Physico-Chemical Properties of Humic Acids Isolated from an *Eriophorum-Sphagnum* Raised Bog

T. Gierlach-Hladoń¹, L. Szajdak²

¹Department of Inorganic and Analytical Chemistry, Karol Marcinkowski University of Medical Sciences, ul. Grunwaldzka 6, 60-780 Poznań, Poland
²Institute for Agricultural and Forest Environment, Polish Academy of Sciences, ul. Bukowska 19, 60-809 Poznań, Poland

Humic acids (HA) isolated from an *Eriophorum-Sphagnum* raised bog have been investigated using X-ray diffraction, FT-IR spectroscopy, ¹³C NMR, and the differential scanning calorimetric method. Peat from *Eriophorum-Sphagnum* raised bogs is commonly utilized for balneological purposes. The size of the HA molecules isolated from the raised bog ranged from 0.05 to 477.01 μm. The smallest percentage of particles equaled 0.1% and pertained to the size range of 0.05 to 0.11 μm. The largest percentage of particles equaled 45.6% and pertained to the size range of 120.67 to 477.012 μm. An increase of the proportion of different size particles with an increase of the size range was observed. The study revealed small values of surface area of HA, and the total pore volume and pore diameter calculated on the basis of BET adsorption isotherm were estimated. It was discovered that ion exchange capacity of peat depends mainly on HA. It was shown that the sorption capacity of peat is determined by the sorption capacity of HA.

**Keywords:** exchange capacity, humic acids, particle size, sorption capacity, textural parameters, X-ray diffraction, ¹³C NMR, DSC, FT-IR.

**INTRODUCTION**

The conversion of higher vegetation and mosses to peat is a process which leads to the formation of lignite, coal, and anthracite. During peat formation, humification of organic substances takes place. A further step of the diagenesis involves dewatering and compaction. The formation of humic substances from plant and animal debris follows no genetic code and occurs stochastically. It is governed by kinetic and thermodynamic restraints; thus, only refractory structures are likely to survive since they are not susceptible to further microbial and chemical degradation (Hertkorn et al., 2002). The stochastic nature of humification is expected to cause an immense structural heterogeneity and
polydispersity of humic substances (Stevenson, 1982). Therefore, variations in peat organic matter appear due to the variety of plants whose residues have impact on peat formation and the environmental conditions in which humification takes place (MacCarthy et al., 1990).

Peat comprises relatively unstable substances whose reactivity contributes to the usefulness of peat. Physico-chemical and chemical properties of peat, in particular surface activity, sorption, and ion exchange, which define its protective function in the environment, depend to a large extent on the composition and molecular structure of the major constituents of organic soils – humic substances. Studies assert that the chemical composition of peat depends on the geobotanical conditions of its formation and the depth of sampling (Maryganova, 2000; Bambalov et al., 2000). Thus, the evolution of hydrogenic peat soils is closely related to the genesis of peat and to the changes in water relations.

HA are the main organic component of peat organic matter, whose chemical structure and concentration depend on the conditions and the duration of the decomposition of plants, the co-occurrence of other organic and mineral substances, redox potential, and pH of the environment (Lehtonen et al., 2001; Stevenson and He, 1990). Stevenson (1982) proposed a hypothetical structure of HA, the main elements of which were later confirmed by chemical analyses (Gonet, 1993). These elements included aromatic systems, free and bound phenol groups, oxygen bridges, and carboxyl groups as substituents on aromatic rings, peptide chains, and carbohydrates. Hitherto studies have not proved the regular and unique structure of HA. It has been only asserted that they are oxocarbonic acids of cyclic structure representing a significant degree of polymerisation. They have an aromatic core linked to amino acids, peptides, sugars, aliphatic acids, and other components of aliphatic structure. The core is composed of aromatic rings of phenolic type or heterocyclic compounds containing nitrogen such as, e.g., indole, pyrimidine, purine, etc. The cyclic macrocomplexes include benzene rings, aliphatic chains, and functional groups such as carboxy, hydroxy, amine, methoxy, carbonyl, ester, ether, amide groups etc (Stevenson and He, 1990; Jansen, 1996; Thorn et al., 1996). The properties and behavior of these elements as well as their compounds determine the nature of peat as a whole. The aforementioned substances, and those which occur in lesser amounts, play an important biological role. Thus, HA represent complex organic substances formed during the biogeochemical degradation of plant debris and animal residue and the condensation of the degraded segments.

