

Study of extracted humic acid from the soil of Naharhoran forest in Gorgan

A.R.Sardashti*, M. Alidoost

Department of chemistry, Faculty of science, University of Sistan and Baluchestan, Zahedan, Iran

*Corresponding author email: sardashti@chem.usb.ac.ir

ABSTRACT: Humic acid is a fertilizing factor of soil. Its composition and actual structure, however, has been unknown up to now. The objective of the present investigation is the study of humic acid structure. In this study, the humic acid was extracted from the soil of a forest in north Iran, according to IHSS protocol. The IR spectrum of this sample was in complete agreement with that of J.L. Gardea. Furthermore, the existence of benzoic-carboxyl and phenol functional groups in it was confirmed. GC-MS analysis showed its structural composition. The XRD pattern of this humic acid indicated the presence of amorphous phases in the sample containing particles. The scanning electronic microscopy image revealed the size of these particles to be about 0.20 μm . Thermal analysis of the sample (TG and DSC) provided the complementary information about its structure. The CHNS experiments indicated that the extracted humic acid from the soil of Naharhoran forest in Iran contained fertilizing factor (C/N=15.01). The molecular weight of humic acid was determined using GPC method. The total cationic exchange capacity and capacities of each functional group were determined by potentiometer titration method using 0.20M NaOH in aqueous media. Potentiometer titration curve with logarithmic scale was used for the calculation of acidic constants (pKa) of benzoic carboxyl and phenol, which were 4.60 ± 0.25 and 8.54 ± 0.35 , respectively. The results of this research demonstrated that the properties of this humic acid corresponded more with the Stevenson model than the other models.

Keywords: Forest soil, Extracted humic acid, potentiometer titration, CHNS, GPC, XRD, SEM

INTRODUCTION

Humic acid is a complex organic macromolecule which forms in the soil by bacterial and chemical phenomena and leads finally to humification. Some researchers including Dragunov, Chrisman, Ghassimi, and Stevenson et al., have already carried out studies to determine the structure of humic acid, but a general agreement on a standard model is still not achieved (Liao et al., 1982; Christmann and Ghassemt, 1966). This acid has a relatively high molecular weight ranging from 10^4 to 10^6 Dalton. Carbon constitutes 50% of its molecular weight (Stevenson, 1994).

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Humic acid is a weak organic acid, which has both principal factors (phenol and benzoic carboxylic groups) and some secondary functional groups such as methoxy, amine, sulphonic and alcoholic (Davies et al., 2001; Sardashti and Mohammadian, 2007).

One of its important features is cationic exchange capacity, whose rate differs based on soil nature and whose quantity is usually much higher in dark brown soils (Sardashti and Rumeau, 1999). This acid forms stable complexes with mediated and heavy metals ions. Hence, it is a proper chelator for eliminating the metal ions in order to remove the pollutions from environment (Schnitzer et al., 1991; Gardea et al., 1996). Complex formation in cation exchange is considered as a secondary reaction. The mechanism of reaction and the nature of cation exchange sites were determined through a series of analyses. However, its bonding nature is not completely known, since a chemical bond forms between the exchanging cation and functional group of humic acid. The velocity of bond formation and decomposition restricts the general velocity of exchange and follows the ordinary rules of chemical kinetics. The velocity of such chemical reactions is obviously independent of functional groups affecting the diffusion (Gonet and Wegner, 1996; Pietro and Paola, 2004). This acid is able to buffer the soil pH in the limits of wide changes (Schnitzer et al., 1991).

Existence of soil-fertilizing elements such as nitrogen and phosphorus makes the acid to be known as geochemical fertilizing factor (Francioso et al.,2004;Lou and Lee,2004).

MATERIALS AND METHODS

Procedure

The samples were derived from the soil of Naharkhoran forest located in 13 km from south of Gorgan city (golestan province in northern Iran). This soil has a high organic content and the Iron level in it is low. Thereafter, it was washed according to the IHSS protocol, first with 0.50 mol L⁻¹ HCl and then several times with DDW, then the soil are dried until their moisture reduced to 20%. Finally the dried soil powdered by mill to bits about 2mm. For dilution, dematerialized water provided by a Milli-Q Plus filter apparatus (Millipore, USA) was used. All chemical were of analytical reagent grade (Merck, Germany). Element test solutions and calibration standards were prepared from commercially available (Titrisol, Merck, Germany) 1 g L⁻¹ stock solution using adjustable micropipettes (Gilson, France). Standards were acidified to 1% with nitric acid.

