THERMODYNAMIC ANALYSIS OF Cu(II) AND Pb(II) ION BINDING TO HUMIC ACIDS ISOLATED FROM DIFFERENT ORIGINS AND STRUCTURAL CHARACTERIZATION USING FTIR AND ^{13}C NMR SPECTROSCOPY

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Abstract

The aim of this work was study molecular and quantitative analysis of metal ion binding to humic acids (HAs). The object of our study were two various samples of HAs. HAs were isolated from South-Moravian lignite from the mine Mír (locality Mikulčice, Czech Republic) and compost which was obtained from the composting plant in Náměšť n. Oslavou, Czech Republic. Isolation of HAs was performed according to the procedure recommended by the International Humic Substances Society (IHSS). The reactivity (thermodynamics) of humic acids (HAs) and metals was studied by isothermal titration calorimetry (ITC). As model metals were chosen Cu (II) and Pb (II) ions. ITC was observed reaction enthalpy, Gibbs energy, enthropy and the amount of metal bound to a specific humic acid. The HAs were characterized and compared using spectroscopic techniques, including FTIR and liquid-state ^{13}C NMR spectroscopy. Infrared spectroscopy is a useful technique in characterization of structure, functional groups and formation modes of HAs. ^{13}C NMR spectra of HAs were obtained with a Bruker Avance III NMR spectrometer. For data analysis, the spectra were divided into chemical shift regions assigned to the following classes of chemical groups: alkyl C (0–45 ppm), O-alkyl C (45–106 ppm), aromatic C (106–165 ppm), and carbonyl and carboxyl C (165–220 ppm), respectively. Aromaticity (f_a), hydrophilicity and hydrophobicity ratio (Hfi/Hfo) and biological activity (BiA) of HAs were calculated from the area of the NMR spectra.

Keywords: humic acids; isothermal titration calorimetry; complexation; metal ions

1.   INTRODUCTION

Humic substances are well known to be one of the most important soil constituents [1,2]. They are the principal components of soil organic matter and have indispensable roles for soil and the environment in general. [3] Due to their colloidal and polyfunctional character, these substances play important roles in the mobility and bioavailability of nutrients and contaminants in the environment [4]. They determine the pH buffering capacity and cation exchange capabilities in natural waters and soils [5].

Humic acids are capable of interaction with wide range of substances, such as metals, organic and inorganic pollutants in soils and waters. Another very important feature of humic acids presence in the soil is their high buffering ability in a wide range of pH values [6]. Humic acids are essentially large system of polyfunctional groups such as lipids, carbohydrates, aromatic compounds, and others. These compounds differ significantly in content of functional groups of the humic acids according to their origin and formation. According to the group of authors [7] these groups are responsible for their reactivity as they are capable of ionization. Among these functional groups oxygen, nitrogen and sulfur forming carboxylic, phenolic, hydroxyl, amine and thiol functional groups are presented.

In view of the frequent use of humic substances in the environment, for example: in remedial technologies, it is important to know the binding interaction of substances with contaminants, pollutants, heavy metals, which are present in the environment. Of explored the properties of humic materials and from known information about the structure we can deduce about possible linkage interaction-ionic bond, coordination bond,

hydrogen bonds, hydrophobic interactions. Extensive studies point to the fact that only few HL occurs in the soil in free form, but many of them are bound to clay or other mineral components of the soil. Interactions with HL compounds in soil is caused by interactions with salts of low molecular weight organic acids, alkali metals and alkaline earth metal complexes [8].

The objectives of this Isothermal titration calorimetry is the only one capable of measuring not only the magnitude of the binding affinity but also the magnitude of the two thermodynamic terms that define the binding affinity: the enthalpy (ΔH) and entropy (ΔS) changes [9].

The general principle of ITC is based on measuring the heat which is generated or absorbed in the interaction between two molecules. ITC was used to determine the stability constants, stoichiometry, interaction enthalpy, under certain conditions, entropy, Gibbs free energy, and this may be change is detected by the thermal capacity [10].

The objectives of this work were determine and discuss the Cu(II), Pb(II) complexes their stability constants and thermodynamics parameters. Humic acids were characterized using Fourier transform infrared spectroscopy (FTIR) and NMR spectroscopy.

2. MATERIALS AND METHODS

2.1. Materials

Humic acids were isolated from South-Moravian lignite from the mine Mír (locality Mikulčice, Czech Republic) and compost which was obtained from the composting plant in Náměšť n. Oslavou, Czech Republic. Isolation of HAs was performed according to the procedure recommended by the International Humic Substances Society (IHSS).