The amphiphilous nature of HA enables them to interact with a variety of inorganic and organic compounds via chemical bonding and less polar organics via nonspecific physical interactions. Therefore, the studies of the molecular structures of humic substances are of great theoretical and practical importance. Macromolecules of humic substances belong to compounds of
variable composition and are probably the most intricate natural compounds (Orlov, 1985).

The development of analytical methods that describe the structure and physico-chemical properties of HA are of great importance for researchers because the structure of HA determines the physico-chemical and biological properties of peat. Therefore, a detailed analysis of HA will probably allow to predict the properties of a given type of peat and its possible application in therapy. Due to numerous useful physico-chemical properties, peat has been used in therapy for physiotherapeutic purposes, as well as in agriculture, horticulture, pomology, gardening, and forestry (Latour and Ponikowska, 1995; Peña-Méndez et al., 2005). The physiological activity of peat is used to increase the growth of particular plants, as well as in peat bath therapy (Chen et al., 2004). The main component responsible for the beneficial role of peat is the organic part of peat mass that is a mixture of different components of well known and unknown structures (Malcolm, 1989; Burdon, 2001).

Balneological peat, an ecologically clean natural substance, is perceived as being more human-friendly than synthetic compounds. Poland has long-lasting traditions of using balneological peat (Latour and Ponikowska, 1995; Latour, 2002). Balneological peat produces a physical effect by altering temperature, and a biochemical effect through biologically active substances (Lukanov et al., 2002). It is mainly used for the treatment of rheumatic diseases that are quite common in Poland. Previous studies have revealed that some extracts of peat, including fractions of biochemically active HA, fulvic acid and hymatomelanic acids, may be successfully used to treat stress and skin diseases (Beer et al., 2000, 2003). They are characterized by very low toxicity for animals and cultivated plants; therefore, their LD_{50} values are very high (Kobec et al., 2008). Moreover, they have relaxing effects and are efficient in cosmetic and sauna procedures. A number of studies have shown that naturally occurring HA prolong the clotting time of blood in vitro and in vivo. This anticoagulation effect is partially based on the inhibition of activated factor II (F IIa = thrombin), activated factor X (F Xa), and activated factor VII (F VIIa) (Klöcking et al., 1999, 2004).

This paper aims to investigate the chemical structure and properties of HA isolated from an *Eriophorum-Sphagnum* raised bog and used in balneology. The results obtained from these experiments are expected to provide a better insight into the molecular structure and chemical composition of peat HA.

**MATERIALS AND METHODS**

Samples of peat were collected from an *Eriophorum-Sphagnum* raised bog located near Kołobrzeg, in the northern part of Poland (Table 1).
Table 1. The chemical composition of the investigated peat

<table>
<thead>
<tr>
<th>Depth of sampling, m</th>
<th>Peat species, decomposition degree, %</th>
<th>pH in 1 N KCl</th>
<th>HA content, %</th>
<th>Ash content, %</th>
<th>Moisture content, %</th>
<th>SiO₂, %</th>
<th>Content of compounds soluble in 10% HCl, %</th>
<th>Content of water-soluble compounds, %</th>
<th>Content of bitumines, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td><em>Eriophorum-Sphagnum</em>, 50</td>
<td>4.0</td>
<td>47.9</td>
<td>5.2</td>
<td>90.1</td>
<td>0.10</td>
<td>0.13</td>
<td>3.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

