Research Article

Complexation of Copper(II) with Humic Acids Studied by Ultrasound Spectrometry

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Copper biogeochemistry is controlled by bonding to natural organic matter. The soluble forms of bonded copper can be more biologically active due to the higher migration in environmental systems and instability of some copper-humic complexes. In this work, the interactions of copper(II) ions with humic acids are studied by means of high-resolution ultrasound spectrometry. It was found that the stoichiometry of the formed complexes is strongly influenced by the organization of humic acid in solution and by the final pH value in equilibrium. Although the ratio between the added copper and humic acids in all used concentrations was constant and the initial pH value was neutral, we observed significant differences between the individual systems. The highest binding ability was determined for a humic content of $0.5\,g\cdot dm^{-3}$. More diluted and more concentrated systems were able to bind lower amounts of copper. The implemented method is very sensitive and can be utilized not only for monitoring copper binding but also as an indicator of conformational changes of humic acid in solutions with varying concentration.

1. Introduction

Complexation ability is a significant property of humic acids that plays an important role in the mobility and bioavailability of contaminants and nutrients in the environment. It is very sensitive to many factors, such as pH, ionic strength, and the content of humic acid in various natural systems. Many authors have demonstrated a dependence of pollutant binding on the concentration of humic substances in studied systems. In the case of copper(II) binding on solid humic particles, varying sorbent content influences strongly the adsorbed amounts and the determined adsorption coefficients [1]. Other works [2, 3] have shown that copper adsorption increases with an increase in the content of suspended humic particles and decreases with low pH values or with an increase in dissolved organic matter. When the pH is high, copper adsorption tends to decrease because of the formation of metal hydroxides or soluble metal organic complexes. Copper partitioning is the greatest at a neutral pH region and decreases at either low or high pH regions. The authors concluded that the fate of copper in natural waters depends on such partitioning characteristics in the solid solution system. A high degree of metal adsorption occurs when the pH value is nearly neutral, the particulate organic matter is high and the concentration of solids is elevated. Under these conditions minor fractions of metals are available to aquatic biota. The study of copper binding to a soil humic acid at pH 4 demonstrated that that copper(II) binding affinity increased when humic acid concentrations were increased from 60 to $250\,mg\cdot dm^{-3}$ [4]. The authors speculated that conformational changes in humic acid were caused by higher humic acid and copper concentrations resulting in increased retention of free copper(II) ions through entrapment.

The results of the size exclusion chromatography and nuclear magnetic resonance spectroscopy indicated that dissolved humic acids may be micelle-like, which are supramolecular assemblies of small entities [5–7]. It was found that the apparent conformation of humic acids at concentrations of $\sim 1\,g\cdot dm^{-3}$ may differ strongly from the conformation in more dilute systems. If micelle-like structures form at high concentrations, a portion of humic reactive functional groups may be less accessible for metal ions as they might be entrapped in the more hydrophobic interior [8].

Contrary to the aforementioned results, some works concluded that the copper binding isotherms obtained at

TABLE 1: Elemental analysis and total acidity of humic sample (normalized on dry ash-free sample).

C (% at.)	H (% at.)	N (% at.)	S (% at.)	O (% at.)	Total acidity (mmol·g^{-1})
43.9	40.2	0.7	0.2	15.0	5.0

constant pH values in the range pH 4–8 are shown to be independent of humic and fulvic acids concentrations [8]. Only a slightly higher copper(II) binding affinity was observed at 1000 mg·dm^{-3} DOM, as compared to 50 mg·dm^{-3} DOM at pH 7, whereas the binding isotherms for 50 and 1000 mg·dm^{-3} were identical for pH 4, 5, and 6 [9].

Based on the published results, we decided to study the interactions of copper(II) ions with humic acids in the dissolved form at neutral pH. A wide range of used humic concentrations (0.01–3 g·dm^{-3}) was chosen in order to investigate the effect of conformational changes in humic acids upon copper binding. High-resolution ultrasound spectrometry was used as an analytical method sensitive to molecular organizations of studied systems, their hydration and conformational rearrangements of the studied systems [10, 11]. The ultrasonic analysis is based on the measurement of parameters of ultrasonic waves propagating through the sample. Ultrasonic attenuation describes the decay of the amplitude of the ultrasonic wave with distance traveled. Ultrasonic velocity is the speed of this wave and is related to the wavelength and the frequency of oscillation of the deformation. It is determined by the density and elasticity of the sample, which is strongly influenced by the molecular arrangement. The applications of ultrasound spectrometry thus include structural analysis, particle sizing [12], analysis of chemical reactions [13], detection of conformational changes in biopolymers [14], and ligand-polymer binding [11].

