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**STUDY ON LIGNITE HUMIC ACIDS**

STUDIUM LIGNITICKÝCH HUMINOVÝCH KYSELIN

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# 1 INTRODUCTION

Lignites develop under anaerobic conditions, and belong to the group of low-rank coals. These caustobiolites present those pools of coal that were subjected to the least amount of metamorphic change during the coal-forming process and contain a greater fraction of a volatile matter from the original peat material and less fixed carbon than high-rank coals (e.g. bituminous and anthracite).

Before 1989, coals of low degree of coalification had been used as a cheap but low-calorific fuel assigned for burning in power plants. However, the environmental policy and ambition of the Czech Republic to incorporate among European Community countries lead to changeover of energy producing raw materials as well as to coal mining inhibition. The latter caused higher unemployment and therefore social tension in locality or evoked discussions about the other possibilities. The main eventuality is the non-energetical application, both direct and non-direct.

The main source of lignites non-energetical utilization idea is based on their low combustibility, very high content of water and especially on the chemical composition and behavior. This fact, associated with an increase of natural gas and crude oil prizes, caused that the coals of low degree of coalification are becoming to be economically extremely important raw material. Moreover, the environmental point of view should not be neglected, as well. It is well known, that for instance the sulphur content, released during combustion as  $\text{SO}_2$ , in lignites varies in range 1.0–5.0 % (w/w) [1]. This comparatively huge amount is supposed to be polluting factor playing an important role in the environmental deterioration of localities where lignites are burnt as a solid fuel.

Generally, OM of coals consists of molecules that contain aromatic and cyclic aliphatic rings linked by aliphatic bridges. Heteroatoms are also present in the structure in the series of miscellaneous heterocycles. In coals with less advanced coalification, the initial woody material that generated the coal can be seen in resulting structures. As a result of low period of coalification, these structures are richer in oxygen containing functional groups comparing with the long- period ones. While the part of decomposed parental phytomass is converted into compounds such as bitumens, the next one resulted in matrix called as humic substances (HS).

Sub-bituminous coals, peats and lignites are known being sources rich in HS content. In spite of their different ways of genesis, these organic materials could exhibit similar chemical properties to those of humified soil organic matter.

Although HS belong among the least understood natural organic matter, their paramount importance in sustaining plant growth and controlling both the fate of environmental pollutants and the biochemistry of organic carbon in the global ecosystem is undisputable [2]. HS obtained from almost any environment (aquatic, terrestrial, etc) or extracted from various substrata (compost, manure, etc.) have been deeply studied for a long time. In literature, thousands of papers dealing with the analytical characteristics of these compounds and with their functions and effects in

the biosphere can be found. Nowadays, the potentiality of humic substances in many fields (e.g. agriculture, industry, environmental, etc.) is well recognized. However, the practical diffusion of these substances seems to be limited to market niches as a consequence of their high selling price, inconstancy of characteristics and limits on supply. Hence, the availability of large humic substances production from an inexpensive source through reliable process appears to be particularly desirable and attractive.

Based on the results and work over last few decades, the application and usage of humic substances have been evident but reserved. Opportunities exist today to further exploit and demonstrate the value of such products. If industry wants to survive or progress further, there must be produced materials beneficial to the end users, containing low levels of undesirable ingredients, improve plant growth and minimize environmental impact. The first step of realization of such idea is good understanding of investigated substrate. It is self-evident, the industrial exploitation of soil or aquatic humic substances is not financially realizable. For instance, the price of humic standards is reported 5-175\$ per 100 mg. On the other hand, the availability of humic substances produced in large amount from an inexpensive source (e.g. peats and low-rank coals) through a reliable process appears to be particularly desirable and attractive. In Japan, some thousands of tons per year of nitrohumic acids are produced via nitric oxidation of low-rank coals and commercialized in local market. To sum up, utilization of such carbon-rich sources can bring not only attractive products but also trade revival in locality.

Lignite, from which the extracted humic acids will be investigated in this work, originates from Neogene field in the Dolnomoravský úval region. South-Moravian lignites (SML) are outputted at present only from a single locality in Mikulčice mine.

## 2 LITERATURE REVIEW

Humic substances (HS) are compounds ubiquitous in waters, soils, sediments, peats and low-rank coals. They develop randomly from the decay of plant tissues, from microbial metabolism–catabolism, or from both. As a result, these natural organic substances comprise an extraordinary complex, amorphous mixture of highly heterogeneous, chemically reactive yet refractory molecules. The amount of C on the earth as humic acids ( $60 \times 10^{11}$  t) exceeds that which occurs in living organisms ( $7 \times 10^{11}$  t). In spite of long-term intensive and extensive study, HS belong to the nature's least understood materials. Most commonly are known as hydrophilic, acidic substances causing brown or black color of rivers or lakes [2].

Operationally, HS are divided into three main fractions based on their acid/base solubility<sup>3</sup>: humic acids (HA), the fraction which is soluble in dilute alkaline solution but is precipitated by acidification of the alkali extract; fulvic acids (FA), which is that humic fraction which remains in the aqueous acidified solution, i.e., it

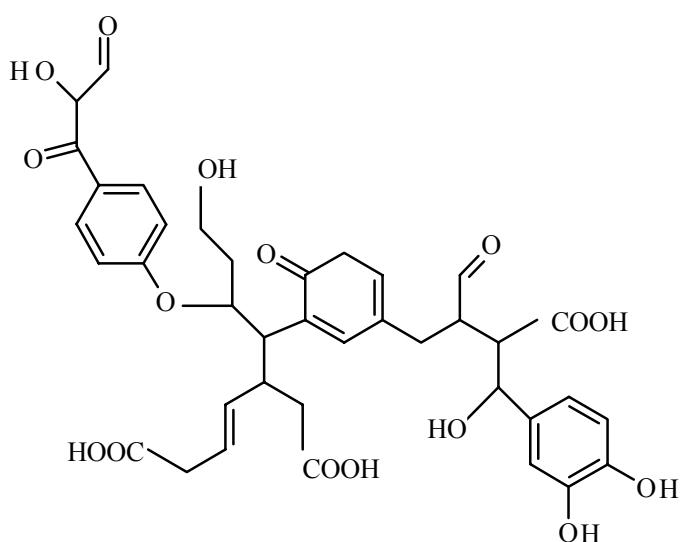
is soluble in both acid and base and the humic fraction that cannot be extracted by dilute base and acid, which is referred to as humin (HU) [2].

Although, at first sight, differences in their feature are obvious (FA yellow, HA brown or gray and HU generally black colored), there exists evidence that the chemical structure and properties of the humin fractions are similar to those of HA and those to FA. Available data [3] suggest similarity to each other except the slight differences in ultimate analysis, functional group as well as aliphatic/aromatic content. FA fraction has higher content of oxygen and is known to be more aliphatic than HA and those more than HU.

## 2.1 CHEMICAL STRUCTURE OF HUMIC SUBSTANCES

Despite the humic substances role is undisputable in sustainability of life, the basic chemical nature and the reactivity awareness of HS is still poor [4].

From the point of chemistry view, humic molecules are composed of aromatic and/or aliphatic chains and with specific content of functional groups. Their number and position depend on the conditions of formation. Elementary analyses data of humic samples originated from miscellaneous sources differ in their elementary composition and reactivity. Although, there exist undisputable differences in the way of genesis, humic substances from different sources should be considered as members of the same class of chemical compounds [5]. In past, many model structures have been suggested, however they could be only considered as “average molecule”.



**Figure 1:** Humic molecule structure proposed by Stein et al. [23]

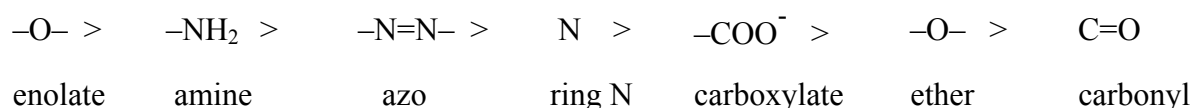
### 2.1.1 Functional groups and reactivity

A variety of functional groups, including COOH, phenolic OH, enolic OH, quinone, hydroxyquinone, lactone, ether, and alcoholic OH, have been reported in humic substances [3]. Except those, nitrogen, sulphur and phosphorus functional groups or bridges can be found in small amount, as well.

The major elements in humic substances are carbon and oxygen. The C content ranges from 53.8 to 58.7 percent in case of soil humic acids, for the coal ones is reported slightly higher. Fulvic acids have lower C (usual range of 40.7 to 50.6 percent) but higher oxygen (39.7-49.8 percent) content (Stevenson, 1994). The rest is as a rule reported being mostly hydrogen (3.2-7.0 percent), nitrogen (0.8-4.3 percent) and sulfur (0.1-3.6 percent) or possibly phosphorus (up to 3 percent). Frequently, the elementary analysis results of humic substances are reported as C/O and C/H ratios. Generally, it can be said, that lower C/O and C/H ratio may reflect higher amounts of COOH and/or carbohydrates and lower “aromaticity” or degree of condensation, respectively.

