circ = -RT \ln K_a, \quad (1)$$

where R is the (universal) molar gas constant, T is the absolute temperature, and K_a is the equilibrium binding constant called the association constant (the reciprocal value of the dissociation constant of the formed metal–fulvic complex). The heat released or consumed during binding is related to the number of binding sites available, the binding constant, and the thermodynamic parameters of the binding equilibrium. The K_a constant can be determined from the slope of the dependence of integrated raw calorimetric data. Peak areas per mole of added metal ions can be plotted versus the molar ratio of metal ions and fulvic acids [26,65]. The molar amount of fulvic acids was determined on the basis of the content of their acidic functional groups (described in Section 2.1). The equilibrium binding constants were determined as $710 \text{ dm}^3 \text{ mol}^{-1}$ for calcium, $429 \text{ dm}^3 \text{ mol}^{-1}$ for copper and $1312 \text{ dm}^3 \text{ mol}^{-1}$ for magnesium. Applying changes in Gibbs energy (ΔG°) and enthalpy (ΔH°) to the principal equation of thermodynamics:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ, \quad (2)$$

the entropy (ΔS°) can be determined [26,27]. The results of the calculations are listed in Table 1. As can be seen, the obtained values are relatively similar. The highest change in enthalpy was determined for copper(II) ions and the lowest for calcium(II) ions. The change in Gibbs energy was lowest for magnesium(II) ions and highest for copper(II) ions. The order in the change in entropy was inverse to Gibbs energy. Comparing our results with values published for the interaction of copper(II) ions with organic matter [23], we can state that the change in enthalpy was lower in magnitude while changes in Gibbs energy and entropy were comparable with published values. In consideration of the heterogeneous character of metal–fulvic complexation where metal ions can interact with more different binding sites [17,18,22], the thermodynamic parameters should be considered as apparent or effective as stated by Kimuro et al. [27].

Table 1. Thermodynamic parameters determined for the interactions of fulvic acids with metal ions.

	ΔH° (J/mol)	ΔG° (kJ/mol)	ΔS° (J/mol.K)
calcium	524 ± 46	-16.3 ± 0.1	56.3 ± 0.4
copper	687 ± 30	-15.0 ± 0.3	52.4 ± 0.9
magnesium	672 ± 47	-17.5 ± 0.3	62.4 ± 0.8

4. Conclusions

In this study, the interactions of fulvic acids with metal ions were studied from the point of view of particle size, charge, and thermodynamic characteristics. It was found that the average particle diameter increased gradually with the added titrant up to 30 mmol per gram of fulvic acid for copper(II) ions and 40 mmol per gram of fulvic acid for calcium(II) and magnesium(II) ions. The average diameter determined for copper(II) ions was much higher in comparison with the other two metal ions. In actual fact, the particle size distributions changed from trimodal for fulvic acids to bimodal for fulvic complexes with calcium and magnesium. Copper–fulvic complexes had only one size fraction. The zeta potential of fulvic particles increased with added titrant as a result of compensation of the negative charge of carboxylic and phenolic functional groups by positively charged metal ions. The charge neutralization was “absolute” in the case of copper(II) ions, where the zeta potential approached zero. Complexes with all three metal ions were colloiddally unstable because of their zeta potentials between -30 and $+30$ mV, which resulted in their visually observable sedimentation. In contrast to quantum chemical calculations and the results obtained for model substances, the interactions between metal ions and fulvic acids

were found to be endothermic, with positive values for changes in enthalpy. Changes in Gibbs energy were negative and changes in entropy were positive as expected and their values were comparable with results published for interactions of copper(II) ions with soil organic matter [27]. The calorimetric titration curves were of a sigmoidal character for fulvic acids, phenol, and pyrocatechol. In contrast, model compounds containing carboxylic functional groups (benzoic acid, phthalic acid, and salicylic acid) provided titration curves with a minimum. The titrations of all model compounds were observed to have an exothermic character. This phenomenon and discrepancy in comparison with fulvic acids (containing both phenolic and carboxylic functional groups similar to salicylic acid) should be investigated in detail in future research. However, our results confirmed that interactions between fulvic acids and the studied metal ions are spontaneous with an endothermic character. Model substances structurally similar to binding sites in fulvic acids gave different results and cannot be used as relevant standards for the investigation of interactions in detail. Fulvic acids are substances containing different size fractions as well as different binding sites with different affinities to metal ions and different bond strengths. In contrast, model substances have a well-defined structure with clearly defined binding sites and bond strengths. The resultant thermal effect is thus the combination of more effects belonging to partial reactions. Some of them can be endothermic, some of them exothermic. As described above, relevant studies presented both negative and positive enthalpies for interactions of humic substances with metal ions in dependence on the type and source of humic substances, which, moreover, can be affected by pH value. Nevertheless, a comparison of the values obtained for the same sample of humic substance under the same conditions can be useful for the analysis of the interactions.

The main conclusions of this study are that the formed complexes are colloiddally unstable which was relatively unexpected. Particle sizes increased with added titrant up to equilibrium values and were highest for copper–fulvic complexes. Particle size distributions differed for pure fulvic acids and their complexes with studied metal ions. The smallest fraction of fulvic ions disappeared by complexation and only one size fraction was detected in the case of copper–fulvic complexes (in comparison with two fractions observed for complexes with calcium and magnesium). The results of calorimetry provided more questions than answers. They indicated that the mechanism of interaction with different binding sites in fulvic structures can be different. Experiments with model compounds provided results corresponding to quantum chemical calculations. In contrast, they differed from results obtained for fulvic acids which have much more possibilities in conformational changes and molecular organization.

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