2. Materials and Methods

2.1. Preparation and Characterization of Humic Sample. Humic acids were extracted from the South Moravian lignite using a mixture of NaOH and Na$_4$P$_2$O$_7$ solutions by the procedure described in our work [15] and characterized previously [15, 16]. Elemental analysis was carried out using a CHNSO Microanalyzer Flash 1112 (Carlo Erba). For the determination of total acidity, humic acids were suspended in water in the ratio of 1 g : 50 cm^3 and were stirred for 24 h to reach equilibrium. The equilibrated dispersion was titrated by a NaOH standard solution ($c = 1.0$ mol·dm^{-3}). Conductometry (Hanna Instruments HI8820N) and potentiometry (Sentron Titan K185-016) were used for monitoring the course of neutralization. The obtained values are listed in Table 1.

2.2. Ultrasound Study. Humic acids for the ultrasound study were used in the form of colloidal solutions (sols) with concentrations of 0.01, 0.1, 0.5, 1, 2, and 3 g·dm^{-3}. The sols were prepared by the dissolution of the humic acids in NaOH (0.1 mol·dm^{-3}) and the addition of the same volume of HCl (0.1 mol·dm^{-3}). The final pH value of the obtained sols was 7 ± 0.05.
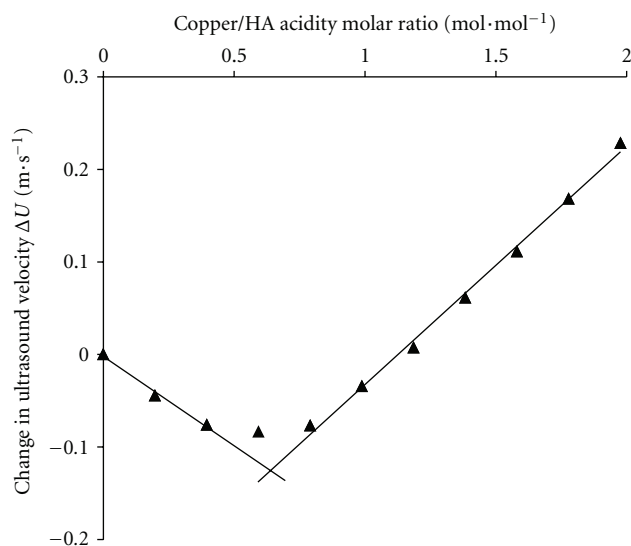

FIGURE 1: Ultrasound data obtained for titration of humic sol at concentration of 1 g·dm^{-3} by Cu(II) ions. The point of intersection corresponds with stoichiometry of formed complexes.

An ultrasonic spectrometer with high-resolution HR-US 102 (Ultrasonic Scientific, Ireland) was utilized for measuring basic ultrasonic parameters at three various frequencies (5110, 8220, and 12 200 kHz). Six values of the ultrasonic parameters were measured and averaged for each frequency. The device consisting of two independent cells tempered at 25°C. Cell 1 was used for measuring the humic sol and cell 2 was used for the blank solution. The ultrasonic velocity (U) and attenuation (N) in both cells were monitored and the resulting values ΔU and ΔN were calculated as the differences between the sample and reference cells. Water was used as the blank solution for determining the ultrasonic parameters of the humic sols.

In the complexation experiments, both cells were filled by the same humic sol (1 cm^3). The sols in cell 1 were titrated by a CuCl$_2$ solution at concentrations of 0.001, 0.01, 0.05, 0.1, 0.2, and 0.3 mol·dm^{-3}, respectively. The six values of ΔU and ΔN were measured after each addition.

The density of the humic sols was determined by means of a DMA 4500 (Anton Paar). The presented results are the average values of the six measurements.

