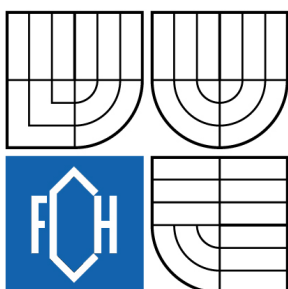# VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

BRNO UNIVERSITY OF TECHNOLOGY

FAKULTA CHEMICKÁ
ÚSTAV FYZIKÁLNÍ A SPOTŘEBNÍ CHEMIE

FACULTY OF CHEMISTRY
INSTITUTE OF PHYSICAL AND APPLIED CHEMISTRY

STABILITY OF SALTS AND COMPLEXES OF HUMIC ACIDS

STABILITA SOLÍ A KOMPLEXŮ HUMINOVÝCH KYSELIN

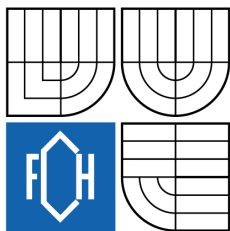## BAKALÁŘSKÁ PRÁCE

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ABSTRACT

The ground of this work lies in the study of influence of metal ions incorporated onto the structure of humic substances during thermal stress in the presence of air oxygen. For these purposes, lignitic humic acid was used. The amount of binding sites, i.e. carboxylic groups, was determined by retitration-conductometric method. Mixing the humic acid with solutions of sodium and cupric ions gave the final products with different saturation degrees of detected binding sites. For the evaluation of thermooxidative stability, thermogravimetric analysis and differential scanning calorimetry were performed. The results bring new view upon the reactivity of humic acid with metal ions in the liquid state, indicate possible formation of "porous" structure of sodium humates and their water-retention ability.

ABSTRAKT

Základem práce je studium vlivu přítomnosti kovů inkorporovaných do struktury huminových látek při tepelné zátěži za přítomnosti vzdušného kyslíku. Pro tyto účely byla použita lignitická huminová kyselina, u které byl nejprve retitračně-vodivostní metodou stanoven počet vazných míst, tj. karboxylových skupin. Smícháním s roztoky sodných a měďnatých iontů byly získány koncové produkty s různými stupni nasycení zjištěných vazných míst. Pro zhodnocení termooxidační stability byla provedena termogravimetrická analýza a diferenční kompenzační kalorimetrie. Výsledky přinášejí nový pohled na reaktivitu huminové kyseliny s kovovými ionty v kapalné fázi, poukazují na možný vznik „porézní" struktury sodných humátů a jejich schopnost retence vody.

KEYWORDS

humic acids, stability, thermal analysis

KLÍČOVÁ SLOVA

huminové kyseliny, stabilita, termická analýza

DECLARATION

I declare that this thesis has been compiled by myself and on my own and I cited all my information sources completely and correctly. The bachelor thesis is in terms of its contents a property of the BUT Faculty of Chemistry and its usage for commercial purposes is subject to a prior consent of the supervisor and the dean.

PROHLÁŠENÍ

Prohlašuji, že jsem bakalářskou práci vypracovala samostatně, a že všechny použité literární zdroje jsem správně a úplně citovala. Bakalářská práce je z hlediska obsahu majetkem Fakulty chemické VUT v Brně a může být využita ke komerčním účelům jen se souhlasem vedoucího diplomové práce a děkana FCH VUT.

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signature of bachelor thesis author
podpis autora bakalářské práce

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1. INTRODUCTION

Soil is from the agricultural and environmental point of view very simply defined as a part of natural environment of the Earth forming from regolith of superficial rock and decaying debris of organisms. It is an open dynamic system with proceeding mass and energy flow. Soil consists of solid, liquid and gaseous phase together with soil organisms, edaphon, their metabollic products and all the organic residues in different state of mineralization. Soil constitutes the base of almost all land vegetation. It is also very important part of nutrition chain for all mankind. [1]

Very important constituent of soil organic matter are humic substances. Being organic ligands they play crucial role in speciation, transport and deposition of variety of compounds ranging from metal ions to lipophilic compounds. Humic substances were found to influence metal transport through soil layers and control their uptake by plants via root systems.

The complexation of metal ions by humic substances is extremely important in affecting the retention and mobility of metal contaminants in soils and waters. Due to their enormous chelation capacity, humic substances are capable of detoxifying lakes that are affected by metal pollution. The role of humic substances in the sequestration of metals by formation of soluble complexes has already been reported. [2] Therefore, in this thesis, the stability of salts and complexes formed by humic acid and metals were studied using thermoanalytical methods.

2. STATE OF THE ART

2.1. Organic matter in soil

The term soil organic matter refers to the whole of the organic matter in soil, including the litter, the light fraction, the microbial biomass, the water-soluble organics and the stabilized organic matter (humus).

The complete soil organic fraction is made up of living organisms and their partly undecomposed, partly decomposed, and completely transformed remains, as well as those of plants. Soil organic matter is a more specific term for the nonliving components that may be described as a heterogeneous mixture composed largely of products resulting from microbial and chemical transformations of organic debris. The transformations are known collectively as the humification process. [3]

Humus can be defined as the total of organic compounds in soil exclusive of undecayed plant and animal tissues, their partial decomposition products, and the soil biomass. Soil organic matter may be partitioned into the active (or labile) and the stable pools. The active fraction contains the comminutive nonliving plant matter (litter) that lies on the surface of the soil, the light fraction, the microbial biomass, and the nonhumified substances that are not bound to the soil minerals. Litter is important for recycling of nutrients. The light fraction is incorporated in the soil but separable from it in liquids. It consists largely of plant residues in various stages of decomposition, has a rapid turnover rate, and hence provides a source of plant nutrients. [4] There is an astounding number of microorganisms in the soil environment, as many as 10^{10} per gram of soil. Microorganisms have a vital role for the turnover and transformation processes involving organic (and some inorganic) materials in the soil. Therefore, these are sources of enzymes in the soil environment, and (as well as plant root exudates) are responsible for water soluble organics in the soil solution. [3] The stable, or passive, humus pool of SOM are humification products, being resistant to some biodegradation processes. [4] Soil organic matter is one of the most complex materials in nature, containing most, if not all of the naturally occurring organic compounds. The major components are considered to be recalcitrant remains of plants and algae, including materials derived from lignins, tannins, sporopollenins, and large aliphatic molecules, such as algaenans, cutans, and suberans. [5]

In most agricultural soils, the bulk of the organic matter occurs as stable humus. The dark brown color of soil is due to their stable humus content. Highly productive soils often have a characteristic rich odor that can be attributed to organic constituents.

Soils vary greatly in organic matter content. In undistalbed soils, the amount present is governed by the soil-forming factors of age, parent material, topography, vegetation and climate. [4]

2.1.1. Soil-forming factors influencing organic matter in soil

2.1.1.1. The Time Factor

Organic matter does not accumulate indefinitely in well-drained soils, and, with time, an equilibrium level is attained that is governed by the other soil-forming factors.

Information on the rate of organic matter accumulation during soil formation has come from studies of time sequences on mud flows, spoil banks, sand dunes and the moraines of receding glaciers. Those studies show that the rate of organic matter accumulation is rapid during the first few years, diminishes slowly and reaches equilibrium in periods of time that

vary from as few as 110 years for fine-textured parent material to as many as 1500 years for sandy areas.

Although several reasons have been given for the establishment of equilibrium levels of organic matter in soil, none has proven entirely satisfactory. Included with the explanation are:
- humic substances (HS) are products that resist attack by microorganisms;
- humus is protected from decay through its interaction with mineral matter;
- a limitation of one or more essential nutrients (N, P, S) places a ceiling on the quantity of stable humus that can be synthesized.

2.1.1.2. *Influence of Climate*

Climate is the most important single factor that determines the array of plant species at any given location, the quantity of plant material produced and the intensity of microbial activity in the soil; consequently, this factor plays a prominent role in determining organic matter levels.

2.1.1.3. *Vegetation*

It is well-known fact that, other factors being constant, grassland soils have substantially higher organic matter content than forest soils. Reasons given for this include:
- larger quantities of raw material for humus synthesis are produces under grass;
- humus synthesis occurs in the rhizosphere, which is more extensive under grass than under forest vegetation;
- the harsh climatic conditions under which grassland soils are formed lead to organic matter preservation.

2.1.1.4. *Parent material*

Parent material is effective mainly through its influence on texture. It has been well established that, for any given climate zone, and provided constant vegetation and topography, organic matter content depends upon textural properties. The fixation of humic substances in the form of organo-mineral complexes serves to preserve organic matter. Thus, heavy-textured soils have higher organic matter content than loamy soils, which in turn have higher content than sandy soils.

2.1.1.5. *Topography*

Topography, or relief, affects organic matter content through its influence on rainfall runoff and/or retention.

2.1.1.6. *Effect of Cropping*

Marked changes are brought about in the organic matter content of the soil through the activities of man. [4]

2.1.2. Function of organic matter in soil

Organic matter contributes to plant growth through its effect on the physical, chemical and biological properties of the soil. It has a nutritional function in that it serves as a source of N, P and S for plant growth, a biological function in that it profoundly affects the activities of microflora and microfaunal organisms and a physical function in that it promotes good soil structure, thereby improving tilth, aeration, and retention of moisture. Other important functions of soil organic matter are:
- formation and the stabilization of soil aggregate structures;
- retention of plant nutrients attributable to its cation-exchange properties;
- absorption of solar radiation (thereby increasing soil temperature);
- complexation of heavy metals;
- retention of aromatic and sparingly soluble anthropogenic (synthetic) organic chemicals;
- release of soluble and colored materials in drainage waters;
- sequestration of C.

More recently, the importance of soil organic matter to considerations of environmental quality has been considered in relation to global climate changes, or the greenhouse effect. It is now accepted that human activity, both agricultural and industrial, has created an imbalance between global sinks and source of carbon, giving rise to increases in atmospheric gases. [3]

2.1.3. Components of organic matter in soil

Both chemical and physical fractionation procedures have been used in attempts to separate the various components of humus and to ascertain their location within the soil matrix. [4] Humus is now known to include a broad spectrum of organic constituents, many of which have their counterparts in biological tissues. Thus, two major types of compounds can be distinguished:
- Nonhumic substances, consisting of compounds belonging to the well-known classes of organic chemistry. Included with this group are such constituents as amino acids, carbohydrates and lipids.
- Humic substances, a series of high-molecular-weight, yellow to black substances formed by secondary synthesis reactions. They can be generally characterized as being rich in oxygen-containing functional groups, notably COOH but also phenolic and/or enolic OH, alcoholic OH and C=O of quinones.

2.2. Humic substances

2.2.1. Basic information

Humic substances are natural organic compounds arised from microbial degradation of decaying plant and animal tissues and synthetic activity of microorganisms. They are the major constituents of humus and largely influence soil quality (e.g. fertility). The most well-known and general definition, as mentioned above, describes them as a series of high-molecular-weight, yellow to black substances formed by secondary synthesis reactions.

HS are among the most widely distributed organic materials on the planet Earth. They are found not only in soils but also in natural waters, sewage, compost heaps, marine and lake

sediments, peat bogs, carbonaceous shales, terrestrial sediments, brown coals (leonardites, lignites) and miscellaneous other deposits.

Humic substances are important in geochemistry and the environment for the following reasons:

- They may be involved in the transportation and subsequent concentration of mineral substances, such as bog ores and nodules of marine strata.
- Humic substances serve as carriers of organic xenobiotics in natural waters. Humic substances play role in reducing toxicities of certain heavy metals to aquatic organisms, including fish.
- HS act as oxidizers or reducing agents, depending on environmental conditions.
- The sorption capacity of the soil for a variety of organic and inorganic gases is strongly influenced by humus. The ability of the soil to function as a "sink" for nitrogen and sulphur oxides in the atmosphere may be in part due to reactions involving organic colloids.
- Humic-like materials in waste waters treated by biological secondary treatment processes create problems at considerable importance in many water works.

There are several ways of classification of HS. One of them divides these substances according to their solubility in acids and alkalis into three basic groups:

- fulvic acids – soluble under all pH conditions;
- humic acids – not soluble under acidic conditions (pH < 2) but soluble at basic pH values; and
- humins – insoluble at any pH value.

Despite the fact that HS are under long-term intensive and extensive study and their importance has been acknowledged for over 150 years, they belong to the nature's least understood materials.

