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Properties of humic substances isolated from different natural sources

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Abstract: The purpose of study was to determine properties of humic substances by combination of modern and traditional methods. Humic substances isolated from three different sources from Mongolia such as peloid from Lake Gurban nuur, coal from Baganuur deposit and oil shale from Shine khudag deposit.

On the basis of determination H/C and O/C atomic ratios in humic substances by elemental analysis, confirmed existing of aromatic structures in the molecules and oxidized functional groups.

Have been studied the structure of humic substances by spectral method. For example infrared spectrums showed that humic substances are characterizing with poly-structural components, with different quantity in the samples. Light adsorption of samples in the UV-Vis region, a decrease on the absorption intensity with an increase of the wave length was observed (Fig. 2). The high ratio H/C, attributed to stretching of C=C bond of aromatic rings in IR spectrums, the high content of functional groups, lower extinction coefficients, confirmed that aromatic fragments to prevail than aliphatic chain fragments in structure of all studied HS

Keywords: peloid, brown coal, humic substance, shale, organic matter

INTRODUCTION

Humic substances (HS) are natural products widely distributed in soil and water as well as in geological organic deposits such as lake sediments, peats, brown coals and oil shale. HS are major components of natural organic matter (NOM) in surface waters and at higher concentrations can impart a dark color, especially in brown fresh water ponds, lakes and streams [1].

Humic substances are complex and heterogeneous mixtures of polydispersed materials formed by biochemical and chemical reactions during the decay and transformation of plant and microbial remains (a process called humification). Plant lignin and its transformation products, as well as polysaccharides, melanin, cutin, proteins, lipids, nucleic acids, fine char particles, etc., are important components taking part in this process .

The properties of humic substances can be determined by instrumental analytical methods. The characteristics properties of humic substances are due to the functional groups situated on the carbon-chain. They could have acidic, alkaline or neutral character. Humic substances represent an extremely heterogeneous mixture of molecules with molecular weight from as low as several hundred to over 300.000 daltons [2, 3]. Due to complex nature of these biopolymers the determination of elemental composition and molecular mass are more difficult.

Have been determined the elemental composition and functional groups of humic substances content, etc. Main difference for studied HS is ratio of hydrophilic and hydrophobic component. The determined results show that the oxygen containing functional groups such as phenolic hydroxyl groups are more active than other bioactive groups.

EXPERIMENTAL

Sampling area: *Lake Gurban nuur in Khentii aimag:* Lake Gurban nuur located in Dadal province of Khentii aimag. It is located at an elevation of 800 m above sea level and [1]. Peloid in middle of lake are widely used in folk medicine. Its coordinates are 49°2'30" N and 111°39'0" E in DMS (Degrees Minutes Seconds) [4].

Baganuur coal deposit: The Baganuur coal deposit locates in 130 kilometers east of Ulaanbaatar, established in 1980, supplying over 70% of the coal required for the Central regional power electric system. It is considered as one of the biggest open coal mines in Mongolia. The mine contains 599.818 million tons of coal and has the capacity to extract 3 million tons of coal per year.

Isolation humic substances from peloid: The isolation process of humic sunstances involved air drying of 10g peloid and dissolved it in 100 ml 10% HCl solution at room temperature for one hour and in water bath heated for 2 hours. Mixture was filtered and washed with distilled water after cooling. Residues were extracted with 0.2N NaOH at 60^0C for overnight

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(Sample:NaOH/ 1:3). Supernatant were recovered by centrifugation and filtration, pH was adjusted to 1 using 0.6 N HCl. Production humic substances separated from supernatant and washed with distilled water until removing of ion $Cl^-$and drying at 50-60^{0}C [5].

Isolation of humic substances from coal and oil shale: 0.25 mm powder coal and oil shale 10 g were digested in 500 ml 5% HCl solution at water heat for 2 hours. Mixture was filtered and washed with distilled water after cooling process. Residues were extracted with 1000 ml 1% NaOH at water bath heated for 2 hours. Supernatant were recovered by centrifugation and filtration, pH was adjusted to 1 using 15% HCl. Production humic substances separated from supernatant and washed with distilled water until remove $Cl^-$and drying at 80-85^{0}C.