3. Results and Discussion

The example of ultrasound data obtained for the complexation of the humic colloidal solution at a concentration of 1 g·dm^{-3} is shown in Figure 1. The copper/acidity molar ratio (*x*-axis) represents the ratio between the total added
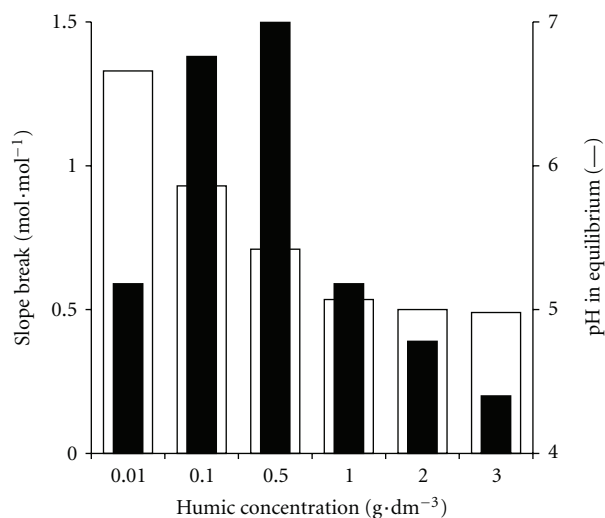
Figure 2: The stoichiometry of formed complexes (black columns) and the pH value in equilibrium (white columns) for various concentrations of humic acids. The stoichiometry corresponds with slope break of ultrasound velocity—see Figure 1.

amount of copper(II) ions in the titration and the content of humic acidic groups in the ultrasonic cell. At the beginning of the experiment, both cells are filled by the same humic sol; therefore the plotted changes in the ultrasonic velocity represent only the interaction of the humic acids with copper(II) ions. The initial decrease in ultrasonic velocity is caused by the release of hydration water from the coordination shell of copper and humic acids. The compressibility of water in the hydration shells is less than the compressibility of bulk water; therefore, transferring hydration water into the bulk water increases the total compressibility of the solution, reducing the ultrasonic velocity. The slope of the measured dependence changes if all available sites on the humic acids are occupied, and it corresponds to the stoichiometry of the formed complexes.

As mentioned previously, the conformation in higher concentrations of humic acids ($\sim 1\,g \cdot dm^{-3}$) may differ strongly from the conformation in more dilute systems. We investigated the influence of humic concentration on the complex stoichiometry. Figure 2 shows how the observed slope break (corresponding to the complex stoichiometry) changes with varying humic content. We can see a maximum of copper/acidity molar ratio in the break for humic sol at a concentration of $0.5\,g \cdot dm^{-3}$ which decreases for all of the other used concentrations. The diluted solutions are able to bind a higher number of copper(II) ions due to the effect of humic organizations with more accessible active sites. The conformational changes of the dissolved humic acids at low concentrations ($<1\,g \cdot dm^{-3}$) were demonstrated by many authors [5, 7, 8]. Some works also demonstrated the increase in copper(II) binding affinity with higher humic concentration, but the increase was always observed in the diluted humic solutions [4].

Notably the copper/acidity molar ratio in the break of the ultrasound data is higher than 1 for humic concentrations of 0.1 and $0.5\,g \cdot dm^{-3}$. This ratio represents the number of copper(II) ions related to the number of acidic groups of the humic acids in the device cell and in the break it should correspond to the stoichiometry of the formed complexes. A value equal to 1 thus means that one copper(II) ion fills one acidic functional group. It is evident that metal ions are bonded also on other humic structures as acidic functional groups at these two concentrations. There are many possibilities for copper interactions with humic substances. Results of a sorption study [17] showed that when the initial concentration of metal is low, the ions form mainly 2 : 1 complexes, which are energetically favored. This is the case for the lowest humic concentration used in our work (copper/acidity molar ratio is ~ 0.5). The same authors reported that when the concentration is sufficiently high to occupy most of the functional groups, the ions are heterogeneously retained over the whole surface of the humic particles. In a recent work [18], copper was found to bind preferentially with high molecular weight fractions of dissolved organic matter. This result corresponds with our previous results showing on copper binding with aromatic structures and other binding sites that cannot lose hydrogen ions and only part of the metal ions bonds to the humic acids through carboxylic or phenolic groups [19]. Although many authors consider the bidentate sites of phthalate- and salicylate-type groups as predominant sites for copper binding [20, 21], recent works have demonstrated that a significant proportion of aromatic carboxylic and phenolic groups do not participate in chelating sites [22, 23]. When the copper-binding properties of the hydrophobic acid fraction and two hydrophilic fractions isolated from a soil solution were investigated, the most hydrophilic fraction appeared to be dominated by weak acid sites. The Cu-binding behavior of the two hydrophilic acid fractions was very similar to that of the hydrophobic acid fraction, despite the differences observed in proton-binding characteristics at pH values of 4, 6, and 9 [24]. XANES and EXAFS spectroscopic studies [25] showed evidence for a five-membered $Cu(malate)_2$-like ring chelate and a six-membered $Cu(malonate)_{1-2}$-like ring chelate as the strongest copper humic complexes. The weaker binding ability of functionalized aromatic rings is explained, as malate-type and malonate-type structures are present only on aliphatic chains. For example, salicylate is a monocarboxylate that forms an unfavorable six-membered chelate, because the OH substitution is in the β position. Similarly, phthalate is a dicarboxylate forming a highly strained seven-membered chelate. The authors also published that aminocarboxylate Cu(II) chelates may form after saturation of the strongest sites or near neutral pH. The important role of N content in the affinity of humic substances towards copper was demonstrated in other works [26–29]. Some authors [20, 21] reported phenolic ethers as important structures in copper binding. Lastly, Li et al. [30] published work on the unknown phenomenon of humics biological activity when carbonylated derivatives are served as copper antagonists.