2.2.2. Structure

From the point of view of elemental analysis, HS consist of carbon (C), oxygen (O), hydrogen (H) and small amounts of nitrogen (N) and occasionally phosphorus (P) and sulfur (S). The C content ranges from 53.8 to 58.7 % in case of soil humic acids, for coal ones is reported slightly higher. Fulvic acids have lower C (usual range from 40.7 to 50.6 %) but higher oxygen (39.7−49.8 %) content. Briefly, elemental analyses data of humic samples originating from miscellaneous sources differ in their elementary composition and reactivity. [4]

From the chemical point of view, humic molecules are composed of aromatic and/or aliphatic moieties and with specific content of functional groups. Particularly carboxyl (COOH), phenolic OH, enolic OH, quinone, hydroxyquinone, lactone, ether, and alcoholic OH groups have been reported in humic substances. Their number and position depend on the conditions of formation. Although, undisputable differences exist in way of genesis, humic substances from different sources should be considered as members of the same class of chemical compounds. [6]

Concerning the structure, many models of HA and FA were suggested, but they should be considered only as models taking into account average composition. Therefore, in the real humic mixture, such a structure may not be necessarily present. Nevertheless the most recent

HA model structure taking into account the system complexity is presented in Figure 1. The spheres represent generic metal cations and dashes lines show H bonds as well as hydrophobic associations, which are shown along with polysaccharides (the R group represents a hydroxyl or proton), polypeptides (R represents a side chain), aliphatic chains, and aromatic lignin fragments.
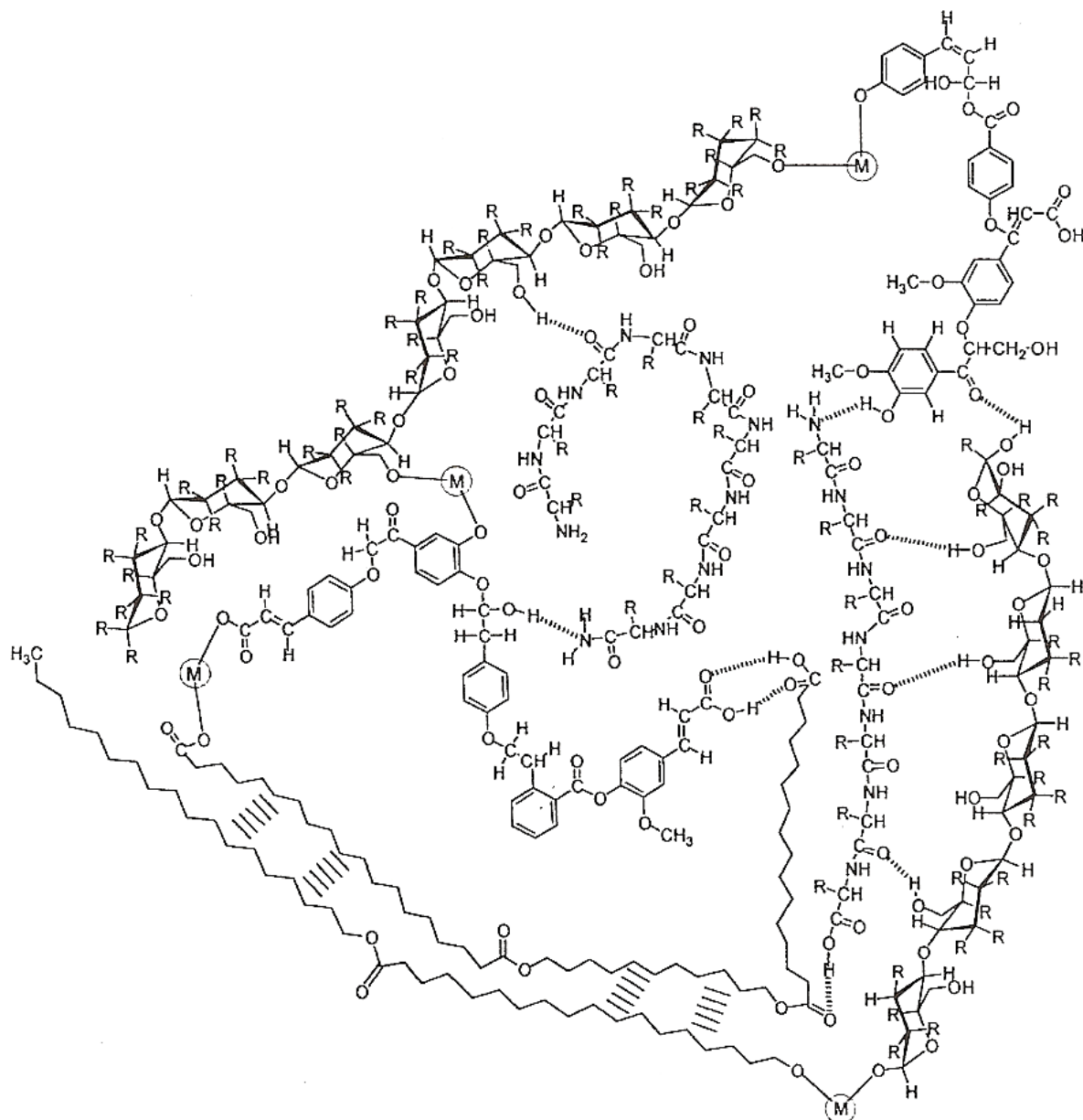

Fig. 1 *Recent model structure of humic acid according to Simpson et al. (2002).* [3]

Up-to-date, there are two well-established models used for description of humic substances structural and conformational properties. Both of them have their supporters and dissenters.

So-called "polymer model" says that humic substances comprise randomly coiled macromolecules, which have distinct properties in dependence on acidity and ionic-strength of their ambience. They have elongated shapes in basic or low-ionic-strength solutions, and became coils in acidic or high-ionic-strength solutions. [4] This concept is based

e.g. on the careful ultracentrifugation study of humic substances by Cameron. [7] The sedimentation-velocity technique was used to monitor the solute-sedimentation boundary of subfraction with reduced polydispersity. The data suggested that the molecules in humic substances had mass-weighted average molecular masses of 20 000–50 000 Da, radii of gyration of 4–10 nm, and random coil conformations.

This "polymer model" of humic substances is not consistent with information acquired using spectroscopic, microscopic, pyrolysis, and soft ionization techniques. Fresh scrutiny of soil processes active in the formation and preservation of humic substances also casts doubt on this model.

More recent model of humic substances is the model of supramolecular associations, in which many relatively small and chemically diverse organic molecules form clusters linked by hydrogen bonds and hydrophobic interactions. [8] This theory is supported by following studies.

Piccolo and his colleagues provide evidence, using both gel permeation chromatography (GPC) and high-pressure size-exclusion chromatography (HPSEC), that the apparent size of humic fragments changes drastically with addition of simple organic acids, as compared to addition of HCl as a reference. Ultraviolet-visible spectra of organic acid-treated humic substances show hypochromism (decreased absorbance due to increased distance between the absorbing chromophores). Evidently, the apparent sizes of humic materials do not change due to tight coiling (or uncoiling), as suggested by the "polymer model", but instead change due to disaggregation (or aggregation) of clusters of smaller molecules. The effect of addition of carboxylic and mineral acids on humic fractions with differing hydrophobicities indicate that aggregate disruption is greatest when more hydrophobic humic materials are combined with organic molecules containing both hydrophobic and hydrophilic segments. This result, in turn, suggests that humic materials are held together by hydrophobic interactions, which are easily disrupted when simple organic molecules penetrate large hydrophobically bonded associations and separate them into smaller, higher-energy H-bonded associations. The low hydrophobicity and high negative charge of fulvic acids, relative to humic acids, would not support large hydrophobically- or H-bonded associations, thus accounting for the observed minimal change in the apparent average molecular size of fulvic acids under variety of solution conditions. [9]

Simpson examined solutions of humic substances with a multidimensional nuclear magnetic resonance (NMR) spectroscopic technique and observed both aggregation and disaggregation behavior consistent with that described above. The diffusivities of aggregates increase with the concentration of HA (mg/ml). [10] Addition of acetic acid to the latter solution caused disaggregation like that observed by Piccolo [8], and made possible the identification of DOSY (two-dimensional diffusion-ordered spectroscopy) signals associated with chemically distinct molecular fragments with differing degrees of mobility. Because acetic acid is known to denature proteins, Simpson suggested that this organic acid may instigate dispersion similarly by triggering conformational changes in humic substance fragments. Examination of spectra of FA solutions suggested typical average molecular masses of ~1000 Da and provided little evidence of aggregation [10], as also suggested by Piccolo. [8]

Soft desorption ionization techniques, such as electrospray ionization (ESI) and laser desorption ionization (LDI), volatilize large ions for identification via mass spectrometry (MS), providing positive or negative ion mass/charge distributions that may represent the true

mass distribution of molecules within humic fractions, if the volatilized organic ions are singly charged, all constituents undergo ionization, and the ionized constituents represent their parent molecules. [11] Humic cations volatilized by ESI-MS have molecular mass distribution with number- or mass-weighted averages near 1000 [12] or 2000 Da [13], respectively. LDI-MS provides mass/charge distributions with number-weighted averages closer to 500 Da, perhaps due to fragmentation, decreased ionization efficiency, or poor resolution of signals representing ions with masses greater than 1000 Da. [11] These ESI- and LDI-MS average masses are far lower than those that have been measured by traditional methods, such as ultracentrifugation. [4] Methylation of an aquatic FA did not change the ESI-MS number-weighted average molecular mass of the sample, but did reduce the mass-weighted average molecular mass, suggesting that methylation of carboxyl groups had reduced the aggregation of humic materials through elimination of H-bonds. [14] Thus an array of independent analytical techniques has consistently revealed small, independent moieties in humic substances, providing significant evidence to support the concept of these organic materials as collections of diverse, low-molecular-mass molecules.

A corollary to this model is the concept of micellar structure. Examination of the same interaction that promote supramolecular associations led to development of the micellar model of humic substances, in which intra- or intermolecular organization produces interior hydrophobic regions separated from aqueous surroundings by exterior hydrophilic layers, analogous to the micelles formed by surfactants in aqueous solution. [15] For example, the fluorescence of pyrene in solutions of humic substances is increased within a few hours by the addition of Mg^{2+}, then decays over a period of several days. [16] The initial increase is thought to result from cation-enhanced formation of micelles featuring hydrophobic domains created by charge neutralization and cation bridge formation, including protection of the pyrene probe from quenching anions such as Br^-. [17] The drop in fluorescence then occurs as Mg^{2+} slowly migrates to higher-energy, directly coordinated position within the humic material, and the previously formed hydrophobic regions disaggregate. Also Nanny and Kontas [18] used the fluorescence of Prodan, to investigate the formation of micelles in humic substances over periods of days to week. Within humic solutions, Prodan experienced a polarity between that of ethanol and cyclohexane, indicating that it had been shielded from water molecules. More micellar structures were formed under neutral to acidic pH conditions than were formed under basic conditions and FA formed the structures more quickly than did HA.

Martin-Neto et al. [19] also detected increasing hydrophobic microsites with decreasing pH using electron spin resonance (ESR) spectroscopy. The width of the ESR absorption line of organic free radical species provides an indication of the relaxation time of these species. The line width of ESR signals produced by stable semiquinone-type free radicals within humic substances decreased with decreasing pH, indicating the presence, under acidic conditions, of protective hydrophobic regions. ESR was also used to monitor humic substances as a function of pH by Ferreira et al. [20] Under acidic conditions (pH<5), ESR spectra indicated that, in a matter of hours to days, the probe was immobilized due to hydrophobic interactions with humic moieties, while at higher pH the probe molecule remained mobile. This response being reversible also suggests that hydrophobic regions from under acidic conditions but disperse under basic conditions.

The work of Chien et al. [21] studied HA solutions containing labeled atrazine employing ^{19}F NMR spectroscopy to assess the effect of paramagnetic probes with differing

hydrophobicities on the intensity of the ^{19}F signal. Their data indicate that atrazine occupied nanometer-scale domains accessible only to hydrophobic molecules, which is consistent with a proposed micellar structure. Simpson et al. [22] obtained high-resolution magic angle spinning (HR-MAS) NMR solution-state spectra of a soil sample containing humic materials that were not fully water-soluble. Humic substances suspended in D_2O showed a reduced aromatic signal, as compared both to those suspended in the more highly penetrating DMSO and to soluble humic extracts-further evidence for hydrophobic regions. Tombácz [23] noted that HA fractions having large hydrocarbon contents, as assessed by NMR, also have high critical micelle concentration values, suggesting that the colloidal properties of humic associations are influenced by the presence of associated alkyl biomolecules such as fatty acids.

Transmission electron microscopy of dissolved organic matter revealed formation of 400–800 nm micelle-like colloids. Images from this study, and those from atomic force microscopic examinations of HA and FA adsorbed to mineral surface from aqueous solutions of varying properties, provide no evidence for coiled structures under either acidic or high-ionic-strength conditions, or for elongated structures under either basic or low-ionic-strength conditions, as would be posited by the "polymer model" of humic substances. [24]

Wershaw [25] further suggests that soil minerals provide surface upon which amphiphilic humic moieties form bilayer, membrane-like coatings, a conclusion based in part on infrared attenuated total reflectance linear dichroism measurements indicating that the carboxylate groups of compost leachate organic acids exposed to model soil surface such as alumina have very limited rotational mobility, and are likely bound to mineral Al. Adsorption behavior of the hydrophobic fraction of this leachate on alumina was similar to that displayed by simple surfactants forming layered structures on alumina surface, suggesting similar self-organization may occur as humic materials adsorb to soil minerals. This broad set of data provided considerable evidence supporting a view of humic substances as large, dynamic associations capable of exhibiting micellar properties in aqueous solutions.

Humic fractions extracted from soils contain recognizable biomolecular fragments that have been specifically excluded from traditional definitions of humic substances. [26] Many of these biomolecular moieties are intimately associated – even covalently bonded – with the humic fraction and cannot be separated effectively. [27] Thus, the assumption of humic material being a supramolecular association of molecules should not exclude an entire category of component molecules simply because they can be identified as biomolecules. A definition of humic components that is relevant to experimental studies should include all molecules that are strongly associated within a humic fraction, i.e., those that cannot be removed without significant alternation of the chemical properties of the fraction. This definition would necessarily include recognizable biomolecules that play important structural roles.