Infrared spectroscopy: The IR spectrum was registered in KBr pellet on VERTEX 70 FT-IR spectrometer (Bruker Optics). The Raman spectrum (the stirred crystals placed in aluminium disc) was measured by RAM II (Bruker Optics) with a focused laser beam of 100 mW power of Nd:YAG laser (1064 nm) from 4000 cm^{-1} to 100 cm^{-1} at resolution 1 cm^{-1} with 25 scans.

Perkin Elmer Lambda 35 UV-Visible Spectrometer: Lambda is a double-beam UV-Visible spectrometer that measures light absorption from the visible to the ultraviolet region (1100 nm to 200 nm, 1.1 eV to 6.2 eV). A sample is usually made up to a known concentration in a non-absorbing solvent (such as water) and loaded into a quartz cuvette (available from the lab).

Diffractometer Bruker D8 ADVANCE: This is a versatile, sensitive, and high resolution X-Ray powder diffractometer. The monochromatic Cu $K_{\alpha1}$ line is isolated by the Vario monochromater at the X-Ray tube. No more need to numerically subtract out the $K_{\alpha2}$ from your data. A variety of sample holders and sample presentation methods are listed below. A Braun position sensitive detector permits up to 8° 2θ of diffracted beam to be measured continuously while scanning, which dramatically increases sensitivity compared with the conventional scintillation detector behind a narrow slit.

RESULTS AND DISCUSSION

Have been isolated humic substances from three different sources from Mongolia and investigated properties of humic substances by combination of modern and traditional methods. In this work, we determined ratio which is one of the important properties of humic substances using elemental analysis, ranged from 0.067 - 0.083%. This result confirmed that exists of aromatic structure in molecules. Usually humic substances of peloid contain 5% nitrogen. But a humic substance from coal has a lower amount of nitrogen (0.83%). The ratio of natural humic substances (peloid, coal and shale) is between 0.65 and 0.86. It is indicating that molecules are more oxidized (Table 1).

Table 1. Elemental content of humic substances

Sample	O/C	H/C	O%	C%	H,%	N,%	Ash,%
HS (from coal)	0.65	0.067	35.6	54.5	3.65	0.83	21.75
HS (from shale)	0.93	0.083	40.1	54.6	4.55	2.6	21.99
HS(from peloid)	0.86	0.011	41.1	47.7	3.42	4.42	19.87

Ash content of humic substances is higher (19.8 – 21.99%), because composition of ashes includes a significant amount of silicium (3.3 – 6.0%), aluminum (1.6 – 3.4%), as a part of alumina silicates (Table 2). The content of sulfur is between 0.1 and 1.4% and this result show that these substances are more sulfonated. The main method for determination of chloride composition is Semi-quantitative analyses. The composition of chloride ranged from 0.2 - 0.7%, maybe it was remains of hydrochloric acid which used in production of humic substances. Also content of Fe^+ in humic substances isolated from pelìod is 0.7%, it belongs to silicate mineral in peloid. These remains of elemental composition in samples to associate structural features of macromolecules of humic substances and adsorbed in space of macromolecules. Also these elements are connected by chemical bonds of functional group and created a complex compound.

Table 2. Ash content of humic substances

Sample	Si	Al	S	Fe	Cl	Na	Moisture, %
HS(from coal)	5.5	3.4	0.3	-	0.6	-	6.56
HS (from shale)	3.3	1.7	0.1	-	0.2	-	4.55
HS(from peloid)	6.0	1.6	1.4	0.7	0.7	0.8	7.92

Have been studied the structure of humic substances by modern spectral method. All IR spectrums confirmed that HS is poly-structural compounds. The absorption spectra in the infrared region presented variable intensities. From IR spectrum, there were signals found at 3424 cm^{-1} (hydroxyl and amino groups), 2922 cm^{-1} (the lengthiest methylene line-ups), 2853 cm^{-1} (methyl groups), 1623-1591, 1383 cm^{-1} (the structures of benzenoid), 1227 cm^{-1} (C-O ester), 1075-1037 cm^{-1} (C-O carbohydrates) respectively, also had the quantitative differences for the samples isolated from different sources.