A major difference between the functional group content of humic and fulvic acids is that a smaller fraction of the oxygen in the former can be accounted for in COOH, OH, and C=O groups. On the other hand, the quinone C=O content of humic acids is universally higher than for fulvic acids whereas the latter seem to be richer in ketonic C=O content. Another practical difference is that practically all the oxygen in fulvic acids can be accounted for in known functional groups (COOH, OH, C=O) whereas a high proportion of the oxygen in humic acids occurs as a structural component of the “nucleus” (e.g., in ether or ester linkages). The absolute values in literature reported in  $\text{cmole kg}^{-1}$  vary in dependence on origin of humic matter.

From the point of reactivity view, the most frequent research in humus chemistry is associated with metal binding and is related to the formation of stable complexes. Study of such ion-humic interactions brings information valuable both for environment and agronomical purposes. Whereas monovalent cations are held primarily by simple cation exchange through formation of salt carboxyl groups, multivalent cations have potential for forming coordinate linkages with organic molecules. The order of decreasing affinity of organic groupings for metal ions is as follows:



However, due to high content of oxygen, metal ions are preferably bounded via oxygen containing groups, especially enolate and carboxylate. The most effective



bounding of metal ion – humic molecule interactions were proposed by Klučáková et al. [6].

Moreover, the interactions humic matter-organic compounds are studied and showed organic matter is being an important factor playing the promoting role in the nonbiological degradation of wide variety of pesticides. For those are responsible the nucleophilic reactive groups believed to occur in humic and fulvic acids (e.g., COOH, phenolic-, enolic-, heterocyclic-, and aliphatic-OH, amino, imino, semiquinones, and others). Of additional interest is that HS have the capability of bringing about a variety of reductions and associated reactions. The known occurrence of stable free radicals in HS further implicates organic matter role in chemical transformations of pesticides. The heterocyclic ring of amitrole, for example, is known to be highly susceptible to be attacked by free radicals [7].

### 2.1.2 Molecular structure

The common view of humic substances (HS) is that they are polydisperse macromolecules created by random polymers of a variety of biological monomers [8,9]. The molecular weight of humic substances is therefore believed to range from 500 Da for some aquatic HS to more than  $10^6$  Da for soil HA [3].

Evidence for this polymeric assumption was reached in many classical laboratory experiments that indicated possibilities for either abiotic or biotic condensations of simple molecules into humic-like materials [10]. The polymeric view of HS has included the general concept of polydispersity [11], in which the HS are made of polymers with different  $M_w$  values, similar to that which applies for other natural biological macromolecules such as proteins, polysaccharides, and lignin.

The amphiphilic nature of HA and a lower extent of FA has given rise to the micellar (membrane-like or detergent) model for these compounds [12]. Other studies [13-15] have indicated that the spontaneous aggregation of aqueous HA can be intramolecular (involving a single polymer chain) or intermolecular (involving multiple chains). In the former case, it is thought that humic polymers fold and coil in a manner that creates structured regions that can be likened to knots in a string. The interior of these assemblies is relatively hydrophobic, while the exterior is more hydrophilic. The structure is similar to that of surfactant micelle or better pseudomicelle, although it is constrained by the intramolecular nature of the arrangement, and the polydispersity of material.

Recent results obtained by size exclusion chromatography [16-19] suggested an alternative model in which relatively small and heterogeneous humic molecules self-assemble in supramolecular conformations stabilized only by weak forces such as dispersive interactions (van der Waals,  $\pi$ - $\pi$ , and CH- $\pi$  bonds) and hydrogen bonding. Such humic associations show only apparent high molecular dimensions which can be reversibly disrupted by either treating humic solutions with low

concentrations of mineral and monocarboxylic acids [20] or slightly varying the mobile phases composition but keeping ionic strength constant [19]. New formation of intermolecular hydrogen bonding and alteration of pre-existing hydrophobic interactions accounted for the disruption of original supramolecular associations of humic matter. The new concept was verified by Buurman et al. [21] as well.

The Multi-dimensional NMR results obtained by Simpson et al. [22], suggested that the major molecular structural components in the mixtures operationally defined as HS are aliphatic acids, ethers, esters and alcohols; aromatic lignin derived fragments; polysaccharides and polypeptides with relatively low molecular weight  $< \sim 2,000$  Da.

## 2.2 EXTRACTION, FRACTIONATION, AND PURIFICATION

The search for suitable extractants has been and still is a matter of high priority. The ideal one should remove practically all of humic material without altering its physical and chemical properties. During the past decades, a number of interesting and useful procedures have been developed. The strong bases (NaOH,  $\text{Na}_2\text{CO}_3$ ), neutral salts ( $\text{Na}_4\text{P}_2\text{O}_7$ , NaF, organic acid salts), organic chelates (acetylacetone, cupferron, 8-hydroxyquinoline), formic acid, mixtures of solvents (acetone- $\text{H}_2\text{O}$ -HCl) etc. have been in past employed with different efficiency. In spite of testing of such large number of extraction agents, dilute aqueous NaOH solution remains the most commonly used and quantitatively the most effective reagent for extracting HS from miscellaneous substrata [25]. Recently, very frequently as the extraction agent the mixture of 0.5 M NaOH and 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  is used to avoid time-consuming operation of decalcification [25].

HS have been traditionally fractionated based on their solubility in alkaline-complexing solutions (HA), in acidic solutions (FA), and in neither of the two solutions (HU). Briefly, after extraction of HS from parental matter, HA and FA (precisely their salts) dissolved whereas HU remains undissolved. Lowering pH of supernatant (after separation) by means of 6M HCl causes precipitation of HA. The precipitate with supernatant consisting of FA is left a few hours in refrigerator to ensure complete precipitation of HA. Then the supernatant is siphoned away. Follows the purification of HA by HCl-HF mixture to lower inorganic impurities content. HA are then washed with distilled water and dialyzed. If necessary, by passing through XAD8 hydrophobic resin column, may be FA separate from co-extracted biomolecules (peptides and carbohydrates), eluted by a NaOH solution, adjusted to pH 7, dialyzed until chloride-free and freeze-dried. The latter is valid for HA, as well. Although the efficiency of alkali extraction is reported high (up to 80%), to obtain whole pool of HS from original matter, it is necessary to repeat extraction procedure at least for three times.

In past many works have been published describing attempts to subdivide humic matter. Several experiments have been made to extract HS by sequential extraction

using different reagents. Another attempt involved partial precipitation with electrolyte under alkaline condition. Japanese workers have applied fractional precipitation to humic substances in aqueous alkali-alcohol system and have separated several fractions. Freezing method for separation have been used by Russian workers and resulted in two discrete fractions for which a number of chemical characteristics were determined. Recently, gels and chromatographic column (filled up with Sephadex,  $\text{Al}_2\text{O}_3$ , activated charcoal etc.) with broad range of molecular exclusion properties have been employed. Some works applied electrophoretic methods have been done, as well [25].

### 2.3 EXPLOITATION OF HA

A perusal of the literature, and particularly of the patent literature, shows many scattered applications of humic matter. Except power-producing application, four main categories can be mentioned: agricultural, industrial, environmental and pharmacological [26].

In addition to its natural occurrence in soils, humus and modified humus materials have been also added to soil for agricultural purposes. A number of different formulations have been used with the aim to increase soil fertility. As the examples can be mentioned: calcium salts of HA and FA in mixture with peat [27]; sodium humate applied directly to the leaves of plants [28]; ammonium salt of HA [29]; trace-element abundant HA [30]; mixture with synthetic polymers [31] etc. The HS and their derivatives were applied with the aim either stimulate plant growth or improve physical properties of soil and in most cases the excellent results were achieved.

Although in 1960 Piret et al. [32] stated, that more consideration should be given to the industrial potential of HA, in the intervening forty years no widespread, systematic industrial application have not been developed. Nevertheless, a review of literature reveals numerous industrial applications. Several examples are given here: improving the characteristics of well-drilling fluids [33]; a cement additive [34]; an agent for tanning leather [35]; a pigment in inks [36]; a plasticizer component for polyvinyl chloride [37], etc.

In environmental applications, HS have been used for removing metal ions, antropogenic organic chemicals, and various other pollutants. It is self-evident, that due to physico-chemical properties of HS (particularly cation exchange capacity and amphiphilic character) they can be used both directly and modified. Briefly, some environmental application of HS in last: removing heavy metal ions, cyanide, phosphates, oil, detergents and dyes from water with more than 98% efficiency [38]; removing phenol from water [39]; sorbent for waste gases from an animal-carcass rendering [40] etc. Despite of very little anion exchange capacity, peat and other humus-based material were converted into anion exchangers [41].

Fuchsman [42] stated that pharmacologically the most important components of peat humus might be steroids and terpenoids. The importance of these compounds classes resides not only in their applications for topical treatments (such as cosmetic creams and therapeutic bath) but more significantly in the isolation and identification of discrete compounds from which new medicinal chemicals may be synthesized. For better information on the subject, the excellent review [26] can be consulted.