Another argument for the classification of associated biomolecular fragments as humic components relies on the fact that biomolecular moieties contribute noticeably to the functional behavior of humic associations. Obviously, biomolecular components of humic fractions affect analytical properties, such as elemental composition and functional group distribution. [28][29] A recent characterization of the optical spectroscopic properties of humic substances indicates that fluorescence absorption and emission spectra arise from a continuum of coupled states formed through charge-transfer interactions of a few distinct chromophores, rather than from a superposition of many independent chromophores. [30] It is

reasonable to suppose that biomolecules associated with humic materials contribute to the charge-transfer interactions that lead to the complex optical properties of humic substances. Thus, the potential role of intermolecular interactions in determining humic behavior indicates that removal of species components, such as biomolecules, may substantially alter the emergent properties of humic associations. All these biomolecules are derived primarily from lipids, lignin, nonlignin aromatic species, carbohydrates, and proteins. [11][31][32]

2.3. Complexes

2.3.1. Fundamentals

Complex compounds are particles composed of central atom coordinated by ligands. Complex is characterized by its composition, category of central atom and present ligands and their conformation. Central atom as an acceptor and ligands as donors are commonly bound by donor-acceptor bonds. Central atom of complex particle is typically a metal cation (or sometimes neutral atom) that contains free orbitals suitable for creation of donor-acceptor, coordination bond. The most frequent central atoms are cations of transition elements. In the formation process of complex compound, the oxidation state of the central atom is considered to be non-variable. Ions and molecules coordinated on the central atom are designated as ligands. Ion ligands are usually anion ones. Molecular (neutral) ligands do not affect the charge of complex particle. The ligand atom, directly bound on the central atom is called the donor atom. In non-coordinate ligand, its donor atom has a suitable free electron pair to form a donor-acceptor bond. The number of used donor ligand atoms is the basis for their classification into groups denoted as mono-, bi- to polydentate. [33]

If bidentate and polydentate ligand form a complex particle and its donor atoms are bound to one and only central atom, the arising complex particle is called a chelate. The central atom, donor atoms and residual ligand atoms form a cycle. If the donor atom of certain ligand is bound by two donor-acceptor bonds to two central atoms, or if the double-donor ligand is is bound by each donor atom to different central atom, so-called bridge configuration is formed. In such case, the arising complex particle has to be at least binuclear. Unusual conformations of some complex particles may emerge, when the donor atom are not exactly specified. Though the axis of donor-acceptor ligand bond and the central atom passes through the central atom but it doesn't proceed directly to a certain atom in the ligand. If the donor atoms in the complex particle are exactly specified, they form a dimensional geometric shape called central atom coordination polyhedron. [34]

2.3.2. Complexes of humic substances

HS are able to chelate positively charged ions. Metal-HS complexation in soil is of practical and theoretical significance for several reasons. For example, chemical weathering of rocks and minerals, and related soil genesis and evolution are known to involve metal complexation reactions with HS. The bioavailability of several metal ions, especially trace elements, is strongly influenced by complexation with the soluble and insoluble fractions of HS. For instance, at pH values commonly found in soils, nutrient metal ions that would ordinarily be converted to insoluble forms may be maintained in solution by complexation with HS, thus increasing their bioavailability. On the contrary, the concentration of a toxic metal ion may be reduced to a nontoxic level through complexation to insoluble fractions of soil HS, whereas soluble HS complexes can function as

metal-carrier in the transport to ground- and surface-water bodies, thereby rendering the water unfit for several uses. Further, complexation of metal ions by HS may represent a challenging problem for their analytical determination in soil samples. [35]

Humic substances contain a large number of complexing sites per molecule, and thus behave as other natural "multiligand" complexing agents like proteins and metal oxides, which are distinguished from "simple" ligands such as inorganic anions and amino acids. The principal molecular characteristics that govern the complexing ability of HS are: polyelectrolyte character, hydrophilicity, and the capacity to form intermolecular associations and change molecular conformation. [36]

The major functional groups in HA and FA that can bind metal ions are O-containing groups, including carboxylic, phenolic, alcoholic and enolic hydroxyl groups as well as carbonyl functionalities of various types. Aminogroups and S- and P-containing groups are also involved in metal binding.

Several types of binding reactions can be visualized between metal ions and HS, the simplest case being the 1:1 binding. Formation also can be expected of mononuclear complexes with the central group being either the HS macromolecule or the metal ion, and of polynuclear complexes. The most common stoichiometries assessed are HS:metal = 1:1 and 2:1, often involving the formation of metal chelates. Aromatic carboxyls and phenolic groups play a prominent role in the 1:1 binding of metal ions by soil HA and FA, by forming chelates that involve two COOH groups in a phtalic-type site (Eq. (1)) and both phenolic and COOH groups in a salicylate-site (Eq. (2)). The most stable complexes involve the more strongly acidic COOH groups, whereas the less stable complexes are believed to be associated with weakly acidic COOH and phenolic OH groups. Other possible combinations involve two phenolic OH, quinone, NH_2, and sulphuryl groups and conjugated ketonic structures (Eq. (3)).

Nonaromatic carboxyl and hydroxyl sites also may be involved in metal ion binding by soil HA and FA. The monomeric analogues of some of these sites, e.g., pyruvic and glycolic acid (Eq. (4) and (5)), have binding constants similar to phtalic and salycilic acids.

Metal ions also may coordinate with ligands belonging to two (or more) HS molecules, forming 2:1 complexes (Eq. (6) and (7)) and/or chelates (Eq. (8) and (9)), and eventually producing a chain structure (Eq. (10)) that may result in the aggregation and precipitation as the chain grows at high metal to HS ratios.

Two main types of complexes may be formed between metal ions and HS, that are:
- inner-sphere complexes, resulting in the formation of bonds with some covalent character between the ligand atom(s) and the metal ion, both completely or partially dehydrated, and
- outer-sphere complexes that result in the electrostatic attraction between the ligand(s) and the metal ion that remains completely hydrated.

For simplicity, all reaction schemes described in Eqs (1) to (10), show formation of inner-sphere complexes, but they may represent outer-sphere HS complexes if the cation is solvated (e.g. Eq. (11)). [35]

(1)

(2)

(3)

(4)

(5)

(6)

(7)

(8)

15

$$(9)$$

$$(10)$$

$$(11)$$
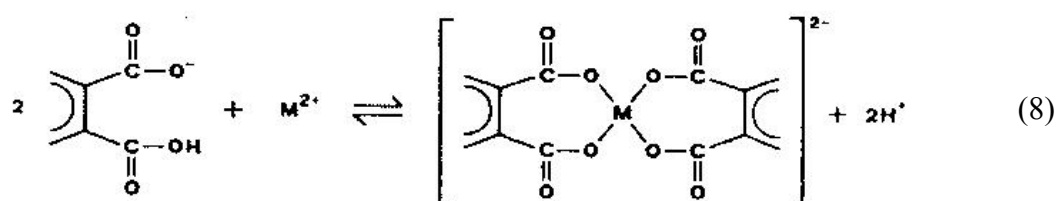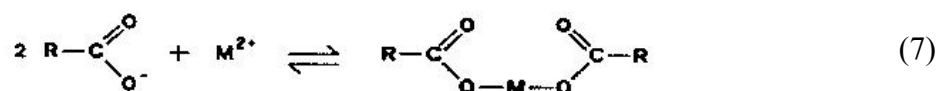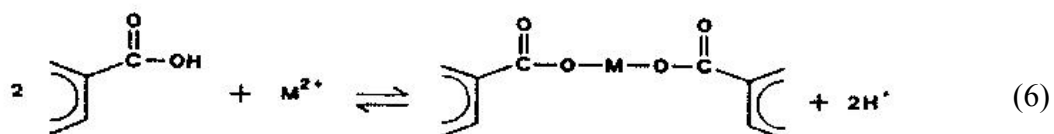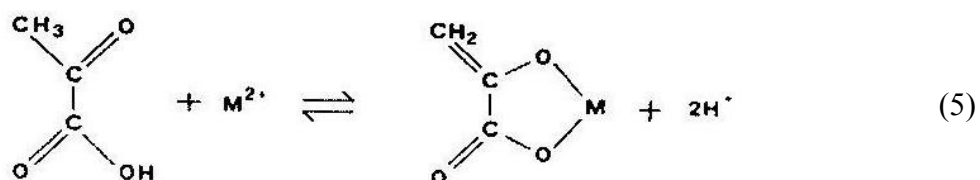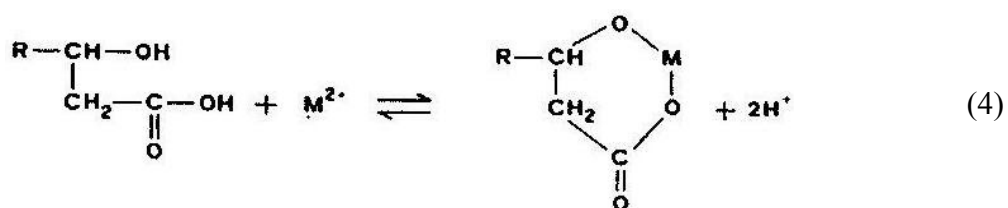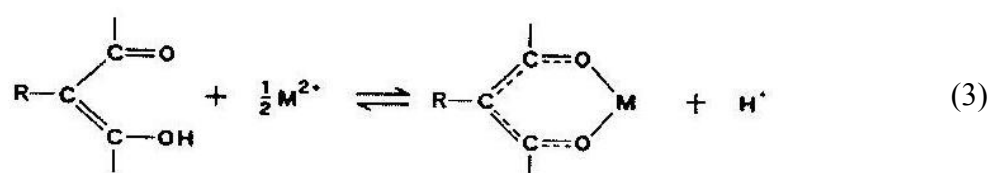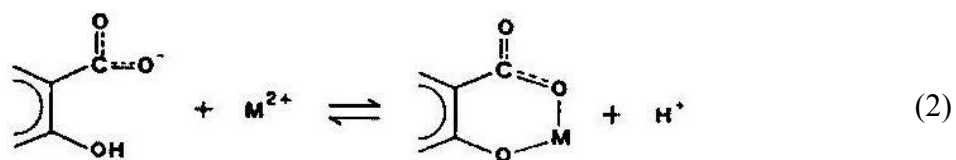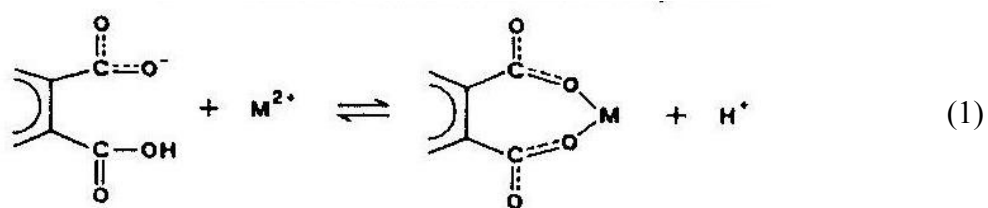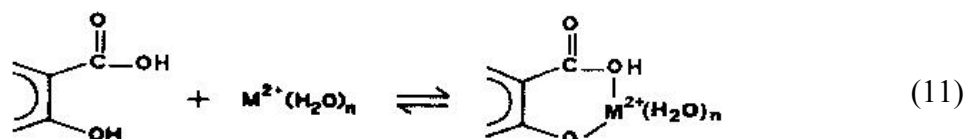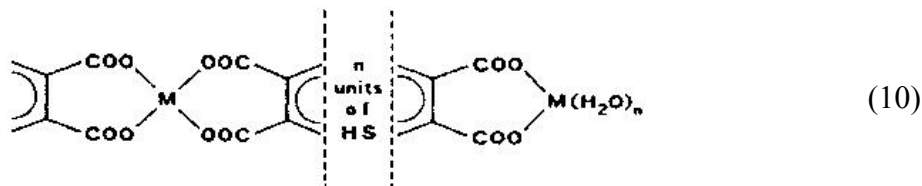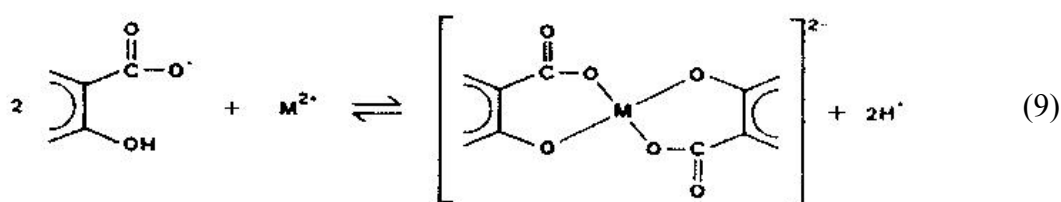
Klučáková et al. has used model systems of metal-humic complexes for calculation of interaction energy of HA/FA with compounds of transition elements. The reactivity and properties were investigated by EPR and FTIR spectroscopy. The reactions of these model systems with metal compounds were described and the enthaplies of these reactions were calculated. The result of this study is that there are many different bonding sites in HA and FA for atoms of transition elements with different bond strengths. [37]

Lishtvan et al. [38] assumed in their studies that the mechanism of metal-humic complex (MHC) formation from solutions consists of two stages, namely, micro- and macrocoordination. The microcoordination term refers to the bonding of metal cations to functional groups of HAs to form coordination sites; the macrocoordination, to the bonding of additional amounts of cations to the developed surface of colloidal aggregates, which was formed at the first stage, to yield macromolecular complexes. The validity of this mechanism of MHC formation is based on the fact that HAs are characterized by the presence of both functional groups and supramolecular formations of colloidal nature with a charged surface. According to infrared (IR) and ESR spectroscopy data, when copper ions interact with HAs, the ions are first bound directly with carboxyl groups. This is evident from a decrease in the intensity of the absorption bands attributed to carboxyl groups and the enhancement of the absorption of carboxyl ions in the course of MHC saturation with the metal, as well as from the disappearance of the ESR signal of bound trivalent iron ions as a result of its inner-sphere displacement by copper ions in the case of HAs of low-moor peat. An additional argument for the penetration of copper into inner-sphere complexes of HA associates is the absence of a solvation shell upon its sorption by carboxyl cationites, which are, actually, represented by HAs. Thus, the formation of copper-humic acid complexes is consistent with the mechanism based on micro- and macrocoordination stages of MHC formation.

The investigation of Lu at al. [39] has shown that the decomposition of the metal forms of humic substances occurs at temperatures that are significantly lower than that of humic

substances. These different thermal stabilities are most likely related to ligands in humic substances. To account for the lower thermal stability of the metal complexes, the interactions between the metal ion and its ligands, together with the surrounding structure need to be clarified and the physical properties of the metal ions should be considered.

It has been known that an intermolecular chelation of metal ions to organic ligands leads to a charge-density rearrangement in the course of a reaction, e.g. for the reaction $AB + C \rightarrow AB - C$, a degree of charge transfer takes place from B (acting as an electronic donor) to C (acting as an electronic acceptor). [40]

According to Schnitzer and Kodama [41] the thermal stability of the sodium and calcium forms appears to be related to the cation radii. This is because the smaller ionic radii and higher charge of the calcium ion can abstract more electrons from the bound ligands so that the calcium-humic substance has a lower thermal stability than the sodium-humic substance. [41]

On the other hand, according to Tan [42], aromatic carboxyl groups and adjacent phenolic OH groups can form a salicylate-like ring, or two COOH groups in close proximity can form a phthalate-like ring. Because of chelation of metal ions to the COOH and adjacent OH ligand in humic substances, a certain amount of charge transfer from oxygen to metal ions and can induce a charge-density arrangement within the functional groups which affects the thermal decomposition behavior during pyrolysis.