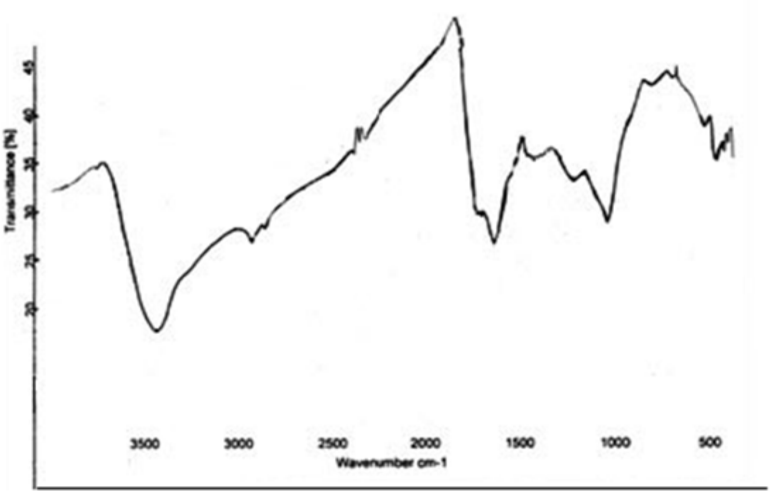

Fig. 1. IR spectra of humic substances isolated from peloid

Semi quantitative assessment of the maintenance of the functional groups carried out on the basis of the relations of the activity in absorption bands of oxygen -containing groups to the optical densities corresponding to aromatic systems (1623cm^{-1}) and aliphatic deputies at 2957 cm^{-1}. Calculation of the structural parameters (Table 3) showed that functional groups are same kind, uniformity by irrespective from the sources of humic acids.

Table 3. Ratio of activity in absorption bands

Ratio of activity in absorption bands	**Humic substances**			
	HS from peloid (stored for 2 years)	HS from peloid	HS from coal	HS from shale
$\text{OH}_{3424}/\text{C=C}_{1623}$	0.84	0.94	1.11	0.83
$\text{Салк}_{2922}/\text{C=C}_{1623}$	1.00	0.70	0.61	0.96
$\text{CO}_{1227}/\text{C=C}_{1623}$	1.10	0.59	0.57	0.58
$\text{OH}_{3424}/\text{Салк}_{2922}$	0.84	1.35	1.81	0.86
$\text{CO}_{1227}/\text{Салк}_{2922}$	1.10	0.84	0.93	0.61

The main oxygen-containing groups in studied HS are hydroxyl groups (OH), ester (C-O) and carboxyl groups (CO-OH). The activity in absorption bands of hydroxyl group compared to aromatic fragments, comparison showed that aromatic fragments are more dominance than hydroxyl group of HS from all sample. Also aromatic is more dominance by activity in adsorption band of amino group compared to aromatic group. Humic substances isolated from coal and peloid are more hydrophilic than humic substances of shale because it contains less amino groups.

Main difference for studied HS is ratio of hydrophilic and hydrophobic component. These study result showed that oxygen containing group including hydroxyl of phenol is more active in reaction than other bioactive groups in HS. Light adsorption of humic substances is higher, based on double bond (C=C and C=O) in molecular structure of humic substances. Adsorption measurement depended on content of polar functional group in sample. Light adsorption of samples in the UV-Vis region, a decrease on the absorption intensity with an increase of the wave length was observed (Fig. 2). The analysis of spectrum showed that humic substances isolated from peloid and coal presents a weak intensity at the field of 274 - 277 nm in ultra-violet and vizible region, attributed to the ionization phenolic hydroxyl groups (or only aromatic structures).