### **3 WORK OBJECTIVES**

In this work, we continue in the previously started research [43] to explore application of SML as a chemical raw material. More precisely, we have devoted to humic substances presented in SML in relatively high content. In the first step the attention is focused on procedures and techniques leading to extraction of HS. As a part of this work, testing of chemical agents and conditions to obtain maximal yield of so-called regenerated humic acids is included. The emphasis is placed on the simplicity and time and financial acceptability, and therefore, products could not be “analytically pure”.

In the next step, the characterization of SML humic acids is done. For the sake of the well-known fact, that the chemical nature of humic substances, in spite of the application of almost all available analytical instrumentations, has not been sufficiently explained, our aim was to obtain data consequently applicable in potential technological applications. Analysis of humic samples purified with standard procedure is carried out.

Due to high complexity and heterogeneity of humic substances, the fractionation procedure resulting in the simplification of analysis should be suggested.

The metal ion/humic acids reactions in sols are studied. Firstly, the main types of interactions are investigated. In the next step, the kinetics of reactions including kinetic parameters determination follows.

FTIR, EPR, fractional centrifugation, elementary analysis, high performance size exclusion chromatography (HPSEC) and methods of thermal analysis (TA) such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were employed.

## **4 EXPERIMENTAL**

### **4.1 EXTRACTION**

20 g of lignite, previously dried to equilibrium moisture content and retained between 0.2-0.3 mm sieves were mixed with 200 ml 0.1 M HCl. The substrate was then washed with distilled water until free of Cl<sup>-</sup> ions. Pretreated lignite was then placed in a beaker and 200 ml of 0.1 M NaOH solution were added. Suspension was

stirred for a few hours. The remaining solids were removed by centrifugation and then rinsed with distilled water. Supernatant solution and the solution obtained during rinsing the remaining solids were mixed and concentrated HCl was added to pH = 1. The coagulated HA were decanted, washed several times, dried and weighted.

## **4.2 OXIDATION OF SML**

20 g of lignite (equilibrium moisture, particle size 0.2-0.3 mm) were mixed with 200 ml of oxidation agent in reaction vessel and for the desired time; concentration of oxidation agent and oxidation temperature were varied. The oxidation by nitric acid and a mixture of hydrogen peroxide with phosphoric acid was used.

## **4.3 ELEMENTAL ANALYSIS**

The ash content was determined by heating at 700°C for 4 hours in air; moisture at 105°C for a few hours to constant weight or by using of Shimadzu DTG-60.

Elemental analysis was kindly made in Pliva-Lachema company, Brno, using standard methods of Carlo-Erba elementary analyzer or in case of pH-fractions by Fisons EA 1108 Elemental Analyzer. Oxygen was calculated by difference.

## **4.4 CHARACTERIZATION OF HA BY FTIR SPECTROSCOPY**

IR spectra were measured using KBr technique with Nicolet Impact 400 spectrometer. The pellets for infrared analysis were prepared by mixing of approximately 5 mg of dry humic substance sample with 250 mg of dried KBr and pressed in press H-418 Trystom Olomouc.

## **4.5 FRACTIONATION**

The HA was treated overnight with a 0.5% (v/v) HCl-HF solution to remove residual ashes, dialyzed (Spectrapore 3 dialysis tubes, 3500 Mw cut-off) against distilled water until chloride-free and freeze-dried. The sample HA1 was obtained.

The freeze-dried HA1 was further shaken in aqueous buffer solutions to sequentially extract fractions soluble at different pHs. First, 1 liter of phosphate buffer at pH 7 (0.062M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  + 0.038M  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) was added to 2 g of HA1, and the suspension was shaken for 24 hours. The supernatant was separated by centrifugation, adjusted with concentrated HCl to pH 1, and freeze-dried as humic fraction HA2. The solid residue was again added with 1L of a phosphate buffer at pH 8 (0.014M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  + 0.086M  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ). The resulting humic extract, HA3, was obtained as for HA2. The solid remaining after extraction of HA3, was further added with 1L of a buffer solution at pH 9 (0.002M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  + 0.098M  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) and the extracted humic acid, HA4, was isolated as for HA2. Finally, the solid residue from the HA4 extraction was treated

with 1 L of a pyrophosphate buffer solution (0.10M) at pH 10. A humic acid sample, HA5, was obtained as above.

For the purpose of HPSEC analysis NA salts of humic samples were prepared as follows: humic sample (50 mg) was suspended in distilled water (60 ml) and titrated to pH 7 with a CO<sub>2</sub> free 0.1M NaOH solution by an automatic titrator (VIT 90, Videotitrator, Radiometer, Copenhagen). The resulting sodium humates were freeze-dried and homogenized in an agate mortar.

#### 4.6 HPSEC ANALYSIS

The HPSEC system consisted of a Perkin–Elmer LC200 pump equipped with two detectors in series: a Perkin–Elmer LC295 UV/VIS detector set at 280 nm for humic analyses, and a refractive index detector from Fisons Instrument (RefractoMonitor 4.0) for calibration with polysaccharides. A rheodyne rotary injector, with a 100 µl sample loop, was used to load HPSEC solutions and a Phenomenex Biosep S2000 (600 x 7.5 mm) column was used for size exclusion separations. The column was preceded by a Biosep Guard column and a 0.2 µm stainless-steel inlet filter. Flow rate was set at 0.6 ml min<sup>-1</sup> while the HPSEC eluent was a 50 mM NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O solution adjusted at pH 7 with 1M NaOH. The salt concentration was chosen to have a constant ionic strength of 50 mM in order to minimize ionic exclusion or hydrophobic interactions with the column [18].

Freeze dried sodium humates were dissolved in the HPSEC eluent to achieve a 0.6 mg mL<sup>-1</sup> humic solution and subjected to HPSEC analysis. The same solutions were then added with HCl, acetic, propionic or formic acid to lower the pH to 3.5 before HPSEC analysis. The small addition of these acids (<10<sup>-2</sup> M) did not change the ionic strength of the humic solutions [19]. All solutions were filtered through quartz filters (Glass Microfibre Filterm Whatman International, LTD) before injection.

#### 4.7 THERMAL ANALYSIS

Differential scanning calorimetry (DSC) and thermogravimetry analysis (TG) of each sample in oxygen atmosphere were performed for three times. For this purpose Shimadzu DSC-60 and DTG-60 were used connected through TA-60WS with computer, where the data were collected and then processed. The furnace was calibrated by using transition temperatures and enthalpies of fusion of indium and zinc (156.6, 419.6 °C and 28.42, 107.5 J.g<sup>-1</sup>, respectively). Samples were carefully homogenized in agate mortar, weighted to approximately 1 mg with accuracy of 0.01 mg and placed into platinum crucible. Flow rate of oxygen was set at 20ml.min<sup>-1</sup> and rate of heating 10°C.min<sup>-1</sup> from room temperature to 600°C was applied.

For the purpose of IP determination Shimadzu DSC-60 was employed, and rate heating 1, 3, 5, 9, 12, 15 °C per minute on were applied. HA sample was prepared as

described previously in case of HA1 with the one exception, for drying the oven heated for 50°C was used.

RHA were prepared by the same way as HA, however the lignite used was treated by 2%, 10%, 15% and 20% nitric acid for 20, 30, 40 and 60 minutes, respectively. The treated lignite was carefully washed with distilled water and the same procedures as in the case of HA followed. By this way, samples RHA2/20, RHA10/30, RHA15/40 and RHA20/60 were prepared (RHA and concentration of nitric acid/oxidation period).

#### 4.8 THE REACTIVITY STUDY

HA were dissolved in NaOH (0.1 M) and then the HCl (0.1 M) was added in ratio 1:1 (NaOH:HCl). The final concentration of sol was 0.101 g<sub>HA</sub>/dm<sup>3</sup> and pH  $\cong$  7. Those were mixed with Cu<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup> solutions in ratio 1:1 and the solution conductivity in dependence of time was measured.

### 5 THE MAIN RESULTS OF THE WORK

#### 5.1 EXTRACTION OF HA AND PRODUCTION OF RHA

As an initial part of whole research aimed to investigate the lignite humic substances, more specifically humic acids, the extraction procedures and the most efficient extraction agent were sought. Results of simple extraction procedure made under different conditions are summarized in Table 1. The content of ash never exceeded 12%.

It is evident, that the most powerful extractant is 0.5M NaOH. As expected, NaF is a mild extractant, on the other hand, it is supposed to cause minimum alteration of humic substances during extraction. Another alkali extraction agents (LiOH, NH<sub>4</sub>OH) were also tested with very low efficiency.

*Table 1: Dependence of yields of HA on the used extraction agent and temperature.*

Extractant/temperature	20°C	50°C	60°C	70°C	80°C	90°C
0.5M KOH	12.5	20.7	21.5	24.0	26.4	30.4
0.5M NaOH	14.9	21.4	24.5	26.6	30.2	38.3
0.1 M NaF	1.6	7.1	8.1	13.1	13.5	–

The possibility of production of the regenerated humic acids (RHA) was tested. Patents and literature suggest many oxidative procedures leading to enrichment of organic matter (particularly low rank coals) by humic acids. RHA gained by this way show similar properties as “common” coal humic acids, as a rule shows slightly higher aromatic content. It was found out, that by means of nitric acid should be

content of humic acids in SML enhanced up to 60%. The other oxidizing agents were tested as well, however, no such high yield has not been reached again.