Decomposition of the Mn(II) and Fe(III) forms of humic substances appears at lower temperatures than the decompositions of the Na(I) and Ca(II) forms. It was proposed by Schnitzer and Kodama [41] that di- and tri-valent metal ion reacts simultaneously with both COOH and phenolic OH groups, forming a complex or chelation structure (as shown in Eq. 2), and thus adding a strain on the complexing or chelation structures. Such a strain could cause thermal decomposition at relatively lower temperature. While sodium ion reacts with one ligand only, there is less strain exerted on the binding structure and this may account for the relatively high thermal stability of this form. [39]

2.4. Thermal analysis

2.4.1. Generalities

The term thermal analysis is frequently used to describe analytical experimental techniques which investigate the behavior of a sample as a function of temperature.

The advantages of TA over other analytical methods can be summarized as follows:

- the sample can be studied over a wide temperature range using various temperature programmes,
- almost any physical form of sample (solid, liquid or gel) can be accommodated using a variety of sample vessels or attachments,
- a small amount of sample is required, typically (cca 0.1–50 mg),
- the atmosphere in the vicinity of the sample can be standardized,
- the time required to complete an experiment ranges from several minutes to several hours,
- TA instruments are reasonably priced. [43]

Temperature program can be described by linear dependence:

$$T = T_0 + \beta t , \tag{12}$$

where T is temperature at time t, T_0 is the initial measuring temperature and β is the temperature coefficient. When $\beta > 0$, the sample is heated, when $\beta < 0$, we speak of linear cooling and when $\beta = 0$, the measurement is under isothermal conditions. [44]

TA data are indirect and must be collated with results from other measurements (for example NMR, FTIR, X-ray diffractometry and so on) before the molecular processes responsible for the observed behavior can be elucidated. The recorded data are influenced by experimental parameters, such as the sample dimension and mass, the heating/cooling rate, the nature and composition of the atmosphere in the region of the sample and the thermal and mechanical history of the sample.

2.4.2. Thermogravimetry (TG)

Thermogravimetry is the branch of TA which examines the mass change of a sample as a function of temperature in the scanning mode or as a function of time in the isothermal mode. TG is used to study processes with decrease/increase of mass, e.g. various types of sorption, sublimation, vaporization, oxidation and primarily for determination the decomposition and thermal stability of materials under a variety of conditions and to examine the kinetics of the physico-chemical processes occurring in the sample. The mass change characteristics of a material are strongly dependent on the experimental conditions employed. Factors such as sample mass, volume and physical form, the shape and the nature of the sample holder, the nature and pressure of the atmosphere in the sample chamber and the scanning rate all have important influences on the characteristics of the recorded TG curve.

TG curves are normally plotted with the mass change (Δm) expressed as a percentage on the vertical axis and temperature (T) or time (t) on the horizontal axis. [43]

The most frequent use of TG is in determining the material thermal stability, relative volatility and also process kinetics. Coupling of TG and DTA enables some other characteristics to be measured and assessed, for example temperatures of first and second order phase transition, namely changes of crystal modification, melting, sublimation, boiling or glass transition. [45]

2.4.2.1. Factors affecting thermogravimetric measurements

The size and shape of crucible can appreciably influence the course of the TG curve. If the volatility of a substance is followed under isothermal conditions, the rate of volatilization depends on the shape of the crucible, as this determines the surface area of the sample. The size of the crucible and the amount of the sample play a major role as they affect the method of heating and also the rate of diffusion of gases liberated from the sample. In view of this, it appears that the best results are obtained with crucibles in the shape of a small shallow dish permitting homogeneous heating of the sample over a relatively large surface area. It is important to take into account other properties of the investigated substance such as decrepitation, or foaming, during heating. If any of these occurs the crucible described is unsuitable and cylindrical or conical or closed crucible should be used. The shallow crucible is also unsuitable in cases where a gas stream is used, as aerodynamic effects cause complications.

Another effect that can influence the course of measurements is the construction of the crucible. The effect of the material is negligible if Pt, Au or Al crucibles are used.

Sample properties influence mostly the course of measurements. One of the most important parameters is its quantity which influence the time required for the reaction to take place throughout the whole volume of the sample.

Thermogravimetry requires heating and weighing of the sample simultaneously. This means there must be no contact between the sample and the furnace wall. Thus the problem of heating the sample is primarily one of heat transfer. This is controlled by a number of factors, e.g. the properties, size and nature of the sample, and the enthalpy change of any reactions undergone by the sample. A temperature gradient is formed between the sample and the furnace wall, which introduces errors into measurement of the sample temperature and determination of the range of temperature reactions being investigated. A temperature gradient may also be formed inside the sample. The temperature difference between the furnace wall and the sample is affected primarily by sample properties, the enthalpy change of any reactions it undergoes, and the rate of temperature increase.

The atmosphere in the reaction space of the furnace is also very important factor influencing the measurements. If the sample is dried or decomposed in air, the atmosphere in the neighborhood of the crucible is continually changing owing to liberation of gaseous products or reaction of the sample with the original atmosphere.

The question of the sensitivity of the weighing mechanism and the recording system is closely related to the amount of sample used. The mass of the sample may exert an appreciable influence on the course of the reactions being followed. Often, in order to follow the reaction under required conditions, as low a sample weight as possible is taken. Great attention must always be devoted to long-term stability of the thermobalance, periodic calibration of weight ranges, quality and strength of the record, etc. These factors will introduce errors into the TG measurement mainly in prolonged experiment and at high sensitivities of measurement.

2.4.3. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry observes the enthalpy changes.[46]

DSC measures the rate of heat flow and compares its difference between the sample and the reference material.

The measurement of sample and reference is carried out in two crucibles and the most common range is on order of ones to tens of miligrams. A substance, which doesn't undergo any thermal changes in the temperature range of the measurement, serves as a reference. Most frequently an empty crucible is used.[44]

2.4.3.1. Heat-Flux DSC

It concerns measurement of temperature difference between the sample and reference as a function of temperature or time, under controlled temperature conditions. The temperature difference is proportional to the change in the heat flux (energy input per unit time).

The thermocouples are attached to the base of the sample and reference holders. A second series of thermocouples measures the temperature of the furnace and of he heat-sensitive plate. The variation in heat flux causes an incremental temperature difference to be measured between the heat-sensitive plate and the furnace. The heat capacity of the heat-sensitive plate

as a function of temperature by adiabatic calorimetry during the manufacturing process, allowing an estimate of the enthalpy of transition to be made from the incremental temperature fluctuation.

2.4.3.2. Power Compensation DSC

The sample and reference holders are individually equipped with a resistance sensor, which measures the temperature of the base of the holder, and a resistance heater. If a temperature difference is detected between the sample and reference, due to a phase change in the sample, energy is supplied until the temperature difference is less than a threshold value, typically < 0.01 °C. The energy input per unit time is recorded as a function of temperature or time. A simplified consideration of the thermal properties of this configuration shows that the energy input is proportional to the heat capacity of the sample. [43]

2.4.3.3. Advantages and drawbacks of DSC

Both types of DSC have their advantages and drawbacks but the final result is the same, they both provide same information.

The benefit of heat-flux DSC is the possibility of measuring larger-volume samples. This type has very high sensitivity and the maximal temperature may reach values over 900 °C. The disadvantage is low heating and cooling rate when using older types of instruments.

Principal advantage of power compensation DSC is no need of calibration because the heat is obtained directly from electric energy supplied to the sample or reference (calibration is still needed to convert this energy into effective units). High scanning rate can be obtained. The disadvantage of such arrangement is the necessity of extremely high sensitive electronic system and his susceptibility to fluctuating environment to avoid compensating effects, which are not caused by the sample. [47]

3. EXPERIMENTAL PART

Humic acid was extracted from South Moravian leonardite-type lignite from the mine Mír in Mikulčice, near Hodonín, Czech Republic. For our purposes, the extraction procedure, according to Piccolo [48], was slightly modified. Briefly, 150 g of previously air-dried and 0.2–0.3 mm sieved lignite was mixed with 1.5 l of 0.5M NaOH and 0.1M $Na_4P_2O_7$ solution and stirred for 2 hours at slightly elevated temperature (50℃). After separation of phases by centrifugation (ROTINA 46R, 4000 RPM, 15 minutes), the supernatant was treated with concentrated HCl until the pH value 1–2 was reached in order to precipitate the HA. Than approximately 40 ml of HF was added and the final solution was diluted with distilled water to 1.5 l and shaken overnight to remove the residual ashes. After that the sample was centrifuged (same conditions as described above), rinsed with distilled water, centrifuged again, dialyzed (Spectra/Por® dialysis tubes, 1000 Da cut-off) against distilled water until chloride-free ($AgNO_3$ test). Minor part of the final solution was freeze-dried (Labconco FreeZone).

The carboxylic acid groups content was determined by two approaches. First one was as follows: 100 ml of concentrated HA solution was titrated with 0.5M NaOH to pH value 7.5. From the consumption of sodium hydroxide the content of carboxylic groups was calculated. The second approach to determine the content of carboxylic groups was conductance titration according to Riggle and von Wandruszka [49]. 0.225 ml of concentrated solution of humic acid (~10 mg) was automatically titrated overnight with 0.1M NaOH to pH value 8 (Schott TitroLine alpha plus) and retitrated with 0.0125M HCl. 0.04 ml of HCl solution was added every 10 seconds (equilibration time). During the retitration, values of conductance (WTW inoLab Cond 730) and pH (Mettler Toledo Seven Multi) were recorded before each HCl addition.

HA was mixed with solutions of metal ions (Na^+, Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+}) in order to reach 50% saturation of binding sites (according to first method of carboxylic groups content determination). Particularly, 15 ml of metal solution (0.03530, 0.01765 and 0.01177 mol/l for uni-, bi- and trivalent ions) was mixed with 4.5 ml of concentrated HA solution (~100 mg HA). Such final solutions were freeze-dried.

HA was mixed with concentration set of solutions of metal ions (Na^+, Cu^{2+}). Particularly, 100 ml of metal ion solution of different concentrations was mixed with 5 ml of concentrated HA solution (~222.5 mg HA) in order to reach 17, 35, 53, 71, 88, 97, 106 and 133 % saturation of binding sites (according to second method of carboxylic groups content determination). After 24 hours the solutions were placed into oven with temperature 30 °C to dry out.

Thermogravimetric analyses of all samples, including unaltered HA, were performed. For this purpose TA Instruments TGA Q 5000 IR was used, connected to the PC, where experimental data were collected. The furnace was calibrated using Curie temperatures of nickel and alumel (358.2 and 152.6 °C, respectively). Samples were carefully homogenized in an agate mortar. Approximately 2–3 mg of each sample was loaded into an open platinum crucible (100 μl). The air (reaction gas) flow rate was set at 25 ml per minute, nitrogen (purge gas) flow rate being 10 ml per minute. The heating rate was 5 °C per minute. The measurement was carried out from room temperature to 650 °C. Obtained results were evaluated by means of Universal Analysis 2000 software.

Calorimetric analyses of all samples, including unaltered HA, were performed employing Shimadzu DSC-60. The instrument is connected through TA-60WS to the computer.

The furnace was calibrated using fusion temperatures of indium, tin, lead and zinc (156.60, 231.93, 327.46 and 419.53 °C, respectively). Approx. 1 mg of homogenized sample was placed into an open aluminum crucible. The air flow rate was 25 ml per minute. Samples were heated (10 °C/min) from room temperature to 600 °C. Obtained results were evaluated by means of TA-60 software.

4. RESULTS

4.1. Determination of reactive groups content

Content of carboxylic groups (acidity) in humic acid determined by simple alkali titration to pH 7.5 provided typical S-shaped curve (not shown) and accordingly it was calculated from the consumption of NaOH solution. As a result the value 3.178×10^{-3} mols of binding sites per gram of humic acid was determined. That data, however, was not in accordance with more sophisticated approach of acidity determination. [49] Obtained specific conductance was plotted against volume of added HCl to solution of HA previously titrated to pH 8 as reported in Figure 2. As it has been stated [49] the conductance decrease at the beginning of dependency corresponds to neutralization of excessive Na^+ ions. After a local minimum, an increase of conductivity was observed. This region (as indicated in the Figure 2) matches to ion exchange of carboxylic groups present in the humic acid from Na^+ to the H^+ form and in this study it was used for the acidity assessment. Final part, where the conductivity increases rapidly corresponds to the accumulation of Cl^- ions in the solution. Accordingly, the total carboxylic acidity was determined to 2.25×10^{-2} mols of binding sites per gram of humic acid.
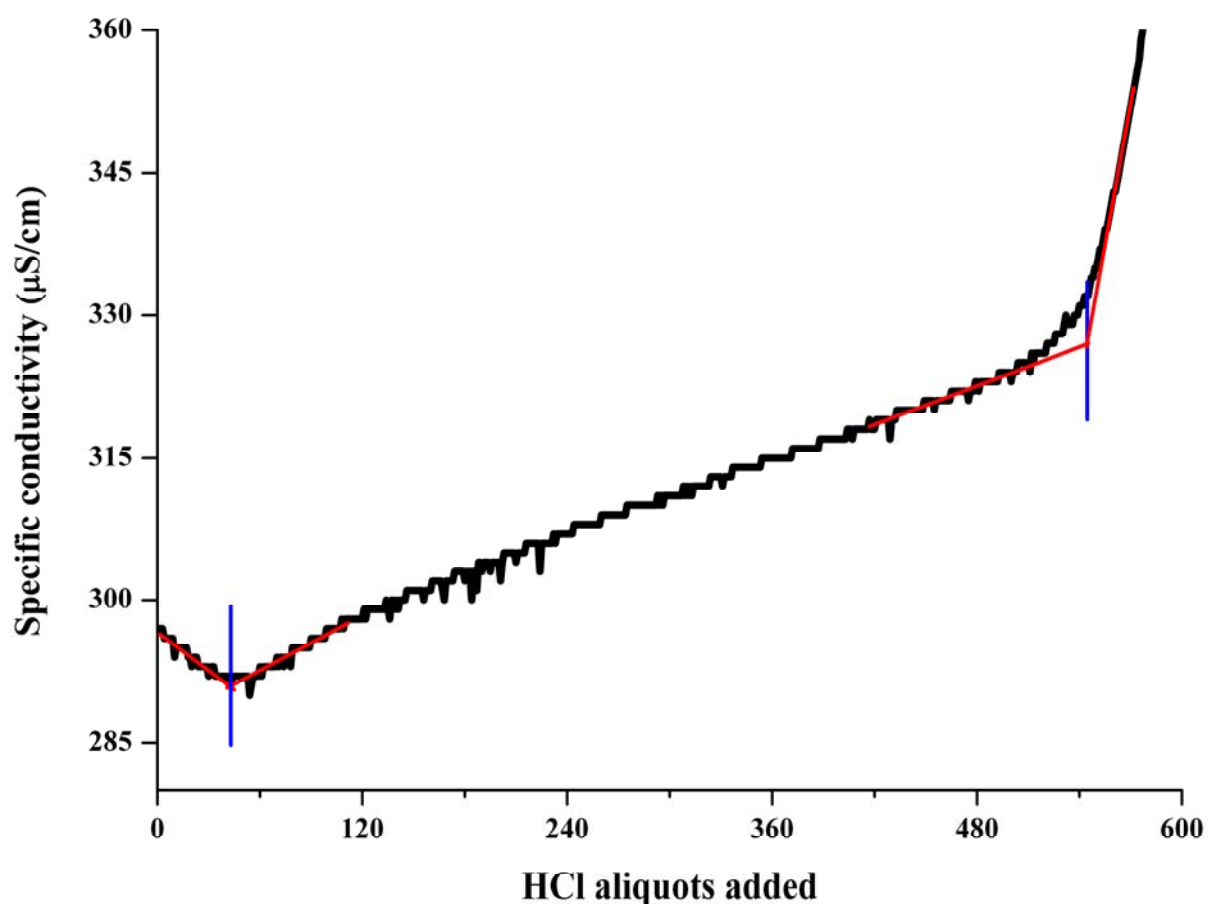

Fig. 2 *Specific conductivity during retitration of HA solution*

After comparison of both results from two different carboxylic acidity determinations, it can be seen that the latter approach gave more than 7 times higher number of possible binding sites in the humic acid. Since the higher values seemed to be more realistic and closer

to the data published already in the literature, the further experiments designing were based on that value.