Apparatus

Extraction of humic acid was performed employing an agitator (ELM 1400rpm, Germany). IR spectrum of the prepared sample was obtained by disk IR apparatus Model 470 plus, made in Shmadzou Company (Japan). Thermal analysis 1500 STA (England). GC/MS analysis of the humic acid were performed using a Hewlett-Packard 6890 Net work GC system, equipped with a HP-5Ms capillary column, and a HP 5973 mass selective detector (HP Agilent Technology made in USA. Elementary analysen system GmbH Varo EL (Germany). X-ray D&Advance diffract meter (Germany). Functional groups of humic acid were determined using a digital pH meter CD620 with glacial calomel electrode (Zag Shimi company , Iran).

Extraction of humic acid

200 g of prepared soil washed by one litter of 0.5 mmol mL⁻¹ HCl and then with double distilled water for several times after drying mix the prepared soil with two litters of 0.5 mmol mL⁻¹ NaOH and stirring alternatively with low speed in the nitrogen atmosphere mixing for 5 h. The mixture put a side for 12 h. until liquid and solid phases are separated completely. After separation we add 6mmol mL⁻¹ HCl to liquid phase that pH reaches to one. The brown residue obtained humic acid that should be dissolved in 0.1 mmol mL⁻¹ KOH and we add KCl until concentration of K⁺ takes 0.3 mmol mL⁻¹. The mixture stirs alternatively for 5 h. under nitrogen atmosphere and then keeping aside and repeats the procedure again. Hence humic acid is obtained from reprecipitation. Then for desalinization, humic acid with solution 0.3 mmol mL⁻¹ HF and 0.1 mmol mL⁻¹ HCl in a polyethylene pot stirring at room temperature within a period of 12 h. Afterward solid phase, humic acid, is separated and is rinsed with double distilled water until it gives no response to color test. Before use, the acid was powdered to 75-150 μ m(Stevenson,1994;Li et al.,2006).

GC-MS analysis

All analyses were performed on a Agilent Technology (USA) HP 5973 mass spectroscopy (MS) system. The GC was fitted with a HP-5Ms capillary column (60m* 0.25mm id, 0.25 μ m film thickness).Helium was used as the carrier gas at a flow rate of 1.7 ml/min.The following temperature programme was employed: initial temperature of 80 °C for 4 min ;increased at 10 °C / min to 150 °C ,held for 1 min;then another increase at 5 °C/min to 200 °C ;yet another ramp 30 °C/min to 250 °C ,held for 2 min.The injector temperature was 280 °C and all injections were madein the splitless mode.

Gel Permeation chromatography

The GPC is performed with on-line detection of fluorescence ($\lambda_{ex}=300$ nm, $\lambda_{em}=400$ nm),UV absorption at $\lambda_{abs}=254$ nm and DOC detection. The column, packed with TSK HW 50 S(Toyopearl I),has a length of 25 cm and an inner diameter of 2 cm.The exclusion volume is 19 ml,(determined with dextran blue) and the total volume is 46 ml. Phosphate buffer with C (K₂ HPO₄. 2H₂O)=1.25 g/l and C(N_aH₂PO₄.2H₂O)=2.5 g/l , pH=6.8, is used as eluent at a flow rate of 1 ml/min. Samples are diluted with phosphate buffer (C(K₂HPO₄.2H₂O)=2.5 g/l and C(NaH₂PO₄. H₂O)=5 g/l) the ratio of 1:1 to adjust the phosphate concentration of the sample and eluent.The system is integrated in an assembly for column experiments with additional detectors for electrical conductivity (LDM/S,WTW) and pH value(Ingold).

RESULTS AND DISCUSSION

The IR spectrum of this sample was in complete agreement with that of J. L. Gardea(Gardea et al.,1996).The presence of benzoic- carboxylic and phenol functional groups was also confirmed in it (Figure1).

Chemical composition was determined in DMF solvent through mean GC-MS technique (Table1). One typical fragment molecule was monitored in addition to the molecular 2,3-Dihydro-1-methylindene, 1, 3-dimethyl benzene, 2- methyl eicosane, N-tridecane, pentadecane, octacosane,4-methyl-3-nitrosalicylic acid, acetaldehyde demethoxy, heptadecane, butanamine (Kappler and Brune,2000).