**Table 2:** Dependence of yields of HA on the concentration of HNO<sub>3</sub> and time of oxidation.

Concentration/time	15 min	30 min	60 min	120 min
5% HNO <sub>3</sub>	39.5	48.5	44.3	43.0
10 % HNO <sub>3</sub>	43.1	43.6	59.4	43.1
20 % HNO <sub>3</sub>	52.5	59.7	48.5	47.4

Results of lignite oxidation experiments are given in Tables 2 and 3. Comparing with Table 1, it is evident that the contents of extractable humic matter can be raised up to about 60%, i.e. two-fold. Optimization of oxidation conditions should include both oxidant concentration and reaction time. Thus, either 10% nitric acid for 60 minutes or 20% for 30 minutes can be used. From Table 3 it is seen that hydrogen peroxide in the medium of 10% phosphoric acid is somewhat less active oxidation agent.

**Table 3:** Dependence of yields of HA on the concentration of H<sub>2</sub>O<sub>2</sub> and time of oxidation in the presence of 10% of H<sub>3</sub>PO<sub>4</sub>.

Concentration/time	30 min	60 min
5% H <sub>2</sub> O <sub>2</sub>	44.6	41.9
10% H <sub>2</sub> O <sub>2</sub>	42.7	42.6
20% H <sub>2</sub> O <sub>2</sub>	42.5	41.9

Using only hydrogen peroxide in the same concentrations as in Table 3 caused substantial reduction in yields (13 – 27% after 18 min. of oxidation). Pure hydrogen peroxide is thus too strong agent causing decomposition of lignite organic matter. The nitric acid and hydrogen peroxide probably did not only show up as an oxidation agent but also as an “opener” of the structure of lignite.

## 5.2 FRACTIONATION OF HA

With regard to the high complexity of extracted humic matter and with the respect of known or accepted theories, the methods of pH-sequential extraction described above have been developed. The idea of the method is derived from the contrary method of separation of polymeric molecules based on sequential precipitation. Molecules are supposed being separated with agreement to their molecular chains lengths and number of functional groups. The original method is based on the stepwise changing of pH causing the precipitation of individual molecular-weight fraction. Firstly, it was supposed that suggested method should lead to similar results, which would be in accordance with “polymeric theory” of humic molecules<sup>3</sup>



and molecules were supposed being separated agreeably to their molecular weight. However, experimental data (below) lead to different conclusions.

### 5.3 ELEMENTAL ANALYSIS

The elemental composition of the bulk humic acid (HA1) and the pH-separated humic fractions (HA2-HA5) is reported in Table 4. HA1 showed a C/H ratio of 12.4, whereas the C/H ratios of the humic fractions were in the order: HA2>HA3>HA4=HA5. Decreasing C/H ratios appears to be related to decreasing aromaticity or degree of unsaturation. The HA2 sample, with the largest carbon amount and the lowest hydrogen content, was extracted from HA1 by a pH 7.0 buffer solution. This fraction may be composed by aromatic systems possessing acidic groups able being de-protonated up to pH 7. In fact, Simpson et al. [44] found that fractions extracted with buffer solution at pH 7.0 was aromatic and had a large content of highly acidic groups. Fractions HA3 and HA4, were extracted with buffer solutions at pH 8.0 and pH 9.0, respectively. Literature [3] reports that decreasing C/H ratios can be attributed to increasing aliphatic contents. Therefore, the aliphaticity of HA3 and HA4 can be considered larger than for HA2.

**Table 4:** Elemental analyses of SML humic acids and its pH fractions.

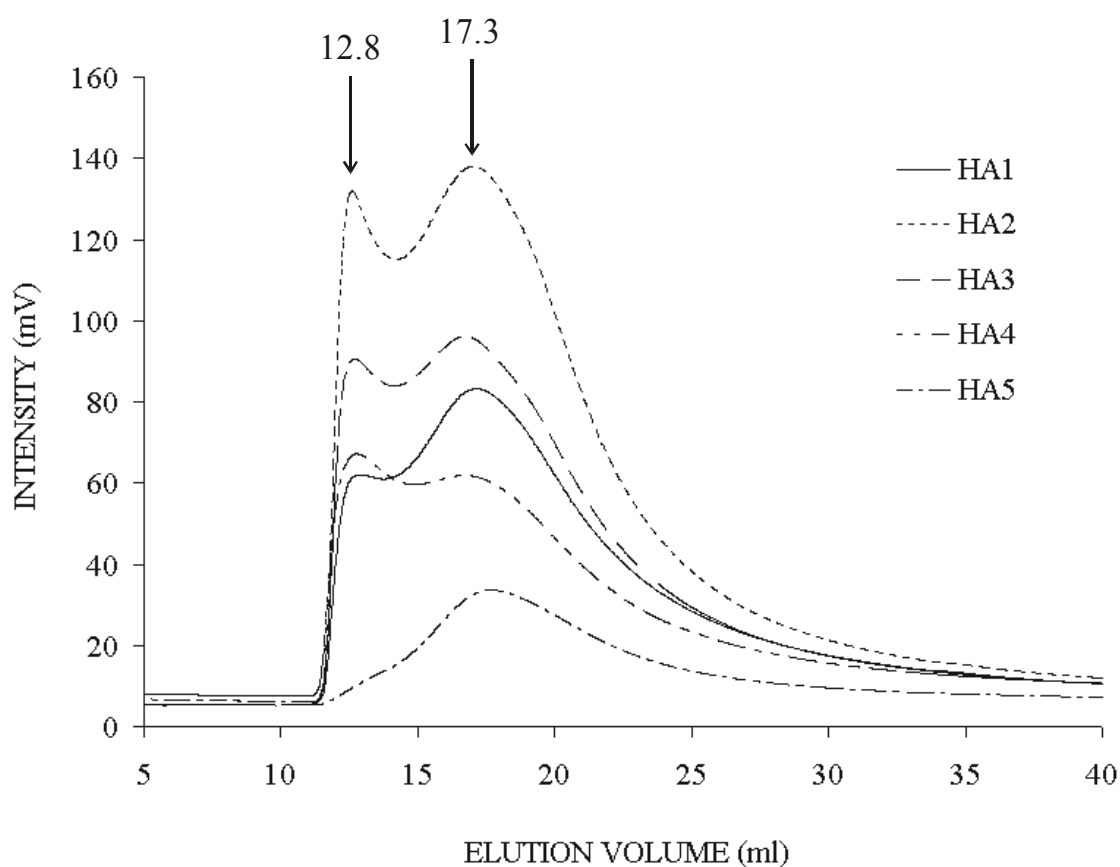
Sample	Ash	C (%)	H (%)	N (%)	O (%)	C/H	C/O
HA1	2.27	57.2	4.6	1.0	37.2	12.4	1.5
HA2	2.11	53.7	4.7	1.0	40.6	11.5	1.3
HA3	1.17	50.1	6.4	1.3	42.2	7.9	1.2
HA4	3.11	47.6	6.8	1.3	44.3	7.0	1.1
HA5	2.44	50.9	7.2	1.1	40.8	7.1	1.3

The oxygen content of HA2, HA3, and HA4 varies in the order HA2<HA3<HA4. Increasing amount of oxygen may be attributed to an increasing polyacidic nature of the humic systems [45]. Fractions HA3 and HA4 are thus not only more aliphatic than HA2, but also more polyacidic. The HA5 fraction revealed the largest amount of hydrogens, whereas carbon and oxygen content was comparable to that of other humic fractions. Simpson et al. [44] reported that humic fractions extracted at pH 10.0 showed the largest amount of hydrogen and related the elemental content of their alkaline-extracted fractions to a large degree of aromaticity. Furthermore, Piccolo et al. [45] reported that phenols were extracted from humic matter more by alkaline pyrophosphate solutions than by sodium hydroxide solutions. We may then assume that the HA5 fraction was selectively enriched in polyphenols as compared to fractions extracted in phosphate buffers at lower pHs.

## 5.4 HPSEC OF HUMIC FRACTIONS

The aim of this part of the work was to verify, if the same mechanism of separation of polymers led to the same results being applied to the separation of humic molecules. Such idea was based on the “polymeric view” of humic molecules commonly accepted by many humic scientists. For the purpose of molecular size distribution determination, the Na salts of HA and its pH fractions were used. The next step was to observe changes in chromatograms induced by addition of small amount of either organic (formic, acetic and propionic) or inorganic (HCl) acid.

The comparison chromatograms of individual HA fractions is given in Figure 2. The profile of size distribution for HA1 shows two peaks. The first one is centered at 12.8 mL, whereas the second peak is at around 17.3 mL of elution volume.