4.2. Influence of a metal presence

As a first step, the influence of various metal ions bound in the HA structure on thermoanalytical behavior was tested.
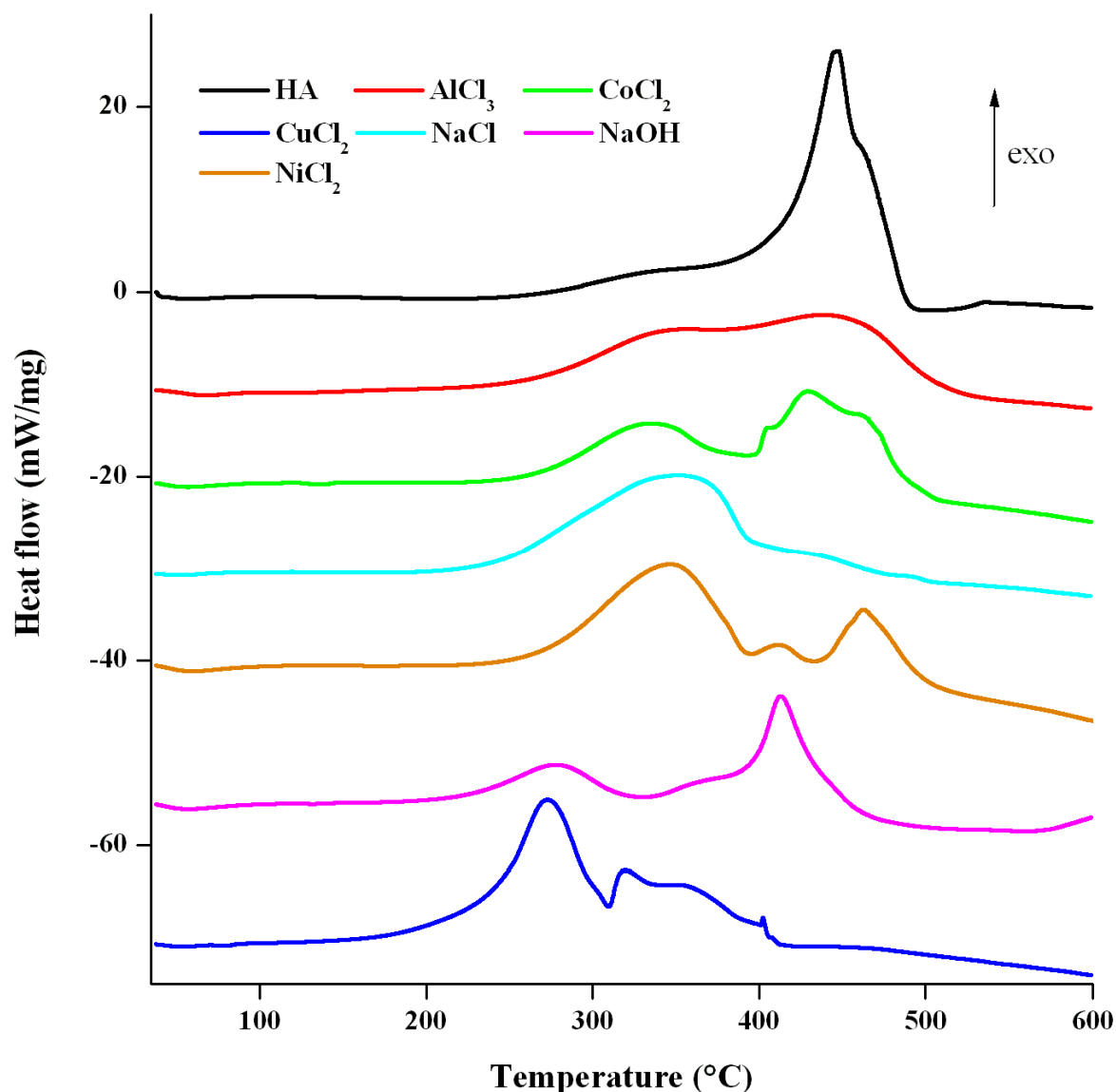

Fig. 3 *DSC curves of HA and its mixtures with metal ions*

Figure 3 shows enthalpic changes in the course of thermo-oxidative degradation of humic acid and samples with 6 different metal ions with 7% saturation of binding sites of the HA. All DSC records showed one endothermic and several exothermic peaks. Endothermic peak observed in the temperature interval 40–120 °C can be attributed to moisture evaporation associated possibly also with sublimation/evaporation of volatile humic molecules. Exothermic peaks reflect the processes of degradation and disclose the difference among tested samples. Pure HA exhibits one major peak at 450 °C. Except of samples with NaCl

and AlCl$_3$, other samples with metal ions show more than one clear exothermic peak. It is noteworthy, all of the exothermic processes of HA salts begin at lower temperatures than that of unaltered HA. The degradation process of mixtures of HA and CuCl$_2$ and NaOH starts at the lowest temperature.
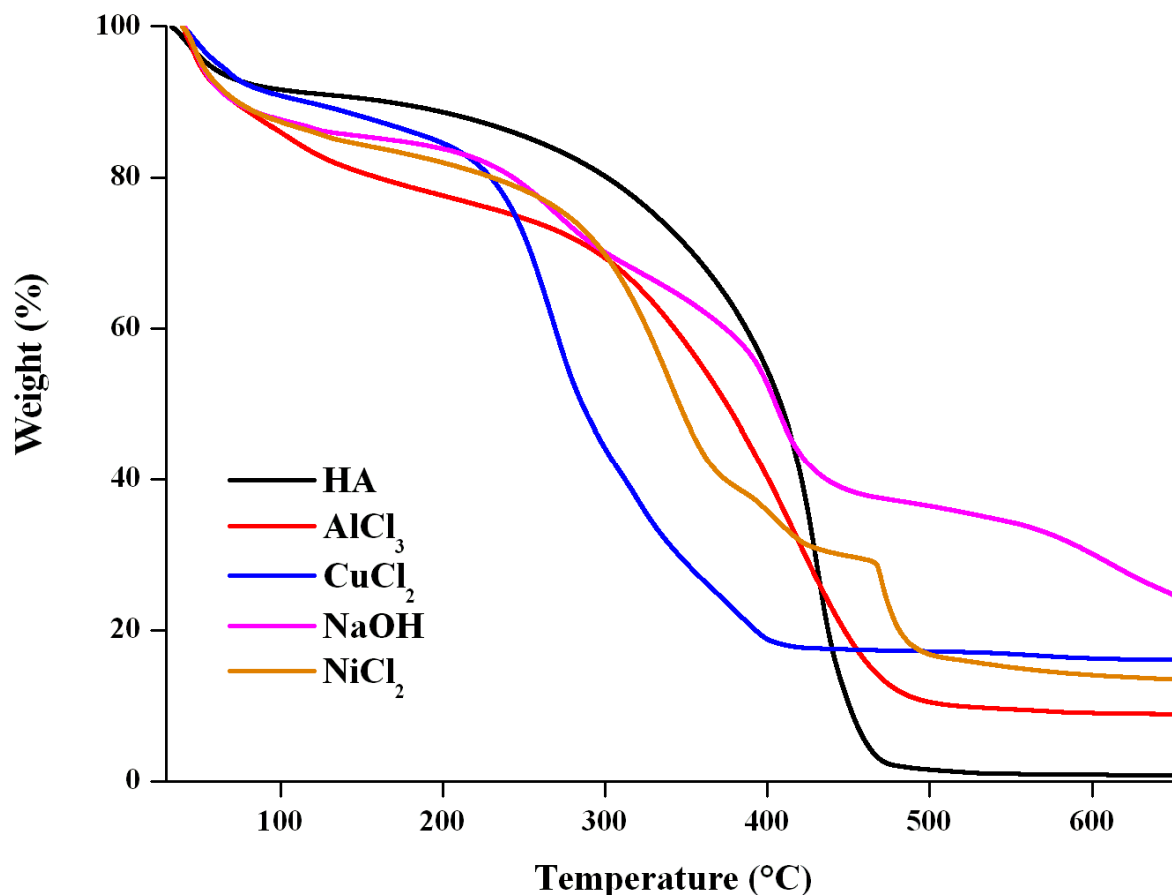

Fig. 4 *TG curves of HA and its mixtures with several metal ions*

Demonstrative thermogravimetric curves are reported in Figure 4 and represent heat-air-induced mass losses of humic acid and mixtures of humic acid with AlCl$_3$, CuCl$_2$, NaOH and NiCl$_2$. The TGA records show one (HA) up to three (HA + NiCl$_2$) degradation steps. At the end of the analyses, i.e. 650 °C, pure humic acid has almost 0 % of its original weight, on the contrary humic acid prepared in mixture with NaOH still has more than 20 % of its initial mass but its degradation is not clearly finished. Weight of the sample with CuCl$_2$ stayed almost constant above 400 °C. Table 1 summarizes the degradation temperatures obtained as the onsets of first DTG degradation peaks.

Table 1 *DTG onset and ash content of HA and its mixtures with metal ions*

	DTG onset (°C)	ash content (%)
HA	287	0.78
HA+AlCl$_3$	268	9.45
HA+CoCl$_2$	201	16.11
HA+CuCl$_2$	212	13.83
HA+NaCl	197	26.31
HA+NaOH	202	24.79
HA+NiCl$_2$	263	13.37

Compounds NaOH and CuCl$_2$ were selected for further investigation of interactions with HA in the liquid state and consequently elucidation of affection of its thermal properties.
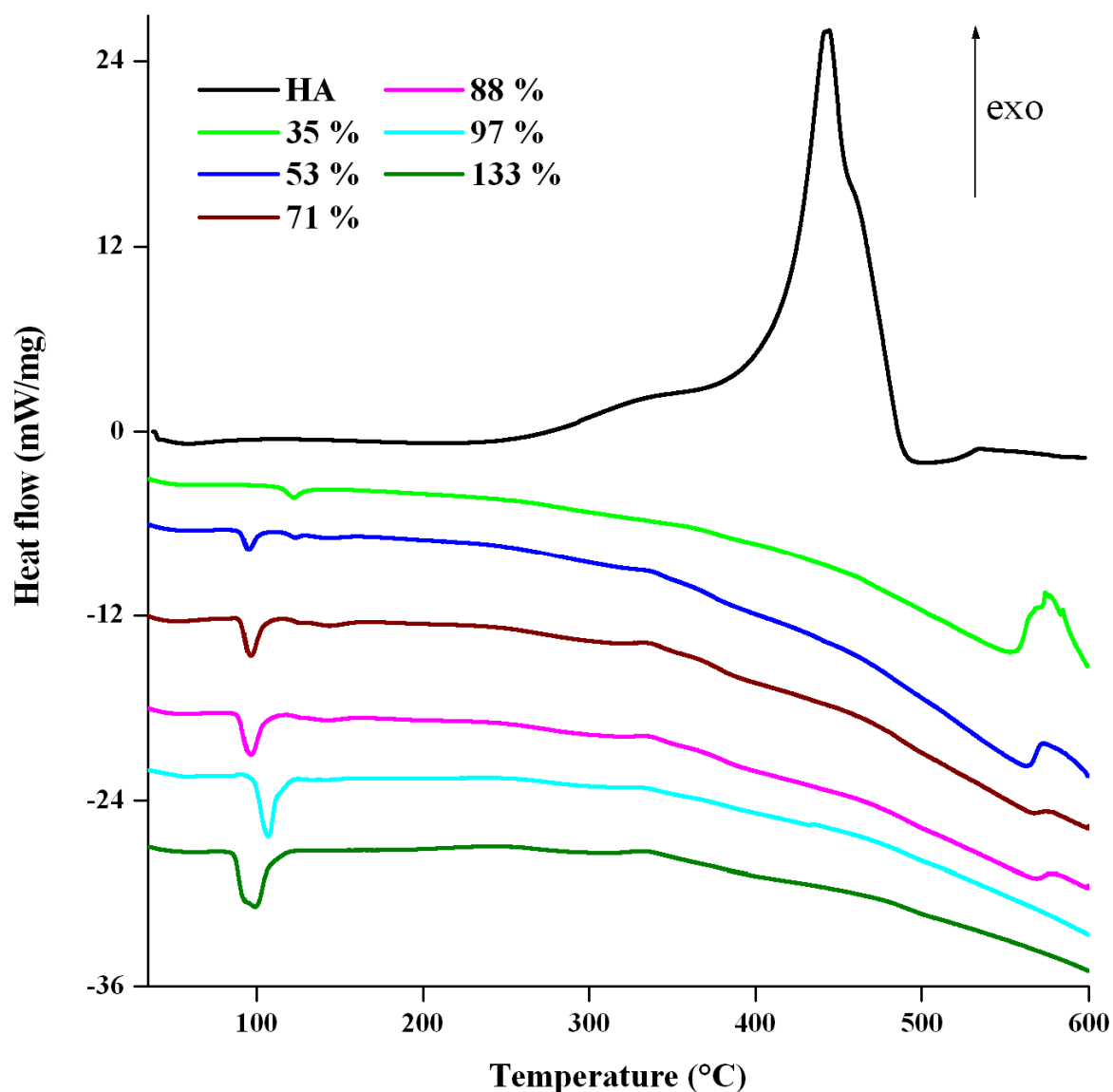
4.3. Influence of Na$^+$ ion