As can be seen, many authors have observed similar behaviors of humic acids in their binding with copper. It is evident that the humic samples are able to bind copper(II) ions on active centers of different acidic functional groups.

If the elemental composition (Table 1) is converted into mmol·g^{-1} of humic acid, the value of 18.2 and 0.8 mmol·g^{-1} for oxygen and nitrogen can be obtained, respectively. Comparing oxygen content to total acidity (5 mmol·g^{-1}), it can be stated that there is a sufficient amount of oxygen for "other" binding sites. The nitrogen content is relatively low but its presence can be important mainly at low copper concentrations [27].

A relatively important role in the stoichiometry of the formed complexes is in the pH value. At the beginning of the experiment the pH of humic sols is ∼7. This value gradually decreased with the titration of humic sol by copper(II) ions. The white columns in Figure 2 show the value of pH at equilibrium of the reaction between copper and humic acid. While the pH decreases only slightly for the lowest concentration of humic acid, more concentrated humic sols (>0.5 g·dm^{-3}) maintained a pH level approximately equal to 5 at equilibrium. Experiments with ion exchange resins of different types showed that the maximum content of labile copper complexes (∼30%) is at a pH bellow 5 and the portion of soluble nonlabile complexes increases with increasing pH [31]. The lower pH values in equilibrium support the decrease in amount of bonded copper for concentrated humic sols.

The ultrasound velocity, attenuation, and density of the used humic sols were measured in order to study conformational changes in the humic acid in solution and their influence on copper complexation. Figure 3 shows the changes in velocity and compressibility of variously concentrated humic sols. It can be seen that the measured ultrasound velocity is higher in comparison with water in the reference cell as expected, and it depends on the humic concentration. The minimum of ΔU was observed for humic content of 1 g·dm^{-3}, which is considered as the important concentration, due to the conformation of humic acids [5–7]. The ultrasonic velocity is determined by the elasticity and the density of the measured sample. Generally, the elastic response, which is extremely sensitive to molecular organization and intermolecular interactions, is dominant. We measured the density of the humic sols and the concentration dependence was strongly linear. The obtained equation is $\rho = 0.3137 \cdot c_{HA} + 999.15$ (g·dm^{-3}); $r^2 = 0.9981$. Therefore, the observed minimum must be the result of sample elasticity.

The compressibility of humic sols as shown in Figure 3 was computed according to the following equation [32]:

$$\beta = \frac{1}{\rho U^2}, \tag{1}$$

where β is compressibility, ρ is density, and U is the ultrasound velocity of humic sol in cell 1. The compressibility in Figure 3 is normalized to water (the computed value is divided by the compressibility of water). The obtained values of compressibility are comparable to humic content up to 1 g·dm^{-3}, and then they decrease with increasing concentration. This confirms that the organization of humic particles in diluted and concentrated humic sols is different. The decrease in compressibility shows the forming of more rigid structures which can cause the decrease in humic binding ability.
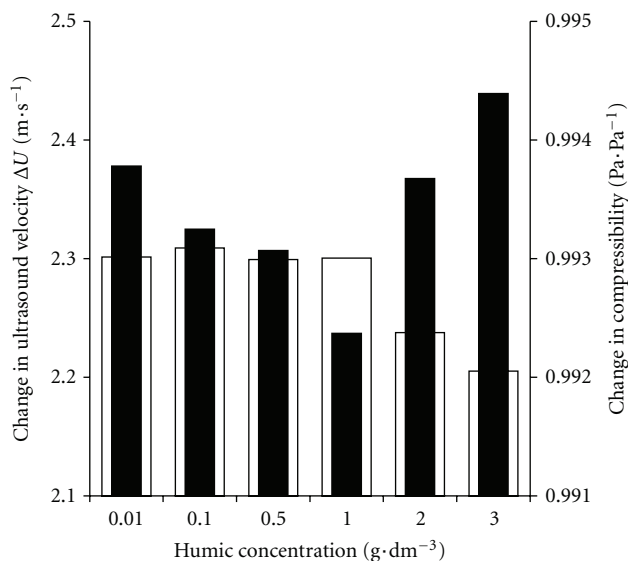

