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Interactions of Metal Ions with Fulvic Acids: Particle Size Distribution, Colloidal Stability and Thermodynamic Aspects

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Abstract: The interactions of metal ions with fulvic acids resulted in changes in the particle size and charge. Particle size distribution was trimodal for fulvic acids, bimodal for fulvic complexes with calcium and magnesium, and copper-fulvic complexes had only one size fraction. The compensation of negative charge of carboxylic and phenolic functional groups by positive charged metal ions resulted in the increase in zeta potential which was getting closer to zero in the case of copper-fulvic complexes. However, all metal humic complexes behaved as colloidally unstable which resulted in visually observable sedimentation. Calorimetric measurements provided positive values of changes in enthalpy which indicated endothermic processes. In contrast quantum chemical calculations as well as experiments with model compounds provided negative values indicated exothermic processes. Changes in Gibbs energy were determined as negative and changes in entropy as positive which meant spontaneous processes.

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

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1. Introduction

Fulvic acids as a soluble fraction of humic substances are highly beneficial molecules promoting several soil functions such as nutrient absorption, transport of metals and improving soil structure. Due their solubility at all pH values they are more active in comparison with humic acids and can participate on the metal speciation as well as their complexation and aggregation. [1–4]. Most studies on migration of metal ions in natural systems are focused on free (hydrated) ions. However, metal ions are often dissolved as complexed 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 the humic acids than for the fulvic acids [4,8–11]. Some authors (e.g. Bertolli et al. [12]) calculated a thermodynamic stability as the change in reaction enthalpy between fulvic anion and metal cation. Thermodynamic aspects of interactions between humic substances and metals can contribute to a detailed understanding of the cation binding properties of humic substances and has particular importance for developing long-term solutions to the problems of waste storage and remediation as well as the metal ion migration and bioavailability in natural systems [13]. Thermodynamic parameters of metal-fulvic interactions are usually based on the experimental data obtained for adsorption [14–16] and the theoretical calculations [12,13,17,18] which can include different model binding sites [13,15,18]. Direct calorimetric measurements are relatively scarce. Bryan et al. [19] used microcalorimetry as aid for the determination of 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+}. Antonelli et al. [20,21] used calorimetry for the determination of binding capacity of fractionated humic acid 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. Qi et. al. [22] determined negative values of Gibbs free energy change and positive values of enthalpy change and entropy change (revealed that copper sorption was

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spontaneous) of interactions of Cu^{2+} ions with organic matter from the decomposition of rice straw. Sheng et al. [23] employed isothermal titration calorimetry and determined similar thermodynamic characteristics of the binding between aqueous metals and extracellular polymeric substances of activated sludge were investigated. Taraba [24] measured adsorption enthalpy of Pb^{2+} ions on natural coal and compared it with calculated value and showed that the calculated values of the adsorption enthalpy can considerably differ from these measured ones. Du et al. [25] 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 changes in Gibbs energy and adsorption enthalpy were negative, the change of entropy was determined as positive similarly as in the case of Qi et. al. [22]. Kimuro et al. [26] combined potentiometry and calorimetry for the determination of the thermodynamic values of complexation of typical humic acid and groundwater humic acid with copper and uranyl ions. They revealed the unique complexation mechanism for groundwater (Horonobe) humic acid based on the comparison of the complexation enthalpies of groundwater humic acid with that 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 [27–33] which affect their mobility. The size, shape, and charge of dissolved humic and fulvic particles and their complexes as well as their secondary structure in water environment can be very dynamic. The 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 as the concentration, pH, ionic strength (in which case the character of ions can be important). They have a strong propensity to aggregate which control their interactions with other components, mobility, and functioning in the environment [30–40]. Liu et al. [39] stated 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. Types of metal ions are an important factor that influences chemical fractionation of dissolved organic matter (and humic substances) in cation-induced coagulation due to their various binding affinities for their special compounds or functional groups [10,41–43]. In general, the carboxylate and phenolic groups are considered as predominate metal coordination sites in humic and fulvic acids [13,28,29,44–46]. Other functional groups can be possible ligands, although they are less prevalent. It is known that dissolved humic substances as fulvic acids can bind easily metal ions due their higher content of ionizable functional groups (supporting their solubility) and more accessible binding sites in water environment [20,41,46–53]. On the other hand, metal ions are able to bind with non-ionizable active centres also on the surface of solid humic particles as was shown in [54] and specific metal ions can interact with humic substances by 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 [39,55]. Calculations based on 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 [39,56].