Fig. 5 *DSC curves of HA and its Na$^+$ salts*

In this part, different concentration of Na^+ was introduced into the HA matrix and the influence on DSC and TGA records was tested. As can be seen in Figure 5, except of parental HA, all DSC records show endothermic peak around 100 °C. Peak area of this process seems to be proportional to the saturation degree. Since the enthalpies of processes in modified samples are weak, the exothermic processes around 330 °C are zoomed and reported in Figure 6. One can see that there is not any relationship with degree of saturation. On the other hand, already 35% saturation degree seems to have a great influence on the stability of HA. Further, whereas in case of pure HA the degradation process is already progressive and culminates at 450 °C, modified samples seem to be more resistant against thermo-oxidative treatment. From 500 °C the unaltered HA doesn't undergo any important enthalpic change, whereas at the end of the measuring process there is possible to perceive an exothermic peak in case of modified samples. This peak decreases with increasing level of saturation; for 97% and higher saturation samples cannot be seen any longer.
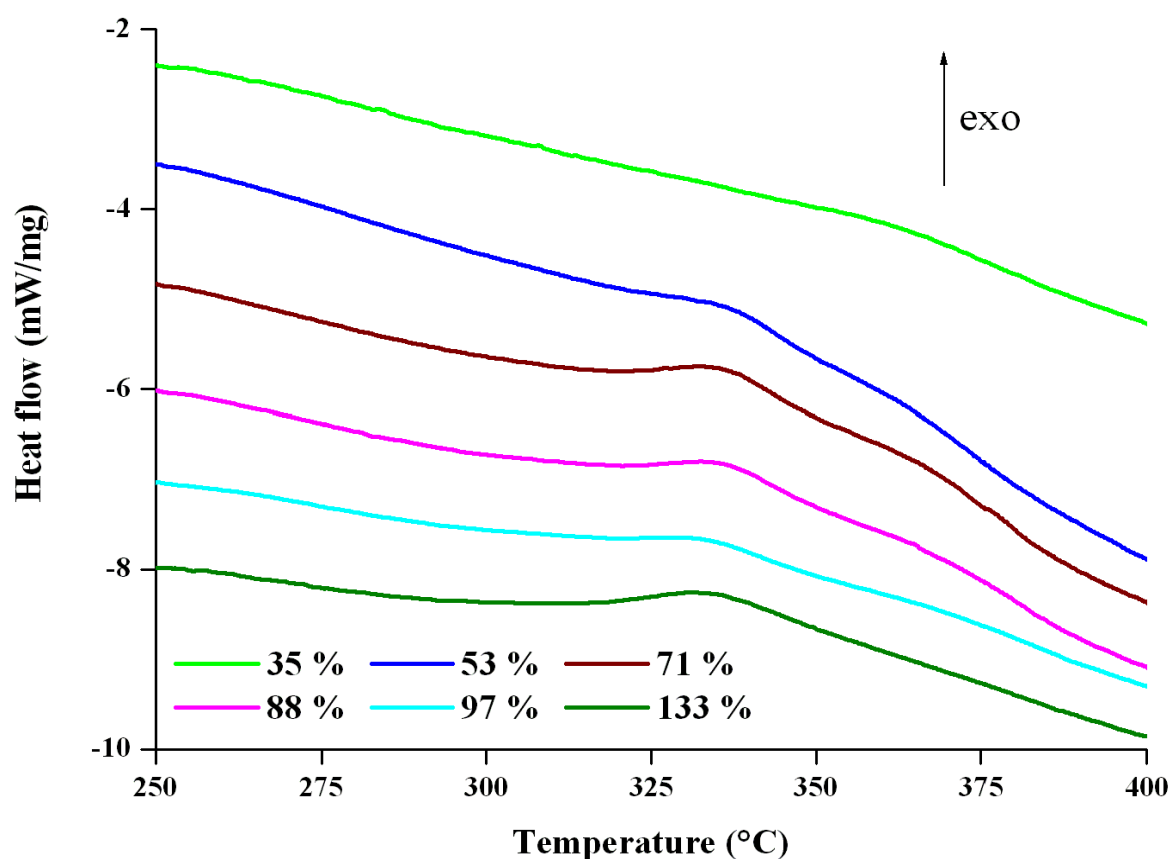

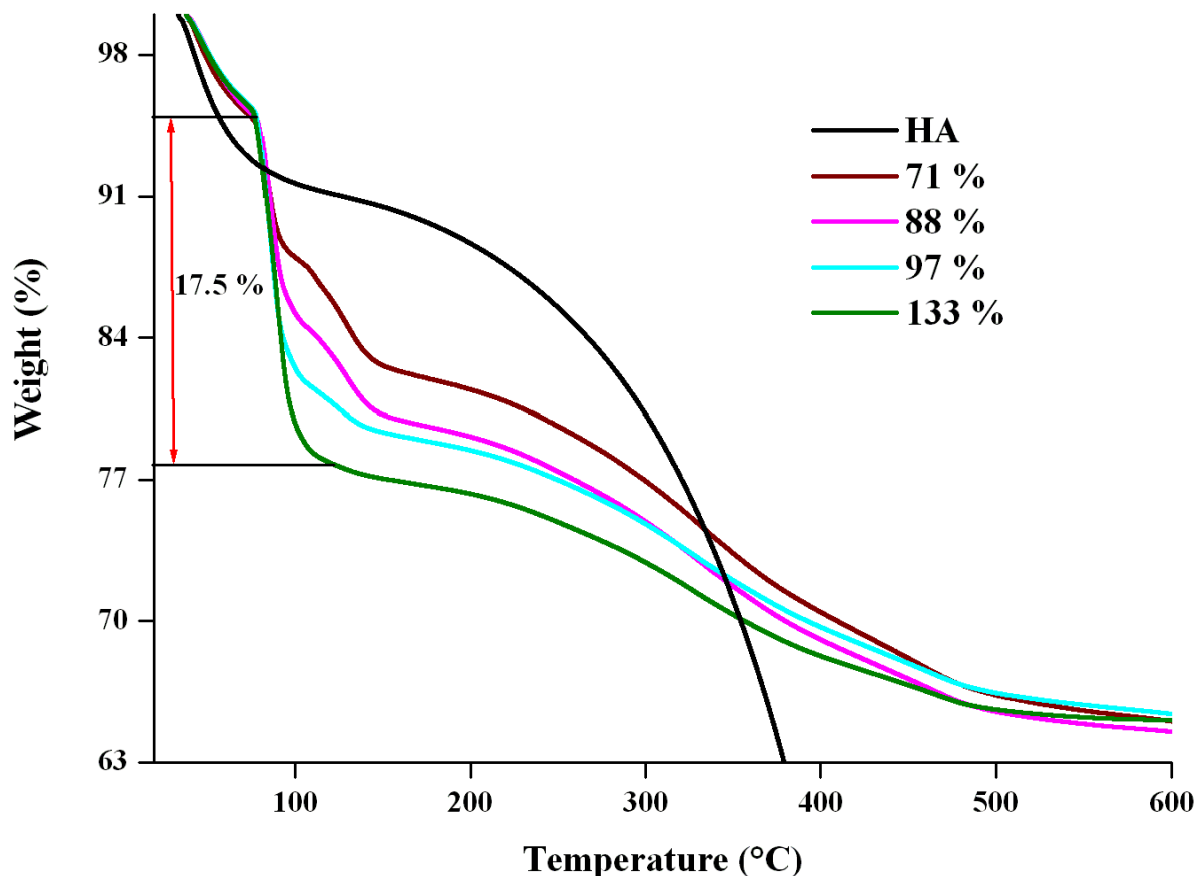Fig. 6 *DSC curves of HA and its Na^+ salts, zoom view*

Fig. 7 *TG curves of HA and its Na$^+$ salts, the whole record of parental HA is reported in Figure 4*

Figure 7 displays selected thermogravimetric records of HA and its Na$^+$ salts with various saturation of binding sites. Concerning the salts, at the beginning of the process at approximately 100 °C one can observe a great mass loss. In the case of oversaturated sample the weight loss is 17.5 %, other samples exhibit minor change in dependence on their saturation degree (see Table 2). The mass of all samples does not decrease below 60 % but it is most probable that the degradation would continue with increasing temperature. The degradation of HA itself has completely different progress. The weight loss at lower temperatures is in comparison with other samples quite low but during the degradation process the mass decreases rapidly and above 500 °C is the sample completely degraded.

Table 2 *Weight losses of Na$^+$ salts corresponding to first endothermic peaks on the DSC record*

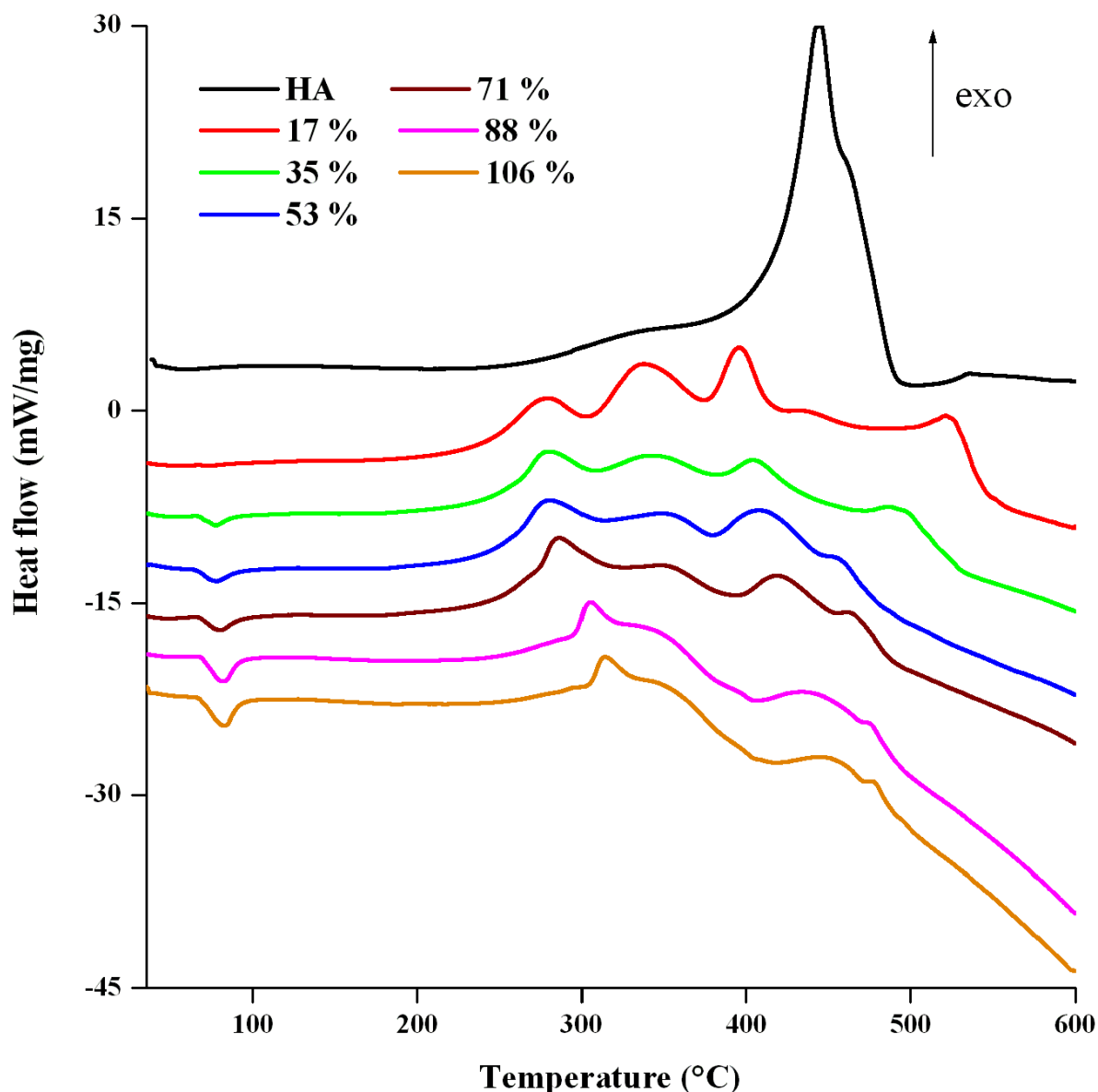| Degree of saturation (%)	Weight loss (%)
35	4.31
53	9.86
71	12.16
88	14.21
97	15.50
133	17.50