Isothermal titration calorimetry

Humic sols were prepared by the dissolution of the humic acid powder (lignite HA, compost HA) in the 0.1 M KOH solution. Obtained samples were stirred overnight. To neutralize KOH [5], the appropriate amount of 0.1 M HNO_3 was added to each solution. The resulting sols had the concentration of humic acids of 1.25 $g \cdot dm^{-3}$.

Metals applied in experimetal works were purchased from Sigma-Aldrich (p.a. purify grade). For the study of the interaction with HAs, metals ($Cu(NO_3)_2$, $Pb(NO_3)_2$) were dissolved in 0.1 M KNO_3. Solutions were also stirred overnight. The concentration of metal solution was 8 $g \cdot dm^{-3}$.

2.2. Methods

The reactivity and complexation with metal ions were performed with an isothermal titration calorimeter (TAM III-thermal activity monitor, TA instruments) with a sample and the reference cells. The reference cell contained miliQ water. The cell (1 ml) contained the solution of HA. In the syringe was titrant – metal solution. Experiment consisted of injecting 4.974 μl (50 injections). The titrant was injected at 10 min interval. Each injection lasted 10 s. The temperature was set on 298, 15 K. For homogeneous mixing in the cell, the stirrer speed was kept constant at 90 rpm. The data were processed with TAM assistant software and NanoAnalyze software, both from TA instruments.

The Fourier transform infrared spectra (FTIR) of HAs were recorded over the range of 4000–400 cm^{-1} on pellets obtained by pressing under reduced pressure a mixture of 1 mg of samples and 400 mg of dried KBr, spectrometry grade. A Nicolet *iS*50 FTIR spectrophotometer operating with a peak resolution of 4 cm^{-1}, and 128 scans were performed on each acquisition. Nicolet Omnic software was used to obtain the spectra.

^{13}C NMR spectra were recorded using a Bruker Avance 500 DRX NMR spectrometer (Bruker, Karlsruhe, Germany) with working frequency of 125.77 MHz (^{13}C). Samples dissolved in 0.5 M NaOD in deuterium

oxide were measured with following parameters: temperature 298.15 K, NMR-tube diameter 5 mm, number of scans 25 000, excitation pulse 10.5 µs (90 °), acquisition time 0.52 s, spectral width 31 250 Hz, pulse repetition delay 2 s, and ^{1}H inverse gated decoupling.X Aromaticity (f_a), hydrophilicity and hydrophobicity ratio (Hfi/Hfo) and biological activity (BiA) of HS were calculated from the area of the NMR spectra.

3.   RESULTS AND DISCUSSION

3.1.   Isothermal titration calorimetry (ITC)

The best results were observed that we used colloid sol from humic acid. We tried titration with powder HA, total dissociation of HA, but results aren´t representative. The representative type of results from ITC is showed in Fig. 1. It demonstrate the isotherm of Pb^{2+} ion binding to compost humic acid. All titration with metals were exothermic-results from heat flow had possitive. In settings of TAM III means positive heat flow - exothermic reaction. The resulted data were fitting with least-squares fitting model for one-site binding model. From fitting results we received thermodynamic parameters – binding constant K_a, reaction enthalpy ΔH, reaction entrophy ΔS, Gibbs energy ΔG (Tab. 1).Both reaction were favorable ΔH, characteristic of hydrogen bond formation, and an unfavorable TΔS. Little bit higher enthalpy had reaction of compost HAs with copper ion. In Fig. 3 are thermodynamic responses between interaction of lignite HAs with metals. Results are in Tab. 3. ΔG is change in free energy, negative value is for spontaneous reaction. More negative means higher affinity. ΔH measure of the energy content of the bonds broken and created. The dominant contribution is form hydrogen bonds. Negative value indicates enthalpy change favoring the binding. ΔS is positive for entropically driven reactions- favoring binding is hydrophobic interactions. Higher Gibbs energy had reactions with Pb.
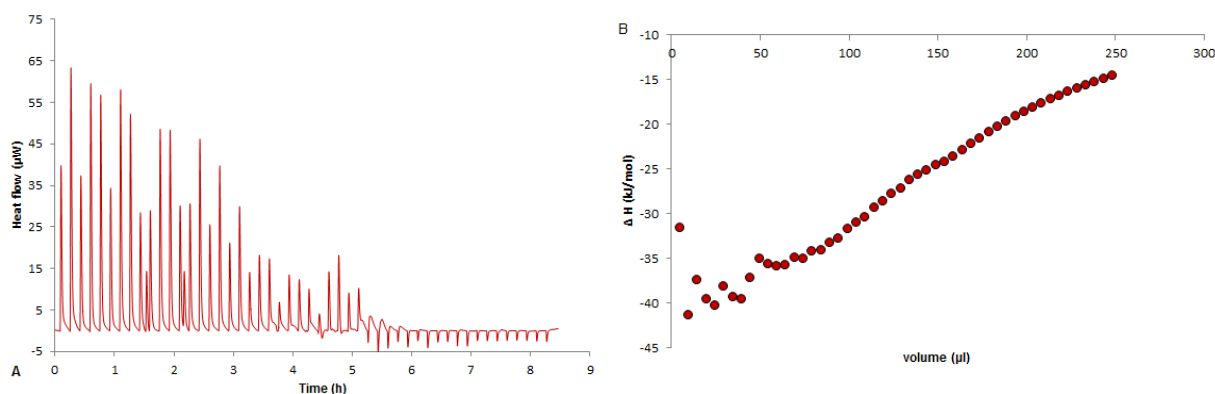