As described above, interactions of humic substances with metal ions can result in heat effects and changes in size and charge of particles. Thermodynamic parameters as enthalpy, entropy and Gibbs energy (or rather their changes) can characterize the interactions from point of view of the ability of metal to bind with humic substances, stability of formed complexes, and 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 diffusion coefficient on the size of diffusing particles and their colloidal stability and conformational changes.

The aim of this study is to investigate the interactions between copper, calcium and magnesium ions from point of view of thermodynamics and size and charge of their complexes with fulvic acids.

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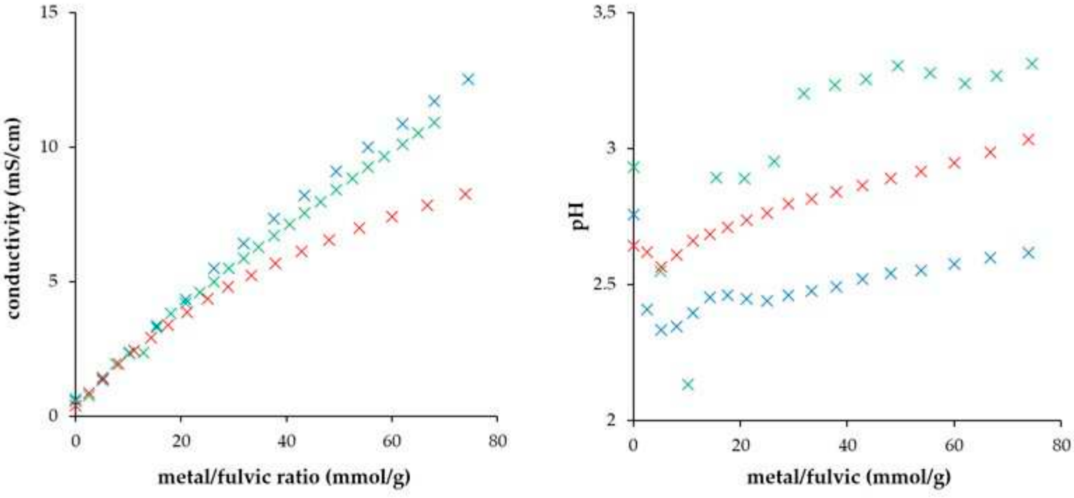
Enthalpy changes are measured directly by isothermal titration calorimetry. Fulvic acids were chosen due to their solubility caused by the higher content of dissociable functional groups and smaller size in comparison with humic acids [1,2,42–44,51] which classes them as the most active fraction of humic substances. Copper was used as metal ion with high affinity to humic substances [4,8,9,12,14,15,18,22,25,45,46,50,52,54]. Calcium and magnesium are known as natural components of soil which have much lower affinity to humic substances in comparison with copper [57–59] but can affect the binding of as copper and suppress its complexation by humic substances [60–63]. Interactions between metal ions and fulvic acids thus can affect their mobility by the decrease in the content of free metal ions in water and soil solution as well as the increase in the size of metal particles caused by the formation of metal-fulvic complexes.

2. Results and discussion

In initial experiments, conductivity and pH were measured during titrations (Figure 1). Measured curves had expected shapes. The conductivity increases gradually as metal ions are added. The strongest increase was observed for calcium, the lowest for magnesium which corresponds with limiting molar conductivities of individual metal ions [64]. Minima at pH curves can be observed. This phenomenon was described in our previous works [46,50]. At the beginning, pH values decreased as a result of the liberation of H^+ ions, thereafter they increased. The minimum is lowest for copper which has the strongest affinity to fulvic acids.