**Figure 2:** UV-detected HPSEC chromatograms of humic samples.

For each of sample, the weight-average molecular weight ( $M_w$ ) was calculated using the PE-TC-SEC 4.01 software and the following relationship:

$$M_w = \frac{\sum_{i=1}^N (h_i M_i)}{\sum_{i=1}^N h_i} \quad (1)$$

where  $M_i$  and  $h_i$  are the molecular weight and the height of each  $i$ th fraction in the chromatogram, respectively [46]. All data were processed using a SEC noise threshold of 100, and a Savitzky-Golay smoothing with a filter size of 5. The calculated  $M_w$  values based on PSS and PSC column calibration are given in Table 6.

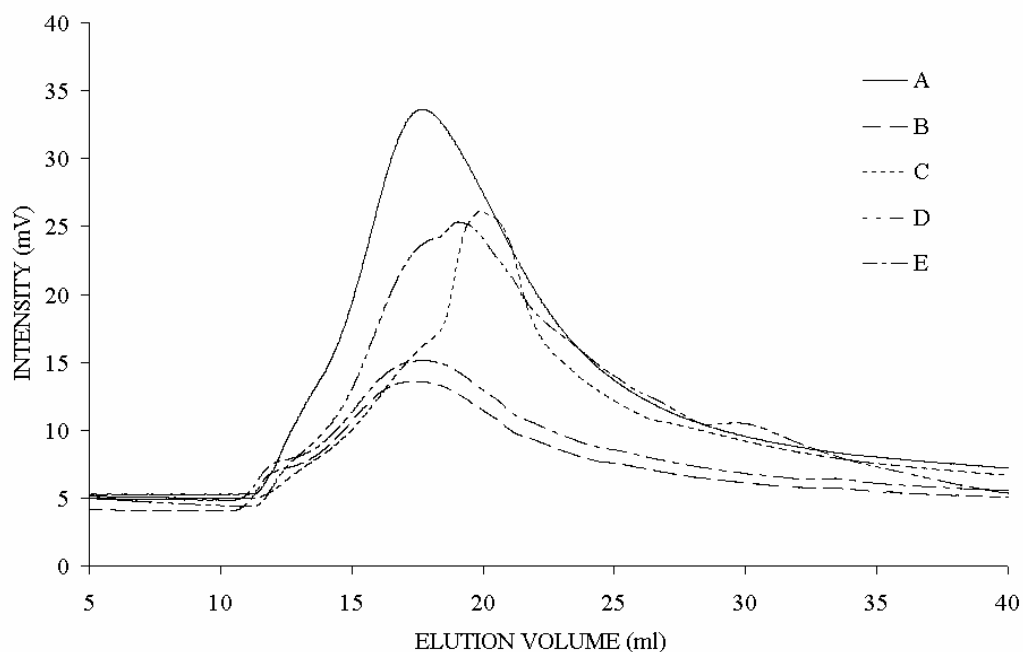
**Table 5:** Weight-averaged molecular weight ( $M_w$ ), in Dalton, of humic samples after treatment with different acids; calibration by polystyrenesulphonates and polysaccharides (PSS/PSC).

Sample	$M_w$				
	Control	HCl	Formic	Acetic	Propionic
HA1	9,961/18,878	10,592/19,475	8,108/14,648	5,133/8,838	3,144/5,317
HA2	11,829/21,437	14,143/26,616	11,820/21,813	9,337/16,650	7,176/12,671
HA3	11,363/20,588	14,467/27,198	13,470/25,235	9,048/16,243	5,402/9,361
HA4	13,515/26,204	13,394/24,944	14,436/27,147	9,444/16,971	6,693/11,758
HA5	4,768/8,036	2,581/4,302	2,663/4,438	2,485/4,030	3,248/5,419

Differences in  $M_w$  values can be attributed to the different chemical composition of the humic fractions. The bulk HA1 contains all the molecular characteristics of HA2, HA3, HA4, and HA5, and its molecular size can be used as a reference to explain the conformational behavior of the humic fractions solubilized from HA1 at different pHs. Moreover, it is obvious that the  $M_w$  values of the fractions derived from HA1 cannot be higher than the bulk material itself as the calculations of  $M_w$  seem to indicate.

The explanation to such a contradiction should be attributed to the hydration of each material in solution that, in turn, depends on its molecular composition. Due to the pH of their extraction, the HA2, HA3, and HA4 fractions had a higher acidic nature than HA5 that was solubilized only at pH 10 and implies a contribution of hydration water to the molecular size. It should be assumed that the hydration sphere of the materials isolated at pH 7.0, 8.0 and 9.0 fractions is larger than that of HA5. In fact, the higher the amount of acidic functional groups, the larger is the hydration sphere of a water-soluble organic system. On the other hand, the polyphenolic character of HA5 confers a larger degree of hydrophobicity to this fraction, thereby preventing the formation of a bulky hydration sphere [46].

The molecular sizes of HA1 and its pH-extracted fractions were altered after addition of small amount of either mineral (HCl) or organic (formic, acetic and propionic) acids (Table 2). The acid groups present in both HA1 and most acidic fractions (HA2, HA3, and HA4) are deprotonated partly (HA2) or fully (HA3 and HA4) at the pH 7.0 of the control solutions. When a strong acid such as HCl or HCOOH is added to the solution until pH 3.5, a protonation of the dissociated functional groups may occur, thereby leading to formation of intermolecular H-bonds and increase of molecular sizes. Conversely, addition of CH<sub>3</sub>COOH and CH<sub>3</sub>CH<sub>2</sub>COOH provided decreasing *M<sub>w</sub>* values (Figure 3). As previously observed [4,47], the loosely-bound supramolecular nature of HA1 and its pH-extracted fractions may account for the decrease of *M<sub>w</sub>* values after addition of acetic and propionic acids. In fact, both protons due to low pH and alkyl chains present in the added organic acids may act together in disaggregating amphiphilic humic matter and lowering its *M<sub>w</sub>*.



**Figure 3:** UV-detected HPSEC chromatograms of HA5. A. HA5 dissolved at pH 7.0. B. As in A but added with formic acid to pH 3.5; C. As in A but added with acetic acid to pH 3.5; D. As in A but added with propionic acid to pH 3.5; E. As in A but added with HCl to pH 3.5

The changes of peak absorbance observed on the HPSEC chromatograms after pH modifications have been reported as a separation of humic chromophores from each other [17-20]. It is well known that the alteration of reciprocal orientation between the transition dipole moment of adsorbing chromophores may cause either an increase (hyperchromism) or a decrease (hypochromism) of the total molecular absorptivity of large biomolecules or aggregates of their components (Cantor and Schnitzer, 1980; Freifeld 1982).

The conformational disruption of humic matter and the separation of chromophores gave rise to hypochromism upon addition of organic acids is due to changes in the energy content of humic superstructures [4]. Lowering pH from 7 to 3.5 induced formation of intra- and inter-molecular hydrogen bonding between protonated acidic functions and complementary oxygen-containing groups present in either humic molecules or organic acids. Hydrogen bonds are relatively strong linkages since they involve an energy gain from 10 to 20 kJ/mol in comparison to van der Waals bonds [4]. As noted before [19, 20] increase in energy content is the thermodynamic drive to pass from unstable conformations, mainly held together by dispersive forces at pH 7, to new conformations predominantly stabilized by hydrogen bonding. Such acid-induced conformational rearrangement of HAs causes disruption of original superstructures into smaller associations and possibly even single molecules.

Therefore, it should be assumed that pH-sequential extraction is not based on the same principle as method commonly used for separation of polymer molecules mixtures and different mechanism appeared being responsible for separation of humic molecules.

## 5.5 THERMOANALYSIS AND STABILITY DETERMINATION

Thermally induced oxidative behavior of humic fractions resulted in gradual decomposition of humic molecules very well identified in DSC and TG thermograms. Changes of humic samples were observed by means of FTIR spectroscopy.

Each thermograms of lignite humic acids and separated samples gave 1 endothermic and from 2 to 5 exothermic peaks. The low-temperature DSC peaks of HA1–HA5 are given in Fig.4 whereas high-temperature ones are shown in Fig 5; all normalized to the sample mass.

**Table 6:** The temperatures of peaks maxima ( $T_p$ , °C) and reaction enthalpy ( $\Delta H$ , kJ/g) connected with well-defined exothermal peaks.

Fraction	Peaks					
	II		IIIa	IIIb	IV	
	$T_p$	$\Delta H$	$T_p$	$T_p$	$\Sigma\Delta H$	$T_p$
HA1	332	0.56	492	-	6.24	-
HA2	319	0.37	432	456	3.82	531
HA3	314	1.57	446	-	4.45	492
HA4	318	1.26	454	-	5.15	507
HA5	313	0.74	456	489	5.10	546

Table 6 reports data such as peaks maxima and enthalpy associated with decomposition steps indicated by the second, third and fourth peak; in two cases the third exotherm showed splitting and therefore was in table marked as IIIa and IIIb.

The reaction enthalpy was calculated as a sum of both peaks. Due to the ill-definable endsets of the last exotherm, only the temperatures of peaks maxima are given in Tab. 6. Experimental TG results are summarized in Tab 7.