Figure 3: The change of ultrasound velocity (black columns) and compressibility (white columns) in dependence of concentration of humic acid.
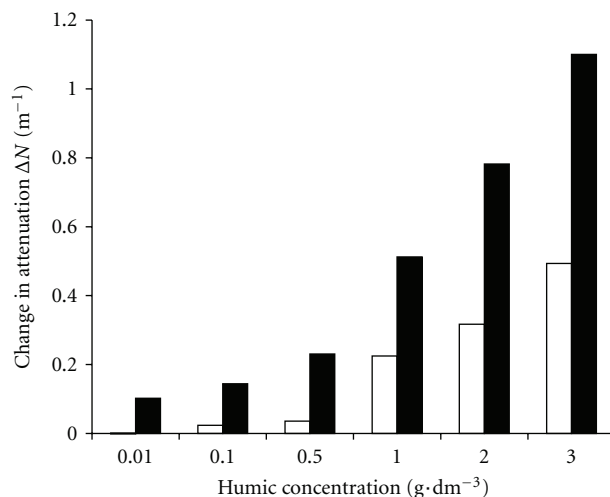

Figure 4: The change of ultrasound attenuation in dependence of concentration of humic acid (white columns for 8220 kHz and black columns for 12 200 kHz).

This conformational change was confirmed also by the strong increase in ultrasound attenuation for humic sols with concentrations ≥1 g·dm^{-3} (Figure 4). The attenuation characterizes the ultrasonic transparency of the sample and can be seen as a reduction in the amplitude of the wave. Its increase is usually related to aggregation of humic acids in more concentrated solutions, which was observed by means of a surface tension measurement [33].

The ultrasound data confirm that the organization of humic acid in solution is strongly dependent on its concentration. The conformational changes influence the accessibility of binding sites, and combined with pH, they are determining factors for copper-humic complexation. The pH

value influences both the conformation of humic acid, for example, electrophoretic mobility, related to the size and conformation of humic particles, which decreases significantly below a pH of 6 [34], and the form of copper(II) ions in solution [35]. The initial pH of all the used humic sols was neutral (~7). Because it decreased only slightly during complexation in diluted humic sol ($0.01\,g\cdot dm^{-3}$), the influence of pH is not important in this case and the stoichiometry of the formed complexes is 2 : 1 (copper/acidity molar ratio ~0.5). An increase in the number of bonded copper(II) ions was observed for humic concentrations of 0.1 and $0.5\,g\cdot dm^{-3}$. The ultrasound measurement showed that the compressibility of these two humic sols was essentially the same as that of the more diluted solution, which implies very similar accessibility of functional groups. Copper can be bound by active centers other than acidic functional groups due to higher concentrations as was published earlier; see, for example, [4]. The more concentrated humic sols have complexation abilities lower than or comparable to diluted humic solutions. The reason for this result is mainly due to the conformational changes detected by the lower compressibility and the decrease in the pH for complexation. The humic sol with a concentration of $1\,g\cdot dm^{-3}$ has the compressibility comparable to more diluted solutions but the pH at equilibrium is ~5 similarly to the more concentrated systems. This results in a complexation ability similar to diluted solutions but the conformation and organization of the formed complexes is probably different.

4. Conclusions

The stoichiometry of copper-humic complexes is influenced by more than one parameter. The first is the organization of humic acid in solution and its conformational changes with varying concentration and pH. The measured ultrasound data are very sensitive to these changes and can be utilized as an indicator of humic organization in solutions. It is known that copper can form with humic acid different types of complexes with humic acids depending on the pH. Although the initial pH value of all the humic sols was the same (~7), its decrease during complexation influenced the stoichiometry of the formed complexes. The highest affinity of humic acid to copper was observed for humic content of 0.1 and $0.5\,g\cdot dm^{-3}$. The organization of more concentrated solutions changes, and the humic particles can aggregate, which causes worse accessibility of the active sites and a decrease in the amount of bonded copper.

Acknowledgment

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