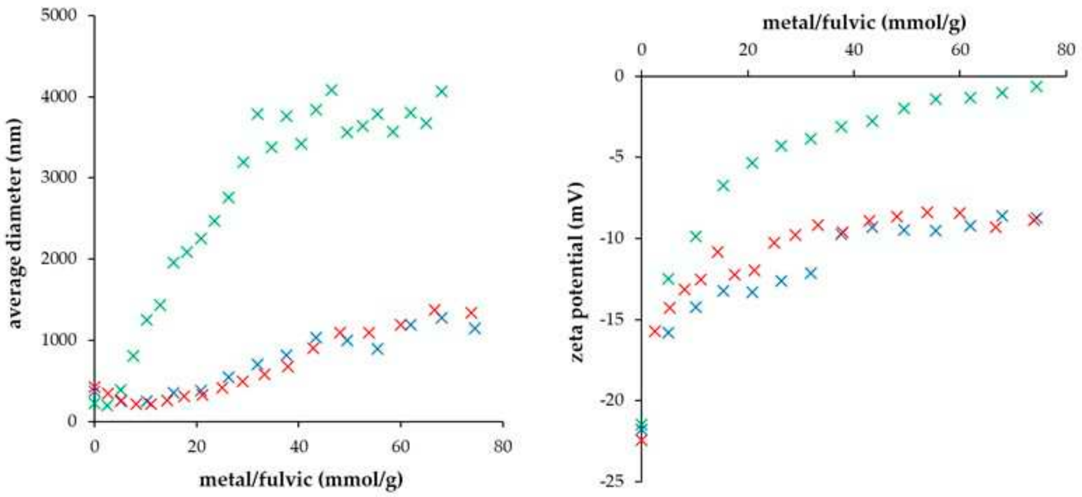


(**a**) (**b**)

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).

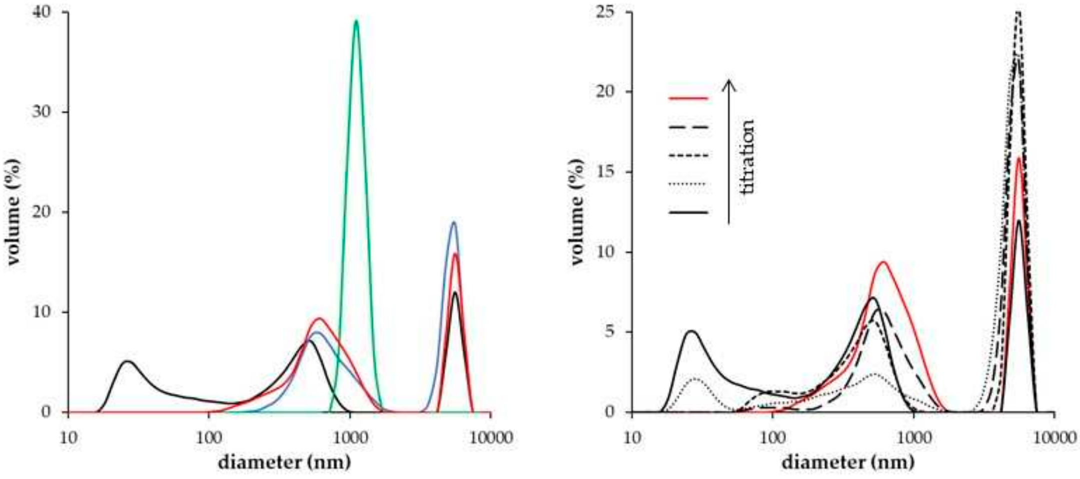
The addition of metal ions to fulvic acids caused the gradual increase in average diameter of metal-fulvic particles as well as the zeta potential. The higher increase in average diameter of metal humic complexes was observed for copper which is known as metal with very high affinity to humic substances. The sizes of complexes with calcium and magnesium are very similar. The average size of copper-fulvic complexes increased up to about 30 mmol 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 the stabilization above 40 mmol per gram of fulvic acids. 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 was getting closer to zero in the case of copper-fulvic complexes. Final zeta potential of fulvic complexes with calcium and magnesium was about -10 mv. It means that fulvic particles became less stable by the interaction with metal ions and their colloidal stability is lower in the case of complexes with copper. It is necessary to explain that particles with zeta potential bellow -30 mV and above +30 mV can be considered as stable ones [34,36,42]. Particles with zeta potential in the range between these two boundary values can be considered as colloidally unstable and can easily undergo the aggregation and sedimentation [33,40,44]. The interaction of fulvic acids with metals ions resulted in the compensation of negative charge of carboxylic and phenolic groups therefore its value increased. In the case of calcium and magnesium some of them probably remained charged due to their lower affinity to fulvic acids therefore the final charge was not closed to zero.