The humification degree (by the von Post scale) and botanical composition of peat samples were determined with a microscope (Maciak and Liwski, 1996). The standard IHSS procedure (Swift, 1996) was used for isolation of HA; other chemical parameters were determined by Rowell (1994). HA series No. 2158096 obtained from the Fluka Factory were used as the standard in the study. The soil profile comprised *Eriophorum-Sphagnum* peat (2 m depth) with 50% of decomposition. Some physico-chemical characteristics of the soil are given in Table 1. Soil samples from 10 sites were air-dried, crushed to pass a 1 mm mesh sieve and mixed together to provide a representative “mean sample” used for the determination of pH (in 1 N KCl), HA, ash, moisture, SiO₂, compounds soluble in 10% HCl, water-soluble compounds, and content of bitumines.

Methods of HA identification

X-ray diffraction (XRD)

X-ray diffractometry measurements of HA isolated from the *Eriophorum-Sphagnum* raised bog were performed with the powder diffraction technique using nickel-filtered copper radiation CuKα (λ = 1.5418 Å). A voltage of 30 kV and current of 30 mA were used. Interplanar distances d were calculated using the Bragg equation:

\[ \lambda = 2d \sin \theta \]  

Differential scanning calorimetry (DSC)

Measurements of HA isolated from the *Eriophorum-Sphagnum* raised bog and of the HA standard with the DSC method were carried out. Thermal properties of HA were investigated in nitrogen atmosphere using a differential scanning calorimeter DSC-50 (Shimadzu, Japan) at a heating rate of 10 deg/min up to 480 °C, with Al₂O₃ as a reference material. The results of DSC measurements were plotted as a dependence of temperature on time using Windows DSC-50 software.
Infrared spectroscopy (FT-IR)

HA isolated from the Eriophorum-Sphagnum raised bog as well as the HA standard were compressed with KBr, and FT-IR spectra were recorded with a Bruker Vector 22 type PS 15 spectrometer.

Solid-state carbon-13 nuclear magnetic resonance ($^{13}$C NMR)

Experiments were performed with a Bruker DMX 300 spectrometer with the carbon resonance frequency of 75.46 MHz at 300 K and the acquisition time of 0.085 s. Amplification of signals from carbon nuclei was obtained with the cross-polarization method. Solid samples weighing about 2 mg were prepared evaporating them from the solution in acetone.

Measurements of the surface area and porosity

Textural parameters of HA were measured from low-temperature (77 K) nitrogen adsorption-desorption isotherms. The specific surface and the parameters characterizing the porous structure of HA are given in Table 2.

Particle size determination

Particle size was measured with a granulometer Mastersizer S (Malvern Instruments). Granulometric analysis is based on the measurement of particle sizes with the scattered helium-neon light technique. A sample was loaded onto a vessel filled with a surfactant, and a stable suspension was obtained. The suspension was placed in the way of the laser beam and the latter was scattered. Calculations were performed using a software, and the results were represented in the form of a particle volume distribution curve.

Sorption capacity determination

The total cation sorption capacity of peat and of HA isolated from the Eriophorum-Sphagnum raised bog was determined according to the procedure described by Hładoń (1980) using buffered 1.0 M ammonium acetate solution as an adsorbate. Adsorbed ions were determined as NH$_3$ by distillation. The results of sorption capacity determination are listed in Table 3.