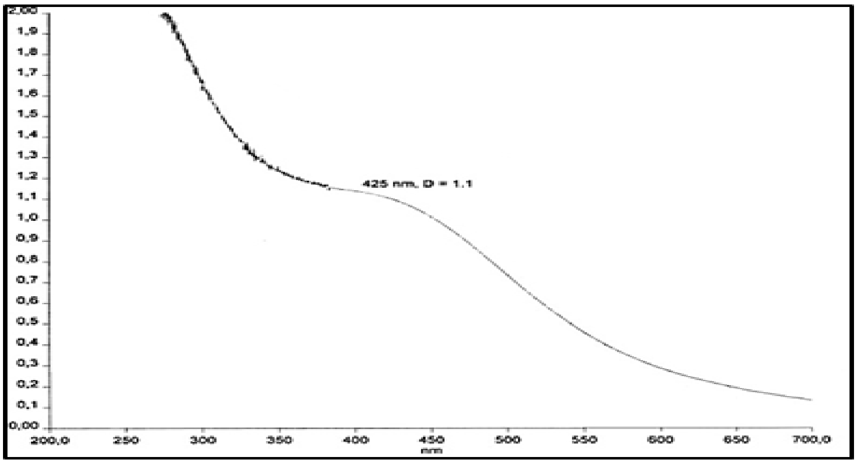

Fig. 2. Electronic spectrum of humic substances isolated from peloid

Color input of humic substances measured using measurement (E_{485}) in wave light at 485 nm. Measurements E for 0.025 mg/100 ml humic substances solution (all samples) were among 0.13 to 0.2. Measurement E for 0.001% solution was 0.003 by standard. We determined coefficient of color input using E_{465}/E_{650} intensive ratio in visible light adsorption.

Coefficient of color input of samples are 2.3 (coal), 3.2 (peloid), 3.9 (shale) respectively (Table 4). Coefficients of color input for humic substances solution to indirectly determine a ratio of aromatic and aliphatic fragments in molecules. High coefficient of color input (for shale 3, 9) seems to occur as less aromatic fragments in molecules. This confirmed by intensity ratio in IR spectrums.

Table 4. Color-input of humic substances

Extention	HS (coal)	HS from coal after hydrolysis	HS (peloid)	HS from peloid after hydrolysis	HS (shale)	HS from shale after hydrolysis
E_{465}	0.13	0.38	0.14	0.26	0.20	0.21
E_{465}/E_{650}	2.3	2.9	3.2	4.1	3.9	4.2

Solubility of humic substances isolated from various resources increased after *alkaline* hydrolysis of *humic substances. Chemical structure of substances not too much changed after alkaline* processes*. Table 5 showed that yield of dry substances is high (93.8 - 98.9%). According to the literature,* after hydrolysis *long-chain structure of macromolecules such as phenolic, quinone and aliphatic group to stretch* into edge. Analysis result confirmed that humic substances isolated from all samples are strong oxidation rate to increase 15 - 20%. On the basis of aluminosilicates component (20 - 22%) of humic substances, concentration of functional group reduced and blocked them; it influenced the reaction, paramagnetic centers and decreased the biological activity. During process for isolation humic substances, functional groups will be unblocked and have an opportunity to participate in various chemical and biochemical processes. In the result of alkaline hydrolysis, all humic substances turn into sodium humate. The amount of sodium in samples reaches 8.1 - 10.6% that can directly characterized the total amount of phenolic and carboxylic hydroxides.

Table 5. Elemental composition of humic substances after hydrolysis

HS	Yield, %	O/C	O, %	C, %	Si, %	Na, %	Al, %
HS from coal	98.78	0.81	37.46	46.05	3.85	8.06	2.38
HS from shale	93.78	0.85	35.29	41.46	7.42	9.99	3.22
HS from peloid	98.98	0.99	38.23	38.54	4.89	10.62	0.96

Carboxyl and hydroxyl groups of sodium humate are in the carbosilikal and phenolyte and to form negative ion when dissociation of these groups. Same charges push away from each other. Therefore, macromolecules of humic substances stretched out and increased the space of fragments in molecules. Such straightened and negative charges influence to increase such as hydration ability of molecules and their stability in water solution, solubility in water and diffusion ability. These abilities can see from their surface and their dryness become more volume and friable. These changes are more visible in their micro photo (Fig. 3).

In the alkaline condition, increases in reaction activity, stability and reducibility of humuc substances influenced positive impact on synthesis nanocomposites.
Humic substances isolated from these samples have a textile and foliation structure. Surface of these substances are similar.