An interesting phenomenon observed in Figure 2a is the local decrease in the average particle size in the beginning of 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. In Figure 3, the comparison of the particle size distribution of final metal-fulvic complexes with pure fulvic acids as well as the development of the distribution for the formation of 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 the 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 is lower than that in the course of titration. Final magnesium-fulvic and calcium-fulvic complexes thus had the 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 interactions between copper and fulvic acids differed from other two metal ions. The system had particle size distribution with only one maximum at 1100 nm, which is smaller in the comparison with bigger fraction of fulvic complexes with calcium and magnesium and it is closer to intermediate fraction of fulvic acids and smaller fraction of calcium and magnesium complexes. In contrast to other two metal ions, the particle size distribution of copper-fulvic complexes is relatively narrow and all particles have similar sizes. The initial decrease of average size was thus caused by the change from trimodal to bimodal character of particle size distribution and the change in particle contents in the individual populations.



(**a**) (**b**)

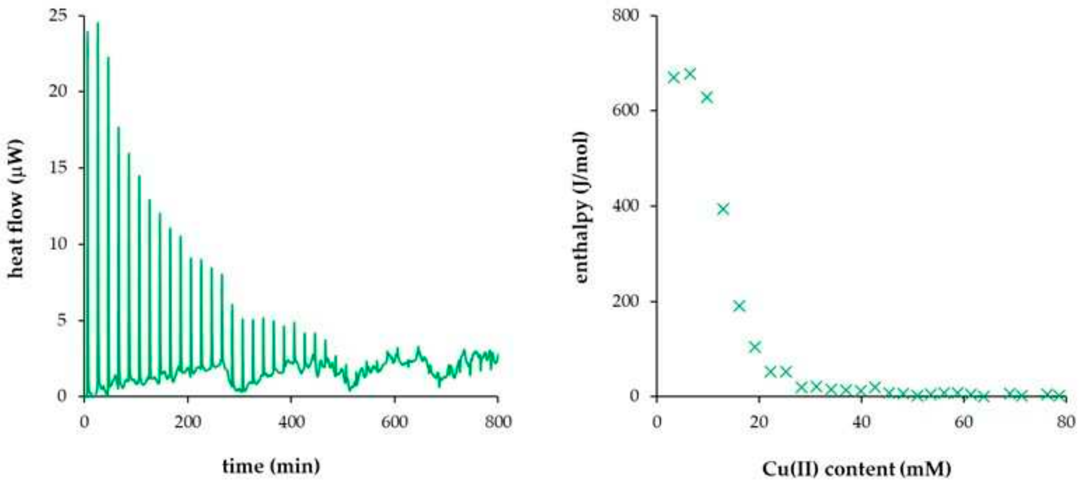
Figure 2. Dependencies of average diameter (**a**) and zeta potential (**b**) on the ratio between metal ions and fulvic acid in the titration: Ca (blue), Cu(green), Mg (red).



(**a**) (**b**)

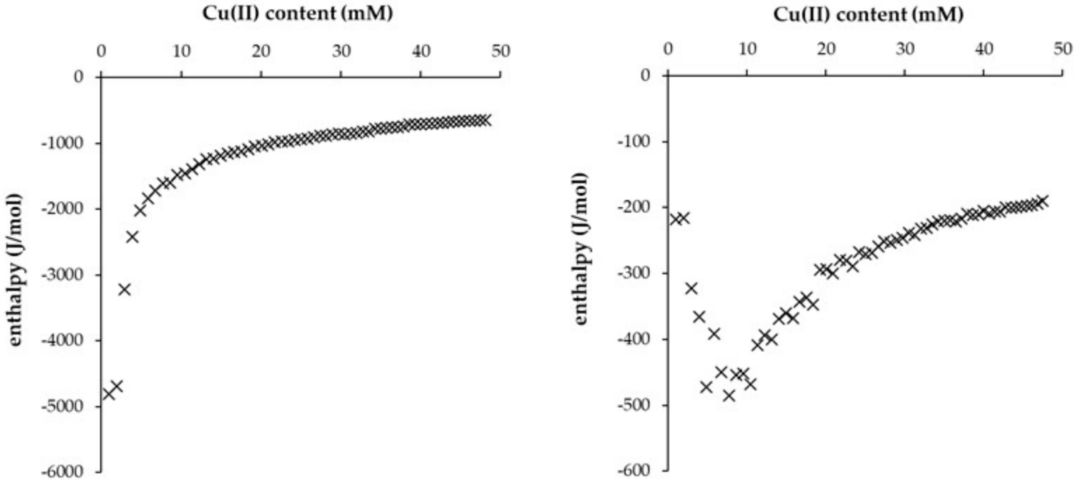
Figure 3. Particle size distributions for fulvic acids (black) and their complexes with Ca (blue), Cu(green), Mg (red) after finishing titration addition (**a**). The development of size distribution in the titration from pure fulvic acids (black) to final magnesium-fulvic complex (red) (**b**).