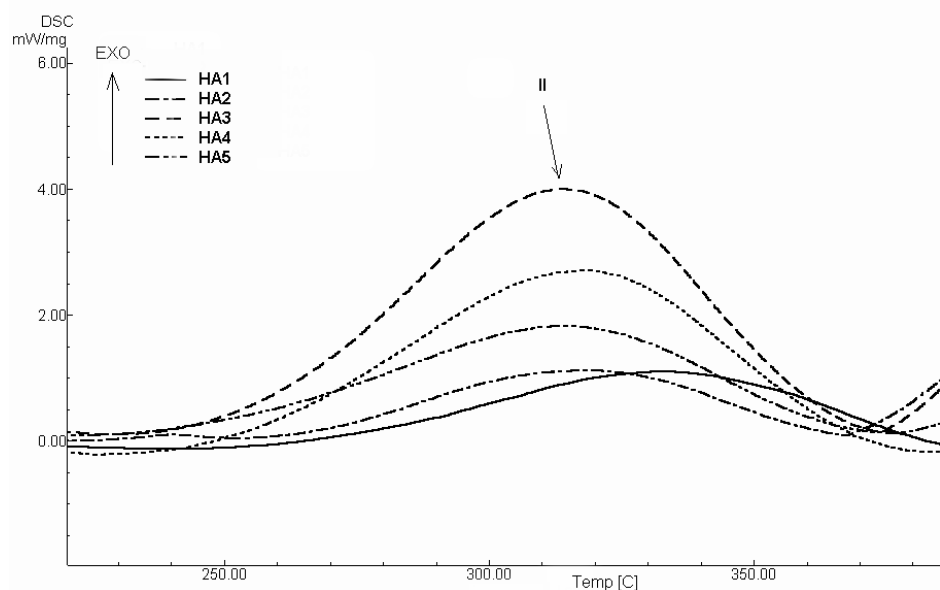
The first endothermic peak occurs in the range 40–110 °C for each sample and is the result of gradual moisture evaporating. The weight loss connected with endotherm did not exceed in all cases value of 9% and corresponds with previously published data [35].

**Table 7:** Percentage weight losses associated with individual decomposition steps; recalculated for moisture free samples.

Fraction	Peaks			
	I	II	III	IV
HA1	8.3	18.0	71.4	-
HA2	9.5	21.1	65.7	1.6
HA3	7.5	32.9	57.9	0.5
HA4	6.9	26.2	63.4	0.4
HA5	7.9	24.0	65.1	0.6

In range 110–210°C there appeared a broad but subtle ill-defined peak associated with slight weight loss (Tab 2). Weight loss in all thermograms did not exceed 10% of total weight and this effect may be related to continuing evaporation of water and small molecular pieces and/or partial destruction of peripheral aliphatic components. FTIR results showed decrease of intensity of broad peak at 3400cm<sup>-1</sup> while peaks in area 2900 and 1400 cm<sup>-1</sup> did not indicate remarkable alteration.

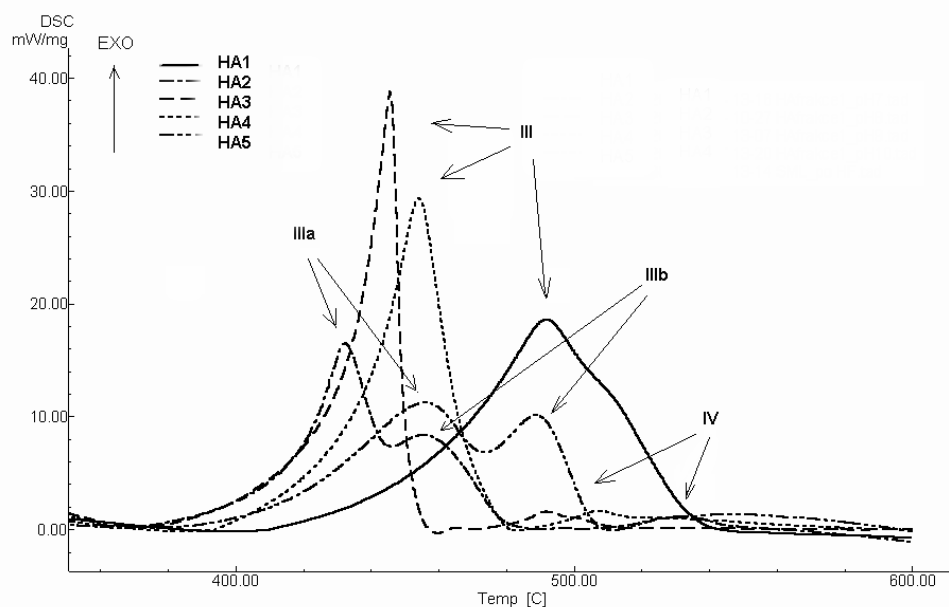
The second broad exothermic peak, with ill-defined maximum for HA2, but well-defined ones for HA1 and HA3-HA5, appeared in the range of 240–380°C. Thermal degradation indicated by exothermic peak occurring in this temperature range can be attributed to the destruction of aliphatic grouping, CH groups, carbohydrate components and to some extent of oxygeneous (alcoholic, phenolic) and amino groups. FTIR spectra (not given here) showed in all cases that the 2860 cm<sup>-1</sup> band disappeared, intensity of 1510, 1430, 1200, 1180 and 1050 cm<sup>-1</sup> decreased. On the other hand, absorption in range 3400–3600 cm<sup>-1</sup> as well as at 1650–1740 cm<sup>-1</sup> slightly increased



**Figure 4:** DSC thermograms of sequential pH-extracted humic samples at lower temperatures

The groups of well-defined but more complicated peaks appeared in range 380–520°C (Fig 4). Results obtained by FTIR showed a dramatic decrease in the intensity of FTIR spectra at 1720  $\text{cm}^{-1}$  that can be attributed to the decomposition of aromatic, mono or polycyclic rings. Likely, the destruction of the rest of COOH groups occurred there, as well. On the other hand, the intensity of 1630  $\text{cm}^{-1}$  band did not change, which indicates the fact that chinoidal moiety has not been decomposed yet.

Small exothermic peak appeared in HA2, HA3, HA4 and HA5 DSC curves in the range 480–550°C. TG results of all samples showed only tiny weight loss (Table 6). Peaks occurring in this temperature range may be attributed to the destruction of the most stable cyclic groupings containing C and N, and the oxidation products of thermal decomposition. In HA1 DSC chart, this peak did not appear likely due to the highest heterogeneity of this humic sample – exotherm corresponding to this destruction might be hidden in the previous broad one. The non-symmetric shape of the end of this peak supports this conclusion. FTIR results showed a dramatic increase of peak intensity in the range 1050–1150  $\text{cm}^{-1}$  (Si-O bonds) and disappearance in region 3400, 1600 and 1400  $\text{cm}^{-1}$ . As can be seen in Tab. 4, amount of nitrogen in all samples varies around 1%. From TG results should be then deduced that most of nitrogen molecules consisting of functional groups destroyed at lower temperatures. The highest weight loss showed in this temperature range fraction HA2, which corresponds to the high aromaticity of this sample predicted from elemental analysis results.



**Figure 5:** DSC thermograms of sequential pH-extracted humic samples at higher temperatures

As can be seen from thermograms comparing individual samples and elemental analysis results, the highest heat released during the oxidation of sequential pH-extracted humic samples showed samples richer in oxygen content. Those results are in contrast with what should be expected, because it is known, that oxygen-containing groups cause decrease of heat released during thermal degradation. In the light of above discussion, it is self-evident, that many physical-chemical processes, which occur during thermal treatment of humic substances, complicate estimation of those kinds of reactions. The most important, as stated Buurman and Aran [48], the evaporation of highly oxygenated low-molecular organic acids and loss of heat used in reconfiguration reactions should be taken into account.

As can be also seen, the original sample HA1 did not show an “average behavior” of its sequential pH-extracted samples. The applied extraction procedure likely affected rearrangement of pattern conformations and chemical alteration of humic molecules occurring during the preparation could not be neglected, as well.

### 5.5.1 The stability evaluation

Biodegradation of organic matter is frequently measured by CO<sub>2</sub> or CH<sub>4</sub> emission in fields plots or by incubation of pretreated samples. The rate of decomposition is related to temperature and moisture supply, to availability of nutrients, and to quality of organic matter. Exact relations with any of these factors, however, can rarely be established and are frequently controversial. In the case of relations with organic matter “quality”, a simple classification of this “quality” is a main problem [48]. The



number-value characterize different quality “types” of humic matter can be established by this method.

As a tool for organic matter stability determination, the non-isothermal method for induction period (IP) estimation was adopted [49]. IP is known as an initial stage of chemical reaction where seemingly no chemical reaction takes place. The time preceding the first exotherm during humic matter decomposition might be considered as the IP of humic matter decomposition.