Determination of ion exchange capacity

1.0 g of peat dried to a constant weight, or 1.0 g of HA was placed in an Erlenmeyer flask of 250 ml capacity followed by addition of 50.0 ml of 1.0 M sodium chloride solution and vigorous shaking of the obtained suspension for 15 min. Then the suspension was filtered through a sintered glass crucible G-4. The amount of hydrochloric acid formed as a result of the ion exchange was determined in the filtrate by titration. The results are given in Table 3. All the measurements were replicated 5 times, and the results were averaged. All chemicals used in this study were of analytical grade.
Table 2. Percent contribution of different size particles in the volume distribution

<table>
<thead>
<tr>
<th>Size range, μm</th>
<th>Standard</th>
<th>Isolated from the <em>Eriophorum-Sphagnum</em> raised bog</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05–0.11</td>
<td>-</td>
<td>0.1</td>
</tr>
<tr>
<td>0.13–1.06</td>
<td>2.3</td>
<td>1.7</td>
</tr>
<tr>
<td>1.24–10.48</td>
<td>3.0</td>
<td>8.9</td>
</tr>
<tr>
<td>12.21–103.58</td>
<td>47.8</td>
<td>43.7</td>
</tr>
<tr>
<td>120.67–477.01</td>
<td>46.9</td>
<td>45.6</td>
</tr>
<tr>
<td>&gt; 477.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. The total sorption volume and ion-exchange capacity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total sorption volume, mol·kg⁻¹</th>
<th>Ion-exchange capacity, mol·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peat</td>
<td>0.27</td>
<td>1.78</td>
</tr>
<tr>
<td>HA isolated from the <em>Eriophorum-Sphagnum</em> raised bog</td>
<td>0.39</td>
<td>3.60</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Peat soil of pH 4.0 (in 1 N KCl) is very acidic (Table 2). HA and ash contents were 47.9% and 5.2%, respectively. The investigated peat revealed a large amount of moisture (90.1%) and low quantities of SiO₂ (0.1%). In the present study, the amount of compounds soluble in 10% HCl equaled 0.13%, water-soluble compounds equaled 3.6%, and the concentration of bitumines (4.1%) was typical of a raised bog (Szajdak, 2002).

X-ray powder diffraction patterns of HA from the *Eriophorum-Sphagnum* raised bog were compared with the data of the HA standard. Debye reflections were measured in 2θ ranged from 2 to 60°. The spectrum of the HA isolated from the *Eriophorum-Sphagnum* raised bog, recorded in the full 2θ range was proved to be very poor in reflections. It suggests that the structure of HA isolated from peat is more amorphous than the structure of the HA standard (Eltantavy and Baverez, 1978), and that the differences in the spectra of HA are related to the qualitative composition of the samples. According to granulometric studies (Table 2), HA isolated from peat and the HA standard are mixtures of particles representing different sizes ranging from 0.05 μm to 477.01 μm. Our study discovered that the percentages of distributions of the different particle sizes are different. The lowest percentage of particles equaled 0.1% and pertained to the size range of 0.05–0.11 μm. The highest percentage of particles equaled 45.6% and pertained to the size range of 120.67–477.012 μm. An increase of the proportion of different size particles with an increase of the size range was observed.
Figure 1. Differential scanning calorimetry curves: a) HA isolated from the Eriophorum Sphagnum raised bog; b) the HA standard
The DSC thermograms of the HA standard and HA extracted from the *Eriophorum-Sphagnum* raised bog do not differ significantly. Endothermic peaks at 57.75 °C and 68.02 °C originate from water evaporation from the surfaces of HA. The thermograms also revealed two exothermic peaks at 381.49 °C and at 460.29 °C. Dehydration and decarboxylation of oxygen-containing functional groups occur up to 300 °C (Rakovskiy and Filimonov, 1967), whereas thermal decomposition of the more thermostable skeleton part of HA takes place from 300 to 500 °C. As a result of the division of aliphatic bridges between aromatic structural units in this region, aromatic compounds are eliminated with the highest rate (Maryganova et al., 1992).