Calorimetric titrations were realized to directly measure the changes in reaction enthalpy in the interaction of fulvic acids with metal ions and subsequently to calculate other thermodynamic parameters as changes in Gibbs energy ($\Delta G°$) and entropy ($\Delta S°$). The simple mathematical model, adapted from previous works [25,26] was used for the calculation of $\Delta G°$ and $\Delta S°$. The 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 endothermic process. It is relatively surprising because of the model calculations which gave negative values of reaction enthalpies [17,18]. Some authors also presented negative values of measured enthalpies [21,23,25,26]. In contrast, other authors measured positive enthalpies of interactions of humic substances with metal ions [21,22]. Antonelli et al. [20] stated that the thermic character of interactions is affected by pH value and changes from exothermic (lower pH values) to endothermic (higher pH values). We realized additional experiments with model compounds of binding sites in fulvic structure as pyrocatechol and salicylic acid (Figure 5). We can see that the shapes of obtained curves are totally different in comparison with fulvic acids. Negative values of enthalpy indicated exothermic processes as predicted by the quantum chemical calculations [17,18]. Similar discrepancy was described by Taraba for the immobilization of lead(II) on natural coal. His measurement provided positive value of enthalpy meaning endothermic process while the calculated value was positive (exothermic process). Calorimetric data obtained for pyrocatechol had sigmoidal character. Similar shape of titration curve was obtained also for phenol. In contrast, the data obtained for salicylic acids had distinct minimum and then the enthalpy returned gradually to initial values. This behaviour was observed also 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 results of quantum chemical calculations published in [18], we obtain value around -4000 J/mol for pyrocatechol and -270 J/mol for salicylic acid from calorimetric titrations contrary to -265 J/mol (acidic pH) and -1848 J/mol (alkaline pH) for pyrocatechol; and -353 J/mol (acidic pH) and -2150 J/m (alkaline pH) for salicylic acid. This phenomenon should be investigated in detail in future work. Measured values differed in magnitude which can be caused by different pH value. While the character of salicylic acid was strongly acidic, the character of pyrocatechol was getting closer to neutral. The character of fulvic acids was also acidic, the measured enthalpies were thus in absolute values in the same magnitude as results obtained for salicylic acid.



(**a**) (**b**)

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**).



(**a**) (**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. [25] and Kimuro et al. [26] where the change in Gibbs energy $\Delta G°$ can be expressed as

$$\Delta G° = -RT\ln K_a, \tag{1}$$

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 (reciprocal value of the dissociation constant of the formed metal-fulvic complex). Applying change in Gibbs energy ($\Delta G°$) and enthalpy ($\Delta H°$) to the principal equation of thermodynamics:

$$\Delta G° = \Delta H° - T\Delta S°, \tag{2}$$

the entropy ($\Delta S°$) can be determined [25,26]. 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, the lowest for calcium(II) ions. Change in Gibbs energy was lowest for magnesium(II) ions and highest for copper(II) ions. The order in change in entropy was inverse to Gibbs energy. Comparing our results with values published for the interaction of copper(II) ions with organic matter [22], we can state that the change in enthalpy is lower in magnitude while changes in Gibbs energy and entropy are comparable with published values. In consideration of the heterogeneous character of metal-fulvic complexation where metal ions can interact with more different binding sites [17,18,21], the thermodynamic parameters should be considered as apparent or effective as stated by Kimuro et al. [26]. Negative Gibbs energy as well as positive entropy indicated that interatictions between fulvic acids and metal ions are spontaneous.