Brief mathematical deduction is as follows: the combination of general mechanism of chemical reaction can be expressed:

$$\frac{d\alpha}{dt} = k \cdot f(\alpha) \quad (1) \quad k = A_k \exp\left[-\frac{E_a}{RT}\right] \quad (2)$$

where  $A_k$  is the pre-exponential factor,  $E_a$  is the activation energy,  $T$  is absolute temperature and  $R$  stands for the gas constant. Equation (1) is supposed to describe the kinetics of the reactions occurring during IP, as well. Although the reactions are not detected by the employed method, they have to be present as a preparatory stage. Combination of Eqs (1) and (2), after the separation of variables, gives the result (3). Further, it is assumed that conversion  $\alpha_i$  is the same for any temperature. Then, after integration of the left hand side of Eq. (3) we obtain (4):

$$\int_0^{\alpha_i} \frac{d\alpha}{f(\alpha)} = \int_0^t A_k \exp\left[-\frac{E_a}{RT}\right] dt \quad (3) \quad F(\alpha_i) - F(0) = \int_0^{t_i} A_k \exp\left[-\frac{E_a}{RT}\right] dt \quad (4)$$

Since the conversion  $\alpha_i$  corresponding to the end of IP is assumed to be independent of temperature, also the value of integrated function  $F(\alpha)$  at the point  $\alpha_i$ ,  $F(\alpha_i)$ , is constant. Therefore, Eq. (4) can be rewritten as (5) where the constants  $A$  and  $B$  are given as (6) and (7).

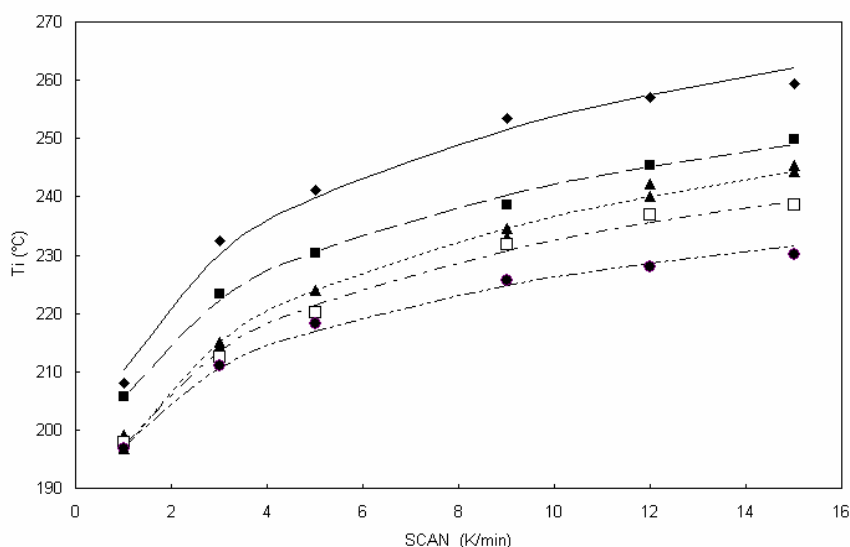
$$1 = \int_0^{t_i} \frac{dt}{A \exp(B/T)} \quad (5) \quad A = \frac{F(\alpha_i) - F(0)}{A_k} \quad (6) \quad B = \frac{E_a}{R} \quad (7)$$

The physical meaning of the denominator in Eq. (5) can be demonstrated very simply for special case of isothermal processes where the denominator is a constant equal to the induction period at given temperature. Thus, the temperature dependence of IP can be expressed as (8). For linear increase of temperature in DSC measurements, the furnace temperature can be expressed as (9). If one assumes that the temperature of the sample equals that of the furnace, combination of Eqs (5) and (8) gives the result (10).

$$t_i = A \exp(B/T) \quad (8) \quad T_f = T_0 + \beta t \quad (9) \quad \beta = \int_{T_0}^{T_i} \frac{dT}{A \exp(B/T)} \quad (10)$$

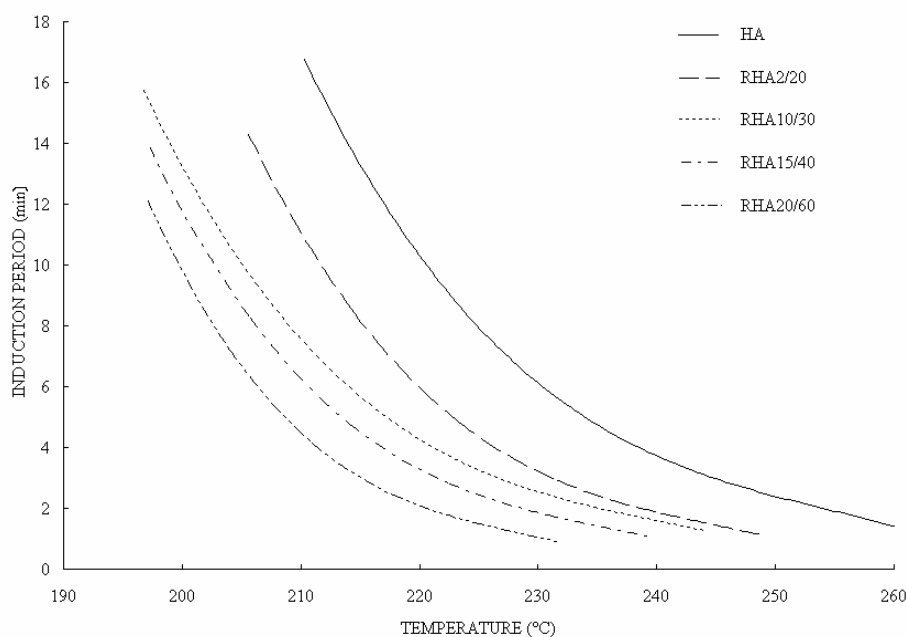
Where  $T_f$  is the furnace temperature,  $T_0$  is the starting temperature of the measurement and  $\beta$  stands for the coefficient of temperature increase (scan).

Kinetic parameters  $A$  and  $B$  (see Eq. 6 and 7) have been obtained from non-isothermal measurements by means of program TIND [49] by minimizing the sum of squares between experimental and theoretical values of the oxidation onset temperatures for various heating rates by the simplex method. The integration indicated in Eq. (10) has been carried out by the Simpson method. All computed data are summarized in Table 1. Although the humic substances are known as highly complex mixture of organic compounds, standard deviations of on-sets temperatures never exceeded 5% (not given here).



**Figure 6:** Dependence of the onset oxidation temperatures on the scan rates. Experimental data (point symbols) and fitted curves.  $\blacklozenge$  HA,  $\blacksquare$  RHA2/20,  $\blacktriangle$  RHA10/30,  $\square$  RHA15/40 and  $\bullet$  RHA20/60.

Humic acids extracted from non-treated substrate shown the highest stability and the dependence of oxidation agent concentration and time of oxidation on onset shifts is clearly seen (Fig. 6 and 7). The dependence of induction period within the given temperature range is showed in Fig. 7.



**Figure 7:** Induction period dependences on temperature

The strong association of humic substances with inorganic soil is regarded as a way of protecting organic carbon against microbial degradation, thereby conferring on humic substances mean residence times in soil which vary from tens to several hundreds years [50]. In agreement with Buurman and Aran [48], we may conclude that thermal behavior of groups decomposed in the first step (alcoholic, phenolic and carboxylic) is closely linked up to the stability of humic matter.

**Table 8:** The computed kinetic parameters *A* and *B* of HA and RHA samples.

Sample	<i>A</i> /min	$10^{-3} \times B/K$	$E_a/kJxmol^{-1}$
HA	$9.89 \times 10^{-11}$	12.5	103.9
RHA 2/20	$8.16 \times 10^{-13}$	14.6	121.4
RHA10/30	$1.88 \times 10^{-11}$	12.9	107.3
RHA15/40	$4.62 \times 10^{-13}$	14.6	121.4
RHA20/60	$5.46 \times 10^{-16}$	17.7	147.2

It is known, that regenerated humic substances are more aromatic in comparison with those obtained from natural environment [24]. The oxidation mechanisms, in ideal case considered as an inverse diagenetic process, i.e. processes leading to either mineralization by microorganisms or alteration to kerogen or coal-like products [3], are able to regenerate the molecules, which originally led to the insoluble structure of the coal. Therefore, on one hand we may expect enhancing of highly oxidized low molecular content and conversely the increasing in aromaticity degree. Hence, the stability of RHA fraction with oxidation time apparently descends, however only in temperature range where the first decomposition peak occurs.

From Eq. (8) it can be found that parameter  $A$  is induction period at infinite temperature. Formally, it has reciprocal dimension of the first order rate constant anticipated in Eq. (1). Parameter  $B$  is directly related to the activation energy of decomposition process – see Eq. (7) and last column in Tab. 1, which contains activation energies computed from the parameter  $B$ . Thus, parameter  $A$  may be interpreted as characteristics of kinetic contribution to stability whereas parameter  $B$  represents energetic or thermodynamic part of stability.

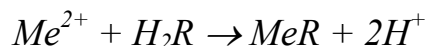
Accepting this interpretation of parameters, it is seen that the original humic acid (HA) is thermodynamically least stable and its stability is mostly controlled by kinetic factors. This nicely corresponds with its origin from very old deposits (coal) in which preferably kinetically stable materials should be found. On the other hand, the fraction isolated from material, which suffered from the strongest preliminary oxidation attack (RHA20/60) possesses highest thermodynamic stability as expected. Although the parameter interpretation is rather operational, the two examples support its justification.