4.4. Influence of Cu²⁺ ion

Fig. 8 *DSC records of HA and its Cu²⁺ blends*

Similar experiments as in case of Na^+ were carried out with Cu^{2+}. DSC records are summarized in Figure 8. However, after sample preparation, i.e. mixing of HA gel-like purified sample with $CuCl_2$, a separation of humic matter occurred from the rest of blue-coloured solution. Therefore, the prepared samples cannot be considered as salts or complexes, consequently the resulted records reported here must be taken as blends of $CuCl_2$ with HA. Again, one can see the same phenomenon as in Figure 5 at temperature about 90 °C. Peak area of this process is again increasing as the concentration rises. Sample with the lowest concentration of Cu^{2+} ions has four major distinct exothermic, i.e. degradation peaks. The first peak maximum is at about 280 °C and the last peak maximum is at about 520 °C. The temperature of the first degradation peak is increasing with increasing

concentration of cupric ions and its area decreases. The heat released is less intensive with increasing metal concentration and the peaks are overlapping.
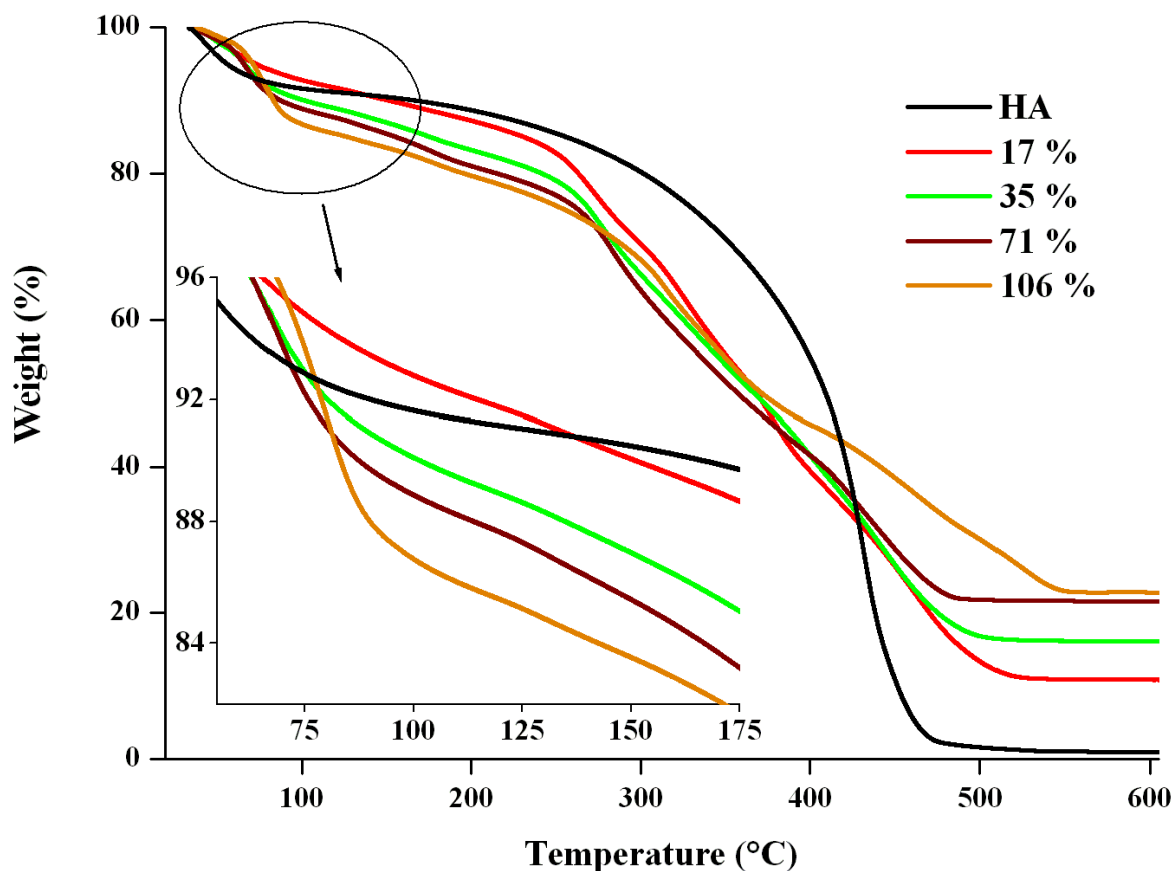

Fig. 9 *TG curves of HA and its Cu^{2+} blends*

Figure 9 summarizes the TGA records of HA with CuCl$_2$ at different concentrations. Salt samples in Figure 9 exhibit the same trend at temperatures above 100 °C as samples with Na$^+$ ions but in minor scale. In this case the decrease of weight is directly proportional to the concentration line of samples, see inner graph for zoomed view. The main degradation step begins at temperatures below 300 °C. At the temperature 600 °C the weight of all samples seems to be stable. Concerning the total mass loss, the highest was observed at unaltered HA and the lowest at sample with highest concentration of CuCl$_2$.

5. DISCUSSION

According to above-presented results, one can state that presumed stabilizing activity of metal ions on HA was not observed. Moreover, DTG onset temperatures of degradation peaks of samples with metal ions incorporated to the structure of humic acid confirm lower thermo-oxidative stability (Table 1).

Both DSC and TG curves of HA samples with various metal ions (Figures 3 and 4) resulted in variable and specific shapes reflecting metals ions' different chemical properties. It has already been stated that thermo-oxidative degradation of HA proceeded in two main stages. The first one is associated with degradation of labile moieties, mainly of those rich in oxygen (therefore relatively reactive) and aliphatic ones. In the second step, stable structures consisting of aromatic molecules are degraded. [50] Such knowledge can be easily identified in Figure 2 for pure HA, where the second degradation proceeded in two steps (see the shoulder of the high temperature peak). Introduction of aluminum into the HA matrix seem to intensify the first degradation stage while second one is significantly reduced. Further, both peaks seem to be more overlapped than in case of HA. This is in line with earlier observation that Al^{3+} ions are frequently sorbed on polar groups present in aliphatic molecules. [51] During the thermo-oxidative degradation of an organic molecule the lower amount of heat is liberated when a polar oxygenous group is present. However, when polar group is in close proximity of a metal ion such as for instance Al^{3+}, only carbonaceous part is decomposed and thus the heat is not quenched by the oxygen. Therefore, the lower stability, as indicated by onset on DTG is caused by re-aggregation of humic quaternary structure. Possibly, system of H-bonds stabilizing humic molecules is corrupted by the presence of Al^{3+}, aliphatic chains are not protected any longer and undergo easier oxidation.

In contrast, the presence of Cu^{2+} in an organic molecule is associated with free radical generation which accelerates the degradation process of HA. This can be supposed also for other ions of transition elements such as Ni^{2+} and Co^{2+}.

The introduction of Na^+ to the structure of HA disturbed the H-bonds between either two carboxylic groups and/or between carboxylic and phenolic/alcoholic OH groups and caused destabilization of whole HA structure. An interesting and unexpected phenomenon such as the endothermic peak at temperature 100 °C was observed on the DSC curves of variously saturated HA with Na^+. This effect comes along with loss of weight, which starts sharply and at the same temperatures of all samples. Moreover, the mass loss is proportional to the degree of saturation (see Table 2). Since the process appeared suddenly, it is clear that this effect cannot be explained as simple evaporation of loosely bound surface water. In such case the DSC peak would be broad, continuous for larger temperature range and only hardly integrable. Furthermore, the weight loss would be slow and gradual. There are two possible options explaining this phenomenon: 1) present Na^+ ion which is hold by COO^- group holds water. Hydratation numbers for a dissolved sodium ions are said to be four tightly bound water molecules. This adds up to eight water molecules for any ion pair of COO–Na. [52] 2) Na^+ ions introduced to the humic matrix caused structural changes associated with formation of new quarternary conformation enabling high water retention. Such water bound in newly formed porous structure needs higher energy input to the sample to evaporate than ordinary moisture. In this case, Na^+ ion plays a role as a bridge which is in accordance with its "hard ion" properties. [53] The temperature around 100 °C is high enough to disrupt such a bridge allowing a water "leaking" and quick water evaporation or boiling.

Figure 10 shows the dependence of heat consumed during the first endothermic process per gram of sample on the saturation of the binding sites with Na^+ ions. As one can see there is no strict linearity but some kind of step phenomenon, which can be related to the heterogeneity of humic structure. Similar picture was obtained when the Y axes was recalculated per moles of Na^+ present in HA structure. Therefore, the option 1) seems to be more realistic.
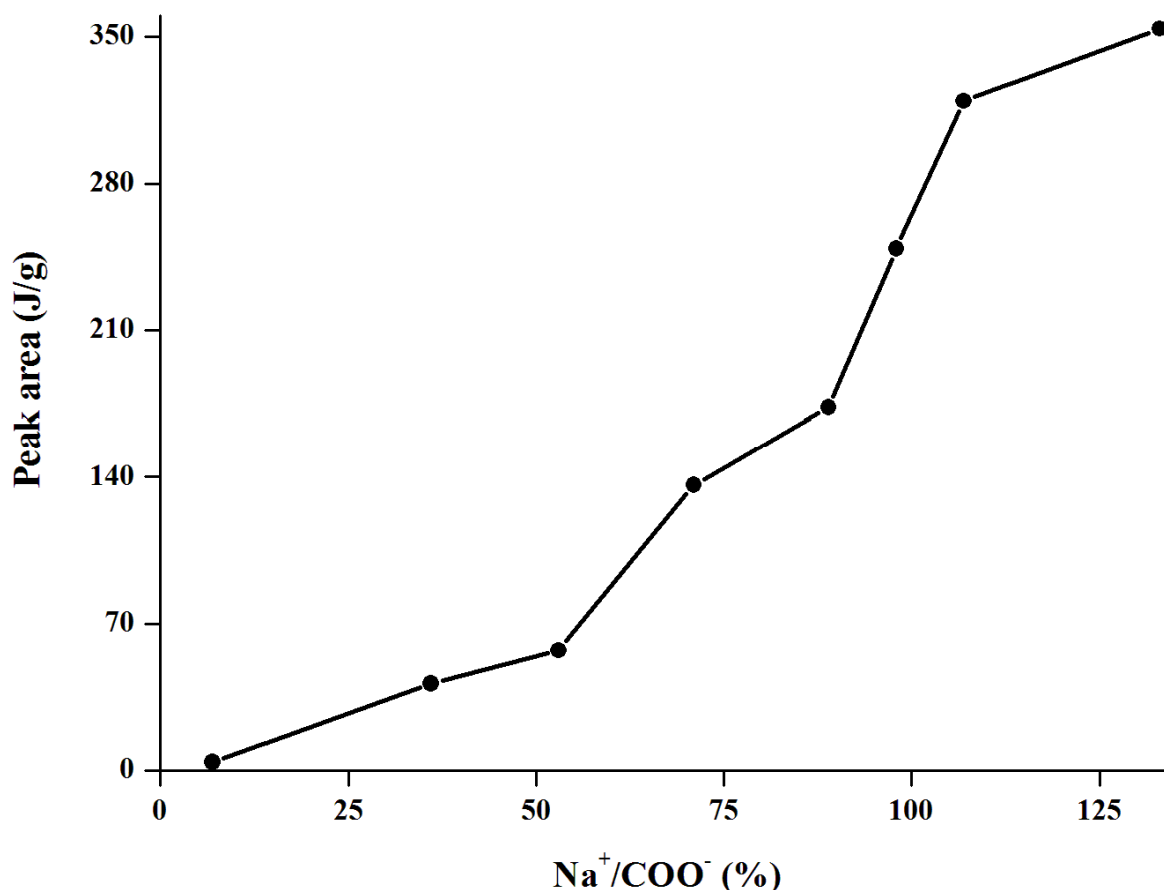

Fig. 10 *Dependence of heat consumed during the first endothermic process in HA/Na$^+$ blends*

Similar dependencies as in Figure 10 were plotted also for first endothermic peak occurring at 90 °C in case of HA/Cu^{2+} blends. In Figure 11 one can observe more-or-less linear trend. But in this case the endothermic process cannot be described as evaporation of water specifically bound onto the HA structure as in previous case. Closer scrutiny of DSC and TG record of pure $CuCl_2 \cdot 2H_2O$ (cf. Appendix I) reveals the same endothermic peak. This process is known to be loss of crystal water from the dihydrate. Since during the process of mixing gelous form of HA and solutions of $CuCl_2 \cdot 2H_2O$ two phases were formed (as described above and furthermore, the more concentrated solution was added the darker coloured phase appeared), we can certainly state that this process under study corresponds to loss of crystal water from recrystalized cupric chloride dihydrate.
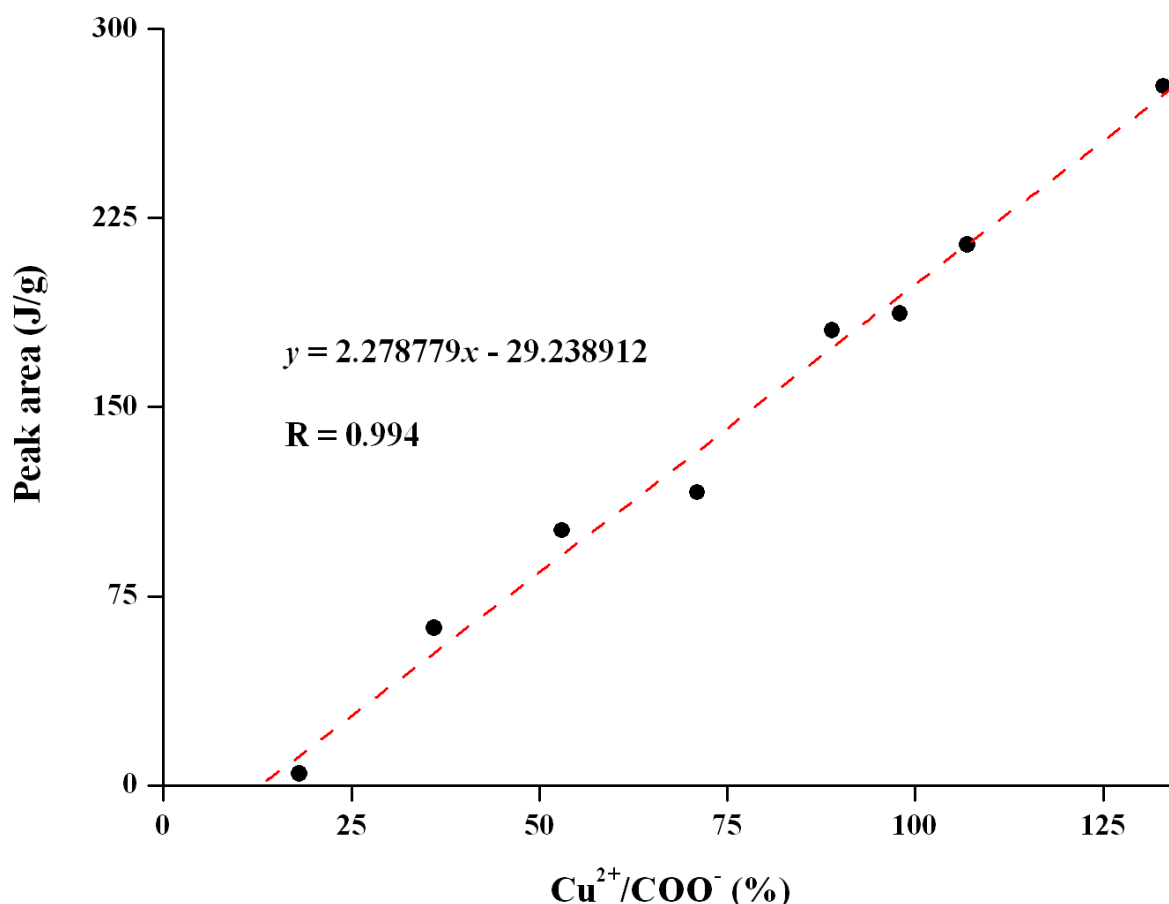
$$y = 2.278779x - 29.238912$$

$$R = 0.994$$

Fig. 11 *Dependence of heat consumed during the first endothermic process in HA/Cu^{2+} blends*