FT-IR spectra of HA extracted from the *Eriophorum-Sphagnum* raised bog and the HA standard are relatively similar. The spectra were analyzed in the range of 4000–400 cm⁻¹. HA exhibit a strong absorption band at 3000 cm⁻¹ that is due to the presence of H-bonds between OH groups, absorption at 2920–2860 cm⁻¹ coming from stretching vibrations of -CH₃ and -CH₂-, absorption at 1720 cm⁻¹ and 1230 to 1200 cm⁻¹ originating from carboxyl groups, absorption originating from amide groups at 1660 cm⁻¹, conjugated C=C bonds at 1610 cm⁻¹, and at 1100–1000 cm⁻¹ originating from aliphatic OH present predominantly in carbohydrates (Orlov, 1983).

¹³C NMR spectroscopy was proved to be a very useful method for studies of soil organic matter (Preston, 1996; Kögel-Knabner, 1997, Bergman et al., 2000). Spectra of the HA isolated from peat were recorded in the range of 220–20 ppm (Figure 2). They show asymmetric signals without any fine structure that is a consequence of the transversal relaxation times T₂. The spectrum of the HA standard revealed a small number of peaks. The chemical shifts in the range from 120.020 to 134.700 ppm indicate the presence of aromatic carbon rings. The chemical shift at 29 ppm corresponds to the presence of the methylene groups in aliphatic rings and chains of various origins such as fatty acids, lipids, cutting acids, and other probably unknown aliphatic biopolymers. The chemical shift signals from 54 to 55 ppm are assigned to carbohydrate residues O-CH₂ and O-CH₃, and C-α of some amino acids. It was determined that the chemical shift at 70–73 ppm corresponds to o-mono-substituted aliphatic structures. The signals at 103–106 ppm suggest the presence of anomeric carbon atoms from carbohydrates and C2 and C6 of lignin, while the signals at 115–117 ppm and 128 ppm correspond to tertiary aromatic carbon atoms. The signal at 125 ppm comes from carboxyl and amide groups in various compounds (Kögel-Knabner, 1997; Maryganova et al., 2004).

Textural studies were also performed in order to evaluate the porous structure of HA. The shapes of the isotherms of low-temperature adsorption and desorption of nitrogen are sigmoidal, representing BET adsorption isotherm Type II (Atkins, 2007). This type of isotherms represents unrestricted monolayer-multilayer adsorption. Moreover, absorption occurs due to very weak interactions of van der Waals type.
Figure 2. $^{13}$C-NMR spectra: a) HA isolated from the Eriophorum-Sphagnum raised bog; b) the HA standard
Figure 3. Low-temperature nitrogen adsorption isotherms: a) HA isolated from peat of the Eriophorum-Sphagnum raised bog; b) the HA standard
Table 4. Specific surface area, the total pore volume, and pore diameter of HA samples

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Standard</th>
<th>HA isolated from the Eriophorum-Sphagnum raised bog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area, m²/kg</td>
<td>500</td>
<td>410</td>
</tr>
<tr>
<td>Total pore volume, m³/kg</td>
<td>0.82</td>
<td>0.69</td>
</tr>
<tr>
<td>Pore diameter calculated from BET isotherm, nm</td>
<td>6.5</td>
<td>7.1</td>
</tr>
</tbody>
</table>

It was discovered that the surface area and total pore volume of the HA extracted from peat are smaller than that of the HA standard by 18% and 19%, respectively. On the other hand, the pore diameter calculated from BET isotherm of the HA isolated from peat was by 9% larger than that of the HA standard. We may suggest that the HA standard and HA extracted from peat contain mesopores (Table 4). Adsorption was estimated to be insignificant in the sorption properties of HA. Due to the small specific surface area of the HA, a small number of organic compounds can be adsorbed.

HA are created in peat as a result of habitat and anthropogenic processes, including the degradation of plants and animal residue. These substances are the sources of energy for many chemical and biochemical processes in soil (Dziadowiec, 1979; Gołębiowska et al., 1996). Therefore, the application of thermoanalytical techniques allows to make a rapid identification of consecutive stages of organic substances in soils. Furthermore, the method helps to estimate the changes of energetic differences in these compounds (Shurygina, 1971; Schnitzer et al., 1974; Leinweber and Schulten, 1992). However, no significant differences between the HA standard and HA from the Eriophorum-Sphagnum raised bog were observed.