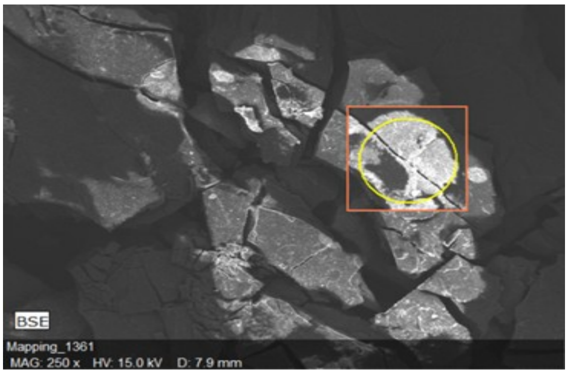

Fig. 3. Scanning electron microscope image of humic substances isolated from shale

Conductivity of all studied HS varied within 10^{-9} - 10^{-11} Sm/cm (Tab. 6), it is shown that these HS are kind of dielectrics.

For example, layer of associates of macromolecules are uneven on SEM photograph of humic substances isolated from shale. For all studied humic substances, associate configuration is uniform during the drying process, because these humic substances contain a lot of minerals.

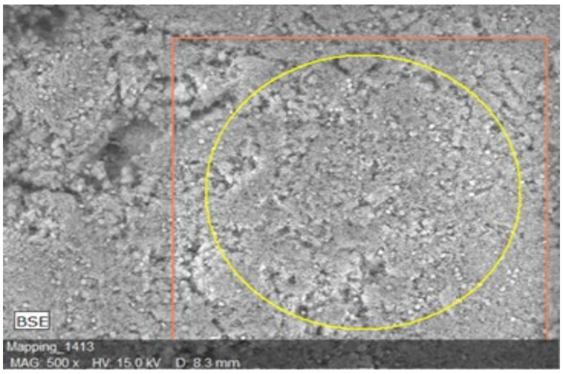

Fig. 4. Scanning electron microscope image of humic substances (isolated from shale) after alkaline hydrolysis

Conductivity of humic substances isolated from peloid were $3.1*10^{-9}$ Sm/cm, it was highest (Table 6).

Table 6. Conductivity of Humic based nanocomposite

Sample	Conductivity, Sm/cm	Moisture, %
HS from shale	$2.3 \cdot 10^{-11}$	4.55
HS from coal	$5.6 \cdot 10^{-10}$	6.56
HS from peloid	$3.1 \cdot 10^{-9}$	7.92

Thus, the molecular structure of HS from the studied sources is characterized by presence of aromatic and aliphatic components. The high ratio H/C, attributed to stretching of C=C bond of aromatic rings in IR spectrums, the high content of functional groups, lower extinction coefficients, confirmed that aromatic fragments dominate than aliphatic chain fragments in structure of all studied HS. The result of paramagnetic resonance showed that there was not a small amount of paramagnetic centers in system, this confirmed that structure of humic substances contain consistent double bond.

CONCLUSIONS

1. Elemental analysis of investigated samples showed that the content of sulfur was between 0.1 and 1.4% and composition of chloride ranged from 0.2 - 0.7%, maybe it was originated from hydrochloric acid which used in production of humic substances, also content of Fe^+ in humic substances isolated from peliod was 0.7%, it belongs to silicate mineral in peloid. These remains of elemental composition in samples to associate structural features of macromolecules of humic substances and adsorbed in space of macromolecules. Also these elements are connected by chemical bonds of functional groups and created a complex compound.
2. Main difference for studied HS is ratio of hydrophilic and hydrophobic component. The analysis of spectrum showed that humic substances isolated from peloid and coal presents a weak intensity at the field of 274 - 277 nm in ultra-violet and vizible region, attributed to the ionization of phenolic hydroxyl groups (or only aromatic structures*). IR and UV/Vis* spectra of these samples showed that oxygen containing groups including hydroxyl group of phenolic origin is more active in the reaction than other bioactive groups.
3. The high ratio H/C, attributed to stretching of C=C bond of aromatic rings in IR spectrums, the high content of functional groups, lower extinction coefficients, confirmed that aromatic fragments are dominating than aliphatic chain fragments in structure of all studied HS

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