Fig. 1 ITC measurement of binding to compost HA.
A) Raw data for injection of 24 mM Pb^{2+} ions into HA.   B) ΔH from titration versus added volume of titrant

Amount of divalent cations is shown in Table 1. The little bit higher amount is in Pb cation in both complexation with humic acids. Also we observed stability constant log K_a. Higher values are in complexes with Cu ions.

Table 1 Thermodynamics parameters from isothermal titration calorimetry and adsorbed amount of ions

Reaction	log K_a (M^{-1})	Δ G (kJ/mol)	-T.Δ S (J/(k.mol))	Δ H (kJ/mol)	adsorbed amount (µmol)
compost HA-Pb	6,1 x 10^3	-22.716	-74.059	-45.29	4.25
compost HA-Cu	1,5 x 10^4	-25.843	-112.095	-59.31	3.79
lignite HA-Pb	1,3 x 10^3	-19.342	-111.924	-51.25	4.72
lignite HA-Cu	3,6 x 10^4	-26.895	-75.322	-47.26	3.56

3.2. FTIR spectroscopy

The FTIR spectra of HAs samples are shown in Fig. 2. Interpretation of the absorption bands was done as described in the literature [11-13]. All spectra of HAs are characterized particularly by bands of aliphatic groups. Bands at 2940–2930 cm^{-1} are ascribed to asymmetric C-H stretching in methylene groups. Bands of symmetric (2855 cm^{-1}) stretching in methylene groups are only observed for compost-HA. The deformation vibrations of methylene and methyl groups occurred in the spectra at 1451 cm^{-1} and 1369 cm^{-1}.