Peat is rich in humic substances, and the ash content is usually low; therefore, it is a common source of the former. In peatlands carbon is accumulated because the annual input of dead plant material exceeds the annual breakdown. Fresh plant litter that accumulates each year has not been altered by microorganisms yet, but carbon from old plants was revealed to be resistant to microbial attack. Transformed carbon, such as microbial polysaccharides, can be further decomposed, whereas lipids are more recalcitrant and accumulate during microbial decomposition of peat organic matter. Selective loss of carbohydrate C (O-alkyl) and net accumulation of lipid C (CH₂) have been reported to occur during the decomposition of both peat and forest litter (Baldock and Preston, 1995). The studies using ¹³C NMR techniques have showed that lipids seem to be the major fraction accumulated (Baldock et al., 1992; Bergman et al., 2000). Our results revealed the presence of aromatic and aliphatic structures, fatty acids, lipids, cuttine acids, amine groups, and some aliphatic biopolymers in HA. The results
Mires and Peat

are consistent with recent studies by Kögel-Knabner (1997) and Maryganova et al. (2004). The results obtained with the FT-IR and $^{13}$C NMR spectroscopic techniques suggest that the structural formula of the HA isolated from the raised bog is similar to that proposed by Peña-Méndez et al. (2005).

Adsorption and desorption of the compounds in peat is described in terms of equilibrium transfer processes between the compounds in the bulk solution exterior to the peat particles and the intraparticle liquid phase. The processes are controlled either by the rate of the diffusion through the surface film or by diffusion through the interstices of the particles in the study conditions. The results of gas adsorption in peat can be classified by parameters characterizing (i) the area available to a particular adsorbate, and (ii) surface roughness or irregularity. Specific surface area is known to characterize the ability of peat to retain and transport nutrients and water. It was revealed that specific surface area of soils significantly correlates with the sorptive capacity and ion exchange capacity, retention and release of chemicals, swelling, water retention, and dry-aggregate stability (Hajnos et al., 2000).

The colloidal fraction, which consists of humified organic matter of peat, is recognized as the set of chemical activity in soils, including cation exchange capacity. The relative contribution of soil organic matter to cation exchange capacity is largely determined by the amount of soil organic matter, the amount and mineralogy of clay, and the soil pH. Therefore, soil organic matter is responsible for all cation exchange capacity in peat soils low in clay (Weil and Magdoff, 2004).

The study asserts that functional groups of HA participate in ion exchange interactions. The total sorption volume of HA and ion exchange capacity were higher than measured in peat by 120% and 202%, respectively. It suggests that the differences of ion exchange properties in peat are almost exclusively determined by differences of properties of HA (Table 3). Moreover, the study indicates that sorption capacity seems to be the quantitative factor of sorption ability of peat and HA. In addition, the study shows that the sorption capacity of HA is higher than that of peat. It indicates that humic substances may have absorbed other cations than HA. The data obtained in the study allow to compare properties of HA representing the main fraction of peat and peat properties.

**CONCLUSIONS**

The size of *Eriophorum-Sphagnum* HA particles extracted from the raised bog ranged from 0.05 to 477.01 μm. *Eriophorum-Sphagnum* HA are characterized by a small specific surface area and the total pore volume calculated from BET adsorption isotherm. Adsorption of non-polar adsorbates on the HA surface is of exclusively physical character. The total ion exchange capacity of *Eriophorum-Sphagnum* peat can be mainly attributed to HA.
ACKNOWLEDGEMENTS

This work was supported by the grant No. N N305 320436 of the Ministry of Education of Poland. Thanks to Mrs Teresa Stachecka for technical support.

REFERENCES