The linear dependence depicted in Figure 11 implies the possibility to determine the real sorption capacity of HA for Cu^{2+} ion. In this work, the non-traditional way of preparation was used. Commonly, Cu^{2+} ion is let to be adsorbed on the solid humic acid and the surplus of salt is later removed away, for instance by centrifugation. [54] However, this approach does not assure that all the Cu^{2+} ion has interact with HA matrix. There exist a distribution of cavities within the HA matrix which can cause entrapment of salt in the structure without any interaction of an ion with HA structure. Approach presented here was aimed to avoid such a danger and humic acids stood in the sol phase (moreover, with no disturbing of other impurities). It seems that approximation of dependence to $Y = 0$ may be used for the evaluation of sorption capacity of HA without the influence of quaternary structure. Accordingly, the content of Cu^{2+} adsorbed on the HA (by unknown interaction) is around 13 %. This value seems to be relatively low, however, the experiments were carried out at pH 3.5 which is not supportive environment for complexation processes. [37]
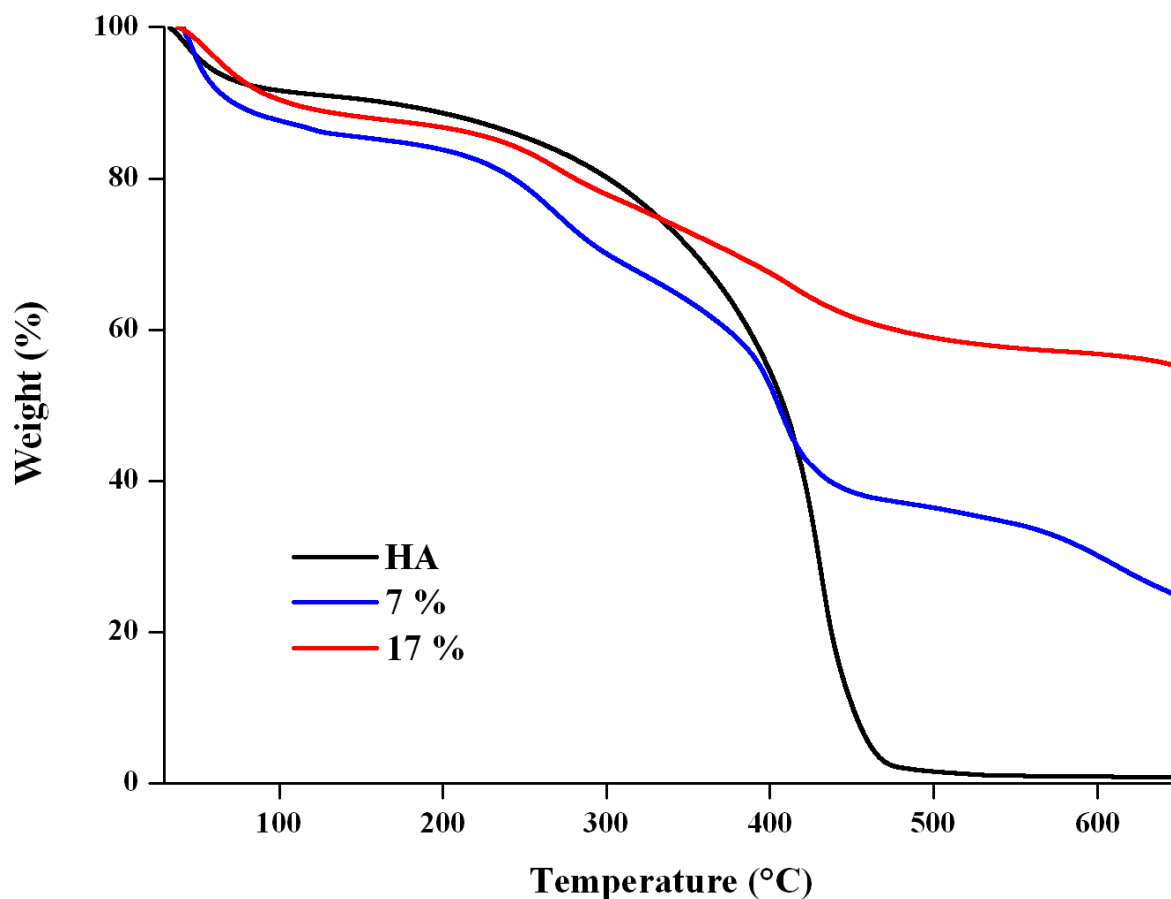
Fig. 12 *TG curves of HA and two differently prepared Na$^+$ salts*

For the better elucidation of differences in methods for determination of carboxylic group content, the 7% Na$^+$ sample, which accordingly to the direct titration to pH 7.5 should give 50% Na$^+$ sample was compared with 17% sample (calculated form retitration method, [49]). As can be seen in Figure 12 the highest and lowest total mass loss was observed for pure HA and HA + NaOH with 17% saturation, respectively. Both two mixtures show higher weight losses than unaltered HA at about 150 °C and degrade in at least three visible steps. Furthermore, none of the mixtures is completely degraded at the temperature of 650 °C. Therefore, it is clear that method described in [49] provide more reliable data. Similar and consistent explanation can be made observing data in Figure 13.
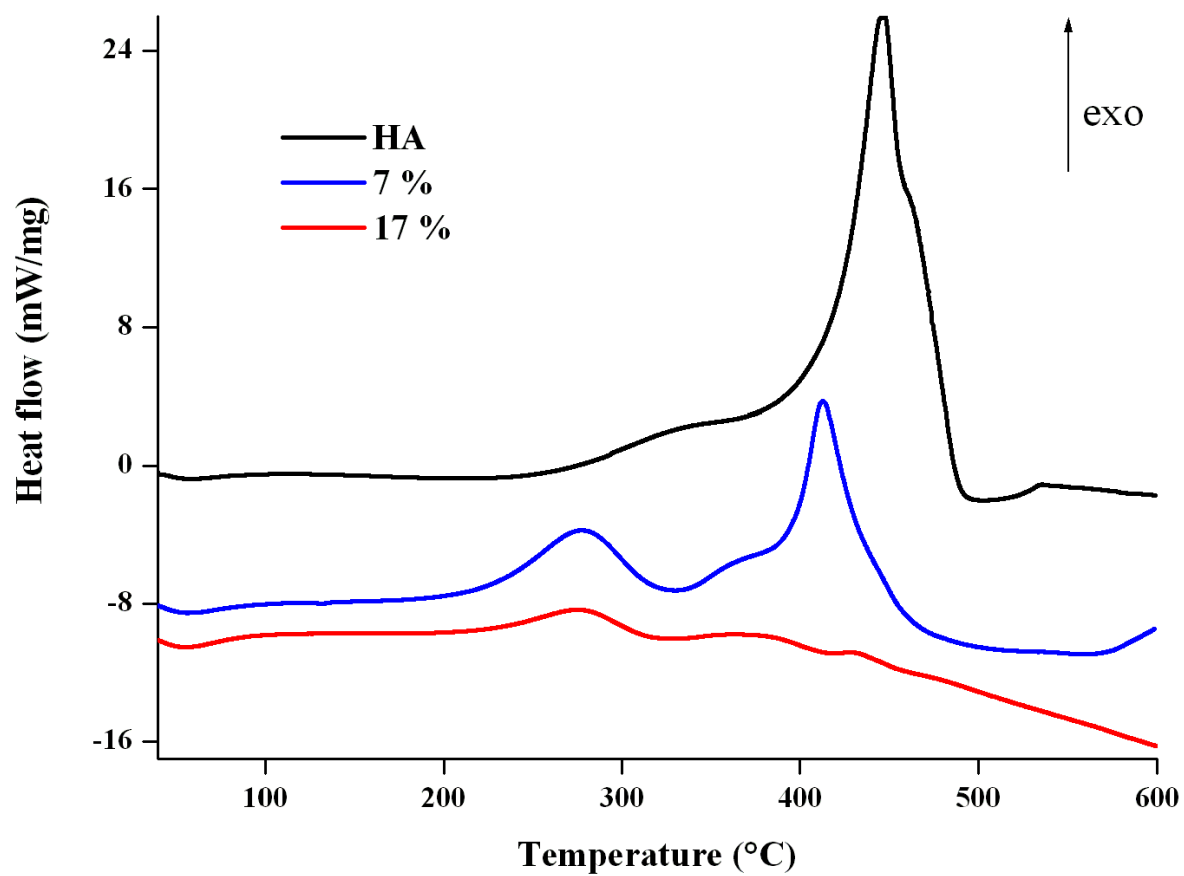
Fig. 13 *DSC curves of HA and two differently prepared Na⁺ salts*

6. CONCLUSION

- Metal ions incorporated to the humic matrix do not stabilize the HA structure.
- Already 7% concentration of metal ions per binding sites of HA constitutes considerable qualitative change in the thermo-oxidative behaviour.
- Na^+ ions showed the ability to influence the HA structure by probable formation of bridges; those are supposed to be responsible for high water retention in HA.
- During preparation of the mixture of HA with $CuCl_2 \cdot 2H_2O$ a separation of humic matter from the rest of blue-coloured solution occurred; we suppose that only 13 % of binding capacity of HA was employed.
- Some additional experiments and application of other analytical techniques are needed to deepen the knowledge of thermal analysis in research of metallic salts and complexes of HA and to shed light on the questions which arisen from this work.

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APPENDIX 1
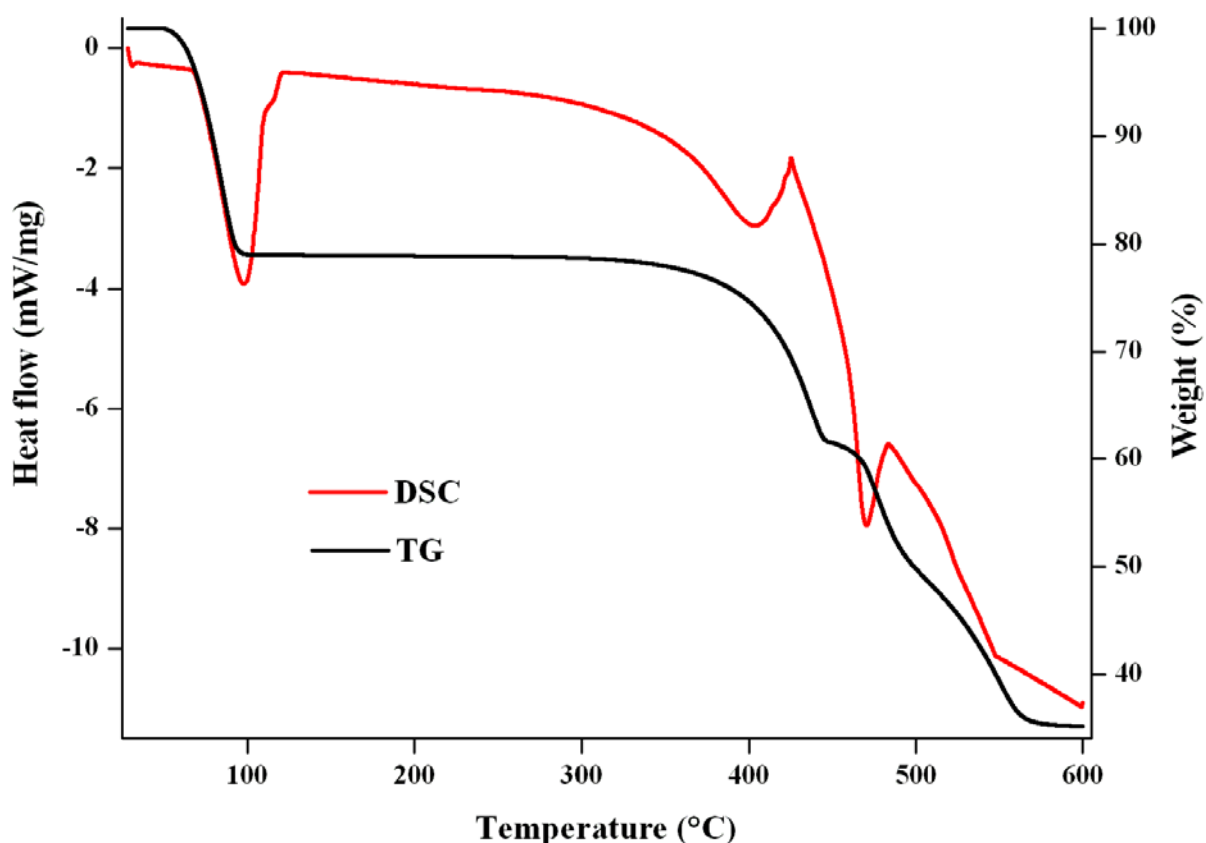
DSC and TG curves of crystallic CuCl$_2$ · 2H$_2$O

This figure displays both thermogravimetric and calorimetric record of pure, crystallic form of dihydrate of cupric chloride. Huge endothermic peak can be observed at 100 °C and comes along with massive weight loss (>20 %). Such behavior can be ascribed to loss of crystallic water from the sample. Another endotherm (400 °C), this time not that sharp and intensive is later overlaid with another very intensive endothermic peak. Both these endotherms are connected with weight losses and their steps are also overlapping. Mass loss is stopped at 35 % of initial weight.