## 5.6 THE REACTIVITY STUDY

The interaction abilities of humic substances to form complexes both with inorganic and organic molecules to cause the effect of crosslinking were studied. The model systems potentially suitable for metal and metal compounds binding were used for calculations of reaction enthalpies by means of semi-empirical version of INDO method. The highest stability constants were found for carboxylic group in presence of phenolic group in ortho-position as well as two intermolecular carboxylic groups. For interaction of HA and FA with transition elements, the deprotonation of carboxylic and phenolic groups and therefore higher pH has been found being an important factor playing a key role in complexation processes. The stabilities of metal/HA and metal/FA complexes have been determined in following order:  $\text{Cu}^{2+} > \text{Fe}^{3+} > \text{Mn}^{2+} > \text{Cd}^{2+} \cong \text{Mg}^{2+} \cong \text{Ca}^{2+}$ . Those results are not in exact agreement with data published by other authors (Stevenson, 1994), however different experimental conditions and samples as well as analytical techniques must be taken into account. Simpson et al. stated [22], among others, metal ions play a crucial role in aggregate formation and stability and therefore, the investigation of humic interactions together with stability estimation can bring information applicable both in agriculture, environmental and industrial exploitation of humic substances.

The kinetics of reactions leading to creation of metal/HA complexes in sol phases was studied. On the base of conductivity of sol system measurement, and for the reactions formally of the second order, the effective rate constants of complexation of HA with  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  were determined. Although, many simplifications were adopted, a good agreement between experimental and fitted results was reached.

Humic acids (HA) behave as weak-acid polyelectrolytes. They determine the pH buffering capacity and cation exchange capabilities in natural waters and soils<sup>1-4</sup>. Metal-organic interactions, including complexation reactions of metal ions and HA or FA, are increasingly becoming recognized as important factors in many natural systems. The binding of metal ions by HA is one of the most important environmental qualities and can generally occur as chelation between a carboxyl group and phenolic hydroxyl group, as chelation between two carboxyl groups, or as complexation with a carboxyl group. Therefore  $H^+$  ions are produced during the complexation of HA with metal ions according to this scheme:



$H_2R$  is the binding site for  $Me^{2+}$  ion in HA structure. The complexation is formally second order reaction with the following kinetics equation:

$$k.t = \ln[a.(b-x)/b.(a-x)]/(b-a) \quad (1)$$

where  $a$  and  $b$  are initial concentrations of  $H_2R$  and  $Me^{2+}$  and  $(a-x)$  and  $(b-x)$  are their concentrations in  $t > 0$ .  $k$  is the rate constant. The solution conductivity would be increasing with the  $H^+$  ions production, because their mobility is much higher than that of other ions in solution. During reaction the concentration of metal ions [ $Me^{2+}$ ] is decreasing, which generally influences the total solution conductivity. If  $b \gg a$ , this influence can be neglected.

On the basis of above-mentioned hypothesis, this equation was derived:

$$k.t + q = \ln[(3G_0 - G_t)/(G_\infty - G_t)]/(b-a) \quad (2)$$

where  $q$ ,  $G_0$ ,  $G_t$  and  $G_\infty$  are constant, initial solution conductivity, its conductivity in time  $t$  and in equilibrium, respectively.

The rate constants determined on the basis of eq. 2 are written in Tab. I. These values are strongly dependent on initial concentration of metal ions ( $b$ ), therefore they can not be considered as the right rate constants. Although, many simplifications were adopted, a good agreement between experimental and fitted results was reached.

**Table 9:** Effective rate constants of complexation of HA with metal ions

B [mol/dm <sup>3</sup> ]	k [m <sup>3</sup> /mol.s]				
	0.05	0.08	0.10	0.15	0.20
Cu <sup>2+</sup>	7.43×10 <sup>-4</sup>	2.02×10 <sup>-5</sup>	1.05×10 <sup>-5</sup>	2.66×10 <sup>-6</sup>	5.21×10 <sup>-7</sup>
Co <sup>2+</sup>	1.17×10 <sup>-4</sup>	6.38×10 <sup>-5</sup>	7.89×10 <sup>-5</sup>	2.25×10 <sup>-6</sup>	8.88×10 <sup>-7</sup>
Ni <sup>2+</sup>	3.32×10 <sup>-5</sup>	1.46×10 <sup>-5</sup>	4.39×10 <sup>-6</sup>	1.13×10 <sup>-6</sup>	2.98×10 <sup>-7</sup>

The highest values of the effective rate constant were determined for Cu<sup>2+</sup> ion, the lowest ones for Ni<sup>2+</sup> ion. In regard to high complexity of miscellaneous reaction

which humic molecules during complexation undergo (combination of the reaction in both directions and other interactions), those values have been called as the effective rate constants.

## 6 CONCLUSION

The goal of the work was to study both extraction and fractionation procedures and chemical and physical properties of lignite humic substances extracted from South-Moravian lignite.

First of all, several alkali compounds have been applied to find out the most efficient procedure leading to extraction of the highest yield of humic acids. 0.5M sodium hydroxide at 90°C have been found being conditions allowed as the most effective humic acids extractant from parental lignite in stirred discontinual reactor.

With the aim to enhance natural content of humic acids, the oxidative pretreatment proceeding alkali extraction has been studied, as well. Both the 10% and 20% nitric acid applied for 60 and 30 minutes, respectively, showed the same potential to increase the content of humic acids in comparison with original value from 40 to 60%. Elementary analysis, FTIR and UV spectroscopy, and membrane centrifugation were used to characterize produced humic acids.

Due to high molecular heterogeneity of extracted humic acids, the technique of sequential pH-extraction was suggested and applied. Four different molecular fractions have been obtained. HPSEC (High Pressure Size Exclusion Chromatography) was applied to elucidate the structural characteristics of individual extracts. It has been found out, that application of phosphates buffers of increasing pH provided samples with decreasing aromaticity and polyacidity while pyrophosphate preferably extracted less acidic and polyphenol-like humic molecules. The changes in chromatographic profiles induced by addition of small amount of either mineral or organic acid proved recent findings, that humic substances could hardly be considered being covalently bounded polymers, but conversely, they consist of randomly self-assembling relatively small molecules, bounded mostly by weak dispersive forces such as van der Waals,  $\pi$ - $\pi$ , CH- $\pi$  bonds, and hydrogen bonding.

Thermooxidative behavior of humic acids was studied by the means of DSC (Differential Scanning Calorimetry) and TG (thermogravimetry). Thermal-induced degradation resulted in two main steps: firstly, destruction of aliphatic grouping, CH groups, carbohydrate components and some extent of oxygenous (alcoholic, phenolic and partially carboxylic) and amino groups was observed. In the second step, the decomposition of aromatic, mono or polycyclic rings and rest of carboxylic groups occurred.

The second goal was to determine the induction period of humic acids degradation e.g. number value correlating their stability and physical-chemical

character. Such value can serve in case of technological and agronomical applications of humic or humic derived products for estimation of their both kinetic and thermodynamic stability.

Humic acids reactivity was studied on the basis of their ability to form complexes with organic and inorganic molecules. The reactivity with transition metals was elucidated by EPR spectroscopy. Several model schemes were suggested and the interactions energies with the aid of semi empirical version of INDO were calculated. The order of stability of tested metals has been established as follows:  $\text{Cu}^{2+} > \text{Fe}^{3+} > \text{Mn}^{2+} > \text{Cd}^{2+} \cong \text{Mg}^{2+} \cong \text{Ca}^{2+}$ . The most probably interactions with high stability constants have been found in case of carboxylic group in presence of phenolic group in ortho-position as well as two carboxylic groups bounded on two different molecules.

The kinetic approach of complexation was performed in sol phase. The effective rate constants of reactions of humic acids with  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  salts were determined. As expected, in alkali media the complexation reactions of humic acids run the most readily in case of interaction with  $\text{Cu}^{2+}$  ions.

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## 8 ABSTRAKT V ČEŠTINĚ

První část této práce byla zaměřena na ověření postupů vedoucích k extrakci maximálního množství huminových kyselin z lignitu těženém v dole Mír Mikulčice (jižní Morava). V dalším kroku byly studovány oxidační postupy po jejichž aplikaci lze z lignitu extrahovat tzv. regenerované huminové kyseliny. Byla navržena metoda sekvenční extrakce vedoucí k homogenizaci huminové matrice. Za pomoci metody gelové chromatografie (HPSEC) byly frakce charakterizovány a experimentální data podpořila nedávno navržený model supramolekulového uspořádání huminových

molekul. Metody termické analýzy DSC a TG sloužily ke korelaci chemických a termodynamických vlastností (především stability) frakcí huminových kyselin a regenerovaných huminových kyselin. V poslední fázi byly počítačově modelovány stabilní interakce huminových kyselin s kovy, které byly následně využity k návrhu jednoduchého kinetického modelu, simulaci komplexačních reakcí a výpočtu zdánlivých rychlostních konstant.

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