The bands at around 3400 cm^{-1} were attributed to OH groups. Bands at 3084 cm^{-1} are assigned to stretching C–H groups in aromatic ring. Bands at 1608–1602 cm^{-1} are assigned to stretching C=C groups in aromatic ring whereas the most intensity peak is observed for lignite-HA. The appearance of C=C aromatic stretching at 1510 cm^{-1} (and probably at 1608–1602 cm^{-1}) pointed out aromatic molecules. Two out-of-plane C-H deformation bands are found in the 900–700 cm^{-1}. Carboxylic groups to the band at 1712 cm^{-1} are observed for all samples of HAs whereas the most intensity peak is observed for lignite-HA. Bands at 1660 cm^{-1} are assigned to stretching C=O in amide groups (amide I band), quinone C=O and/or C=O of H-bonded conjugated ketones only observed for compost-HA. Bands of asymmetric (1580 cm^{-1}) and symmetric (1370 cm^{-1}) stretching in benzoate groups are observed for compost-HA. The bands at 1405 cm^{-1} and 1220 cm^{-1} are attributed to O–H bending in phenols. In the range 1300–1000 cm^{-1}, several bands is situated which can be attributed to other oxygen functional groups. In the zone, the most intensity band at 1260–1220 cm^{-1} is related to C–O stretching of phenols and ethers. The appearance of bands at 1130 cm^{-1}, 1080 cm^{-1} and 1040 cm^{-1} points out alcohols (primary and secondary), aliphatic ethers and C–O groups in polysaccharides.
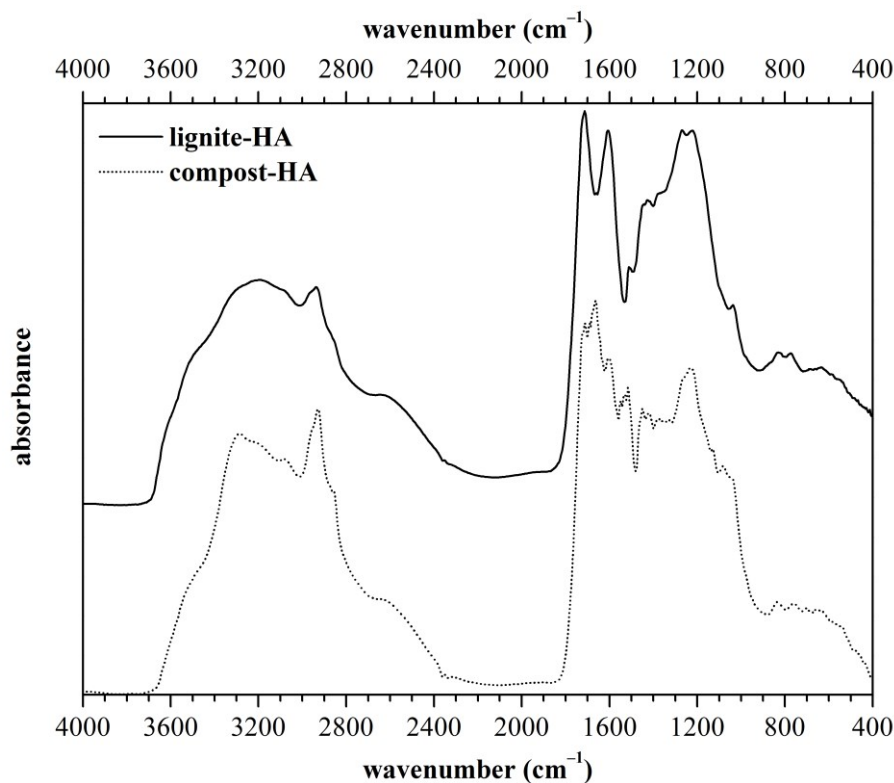
Fig. 2 FTIR spectra of lignite-HA and compost HA

3.3. ^{13}C NMR spectroscopy

The quantitative ^{13}C NMR spectra of the studied samples showed typical resonances of HAs: aliphatic carbon (0–45 ppm), carbohydrates and/or aminoacids (45–106 ppm), aromatic and phenolic carbon (106–165 ppm) and carboxyl and carbonyl carbon (165–220 ppm) are listed in Table 2. The ^{13}C NMR spectrum of lignite-HA was characterized by a low content of aliphatic and *O*-alkyl functional groups, the presence of a sharp signal of lignin O–CH₃ groups at a shift of 56 ppm, and signals of the aromatic carbons near 115–130 ppm, separated from the area of phenolic carbons. The ^{13}C NMR spectrum of compost-HA contained in addition to the signals typical for HAs derived from natural compost (aliphatic, *O*-alkyl and aminoacids) also many sharp signals of carboxyl carbons in the area of 165–190 ppm. **X** From the ^{13}C NMR spectrum, it is also evident that the compost-HA is characterized by a high content of aliphatic (0–45 ppm) and alkoxy (45–106 ppm) carbons. The values of the different indexes calculated from the ^{13}C NMR spectra (f_a, BiA and Hfi/Hfo) are presented in Table 2. The values of f_a, Hfi/Hfo and BiA of different HAs are typical for terrestrial origin and autochthonous sources. The higher values of f_a and BiA of lignite-HA may be indicative of greater humification degree and biological activity.

Table 2 Structural coefficients (f_a, BiA and Hfi/Hfo) and average distribution of individual carbon types and in the studied HAs from the ^{13}C NMR spectra

Sample	0-45 ppm	45–106 ppm	106–165 ppm	165–220 ppm	BiA	Hfi/Hfo	f_a (%)
lignite-HA	0.21	0.16	0.48	0.15	1.70	0.45	56
compost-HA	0.25	0.30	0.29	0.16	0.82	0.85	35

4.   CONCLUSION

The characterization of the humic acids was performed by NMR and FTIR spectroscopy. The thermodynamics of complexation of metal ions – Pb, Cu with humic acids in the sol form was studied using isothemal titration calorimetry. These metals were selected because they are pollutant in environment. We have developed optimization procedure for ITC experiments that can be applied in humic research for study their nature interactions (organic and inorganic). Thermodynamic quantities – enthalpy, entropy, Gibbs energy and the binding constant were obtained by calculation. It was found that metal ions can be adsorbed not only by acidic functional groups. The interaction of divalent cations and humic acids are entropy driven.

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