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

	D*H*° (J/mol)	**D*G*° (kJ/mol)**	**D*S*° (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

3. Conclusions

In this study, the interactions of fulvic acids with metal ions from point of view of particle size, charge, and thermodynamic characteristics were studied. It was found that the average 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 other two metal ions. In actual fact, the particle size distributions became from trimodal for fulvic acids to bimodal for fulvic complexes with calcium and magnesium. Copper-fulvic complexes had only one size fraction. Zeta potential of fulvic particles increased with added titrant as a result of the compensation of negative charge of carboxylic and phenolic functional group by positively charged metal ions. The charge neutralization is “absolute” in the case of copper(II) ions where zeta potential was getting closer to zero. Complexes with all three metal ions were colloidally unstable because of their zeta potential between -30 and +30 mV which resulted in their sedimentation (visually observable). In contrast to quantum chemical calculations and results obtained for model substances, the interactions between metal ions and fulvic acids were found as endothermic with positive values of 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 [26]. Results confirmed that interactions between fulvic acids and studied metal ions are spontaneous with 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 affinity to metal ions and bond strengths. In contrast, model substances have well-defined structure with clearly defined binding sites and bond strengths. The resultant thermal effect is thus 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 of interactions of humic substances with metal ions in the dependence of ty and source of humic substances which can be moreover affected by pH value. Nevertheless, the comparison of values obtained for the same sample of humic substance under the same conditions can be useful parameters for the analysis of the interactions.

4. Materials and Methods

4.1. Chemicals

Copper(II) nitrate was purchased from Sigma-Aldrich (St. Luis, 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 – 1 mol dm^{-3}.

Suwanee River Fulvic Acids (2S101F) were purchased from International Humic Substances Society which provides their basic characteristics as the elemental composition, contents of functional groups etc. (www. https://humic-substances.org/#products). This fulvic standard was also studied from point of view of their content of acidic functional groups, interactions with metal ions and conformational changes, e.g. [2,5,34,51,52,65,66]. Fulvic acid was used in the form of the aqueous solution with concentration 1 g dm^{-3}. It means that 1 g of the sample contains 5.02 mmol of carboxylic groups and 1.22 mmol of phenolic groups [66]. The total content of acidic functional groups thus was 6.24 mmol dm^{-3}.

4.2. Determination of size, charge, and colloidal stability of formed complexes

Aqueous solution of fulvic acid (10 cm^3) was titrated by metal ion solutions (á approximately 0.25 cm^3). The total addition was 15 cm^3. Conductivity and pH values (multimeter Mettler Toledo SevenGo Duo, Mettler Toledo, Küsnacht, Switzerland), zeta potential and particle size distributions (Zetasizer Nano ZS with backscattering detection, Malvern Panalytical, Malvern, GB) were measured after each addition. For homogeneous mixing in the cell, the stirrer speed was kept constant at 90 rpm. Measurements were triplicated. Data are presented as average values with standard deviation bars. Measurements were realized at 25.0 ± 0.1 °C.

4.3. Isothermal titration calorimetry

Microcalorimeter TAM III (TA Instruments, New Castle, DE, U.S.) was used for calorimetric measurements. Aqueous solution of fulvic acid (0.75 cm^3) was titrated by ion solutions (á 0.005 cm^3). The total addition was 0.25 cm^3. The titrant was injected at 10 min interval. Each injection lasted 10 s. The temperature was set on 25.0 ± 0.1 °C. For homogeneous mixing in the cell, the stirrer speed was kept constant at 90 rpm. Microcalorimeter TAM III (TA Instruments, New Castle, DE, U.S.). The data were processed with TAM assistant software and NanoAnalyze software, both from TA instruments. Measurements were triplicated. Data are presented as average values with standard deviation bars.

Author Contributions: Conceptualization, M.K. (Martina Klučáková) and J.K.; methodology, M.K. (Michal Kalina) and J.K., validation, J.K. and M.K. (Martina Klučáková); formal analysis, J.K. and M.K. (Michal Kalina); writing—original draft preparation, M.K. (Martina Klučáková). All authors have read and agreed to the published version of the manuscript.

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