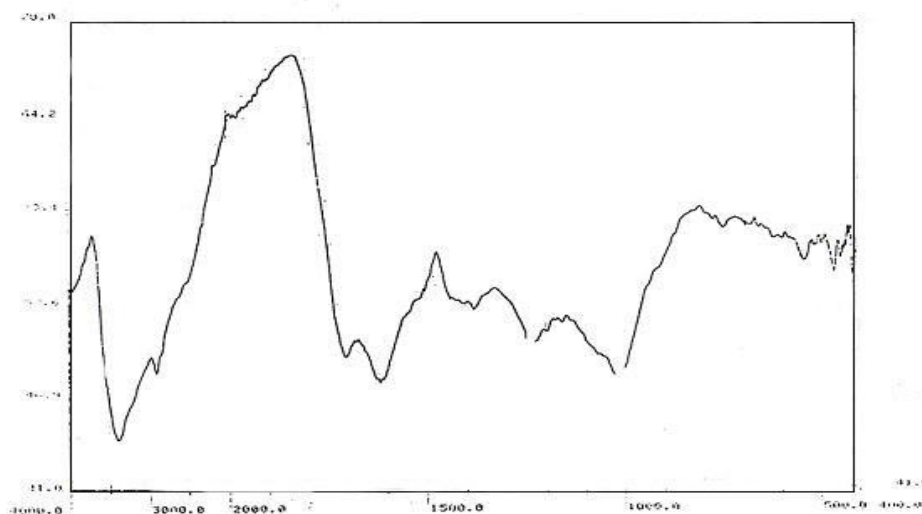


Figure 1. IR spectrum of extracted humic acid

Table1. GC-MS analysis of extracted humic acid

| N ₀ | compound | Chemical formula |
|----------------|----------------------------|--|
| 1 | 2,3-Dihydro-1-methylindene | C ₁₀ H ₁₀ O ₂ |
| 2 | 1,3-dimethyl benzene | C ₈ H ₁₀ |
| 3 | 2-methyl eicosane | C ₂₀ H ₄₂ |
| 5 | n-tridecane | C ₁₃ H ₂₈ |
| 6 | pentadecane | C ₁₅ H ₃₂ |
| 7 | octacosane | CH ₃ (CH ₂) ₂₆ CH ₃ |
| 8 | 4-methyl-3-nitrosalicylic | C ₈ H ₇ NO ₅ |
| 9 | acetaldehy dimethoxy | C ₃ H ₆ O ₂ |
| 10 | heptadecane | C ₁₇ H ₃₆ |
| 11 | butanamine | C ₄ H ₁₁ N |

Mass spectrum

On the mass spectrum (Figure 2), there are three peaks at 603, 577 and 551 with the relative magnitudes of 3.8%, 12.3% and 19% respectively, whose differences in mass number is only 26 times of mass unit, which is equivalent to one C₂H₂. These peaks belong to contracted part of structure. The mass number difference of two peaks at 523 and 495 with the relative abundance of 13.8% and 5% respectively, is 26 times of mass unit. The difference between the peaks at 479 with 2.00% relative abundance and the previous peak is only 16 times of mass unit. peaks at 367, 339 and 313 have the relative abundance of 17.00%, 10.00% and 27.00%, respectively.

The peak at 44 with 100% relative abundance, belongs to acetic aldehyde and the peak at 69 having a relative abundance of +65% and 72 belong to CH₂=CH-COOH. The molecular ion at m/z 73, belongs to butanamine and 74 belongs to acetaldehyde demethoxy, which is separated from aliphatic parts. The molecular ion at m/z 94 and 97 are the base peak and can be tentatively assigned to phenol. To confirm pesticide ions tentatively identified by SIM, one characteristic fragment ion was monitored in addition to the molecular ion; m/z 105, 106 (1,3-dimethyl benzene) 110, 111, 115 (Catechol and Resorcinol), 122, 123 (benzene carboxylic acid) 125, 126 (Pyrogallol) 133, 138, 141 (salicylic acid) 149, 152 (methoxy benzene carboxylic acid) 161, 162 (2,3-Dihydro-1-methylindene) 163, 166 (benzene dicarboxylic acid) 167, 170 (Gallic acid) 175, 177 (methyl-3-nitro salicylic acid) 179 (n-tridecane) 182, 185 (dicarboxylic acid phenol) 211, 212 (pentadecane) 254 (2-methyl heptadecane) 282, 285 (2-methyl eicosane) 394, 395 (octacosane) (Li et al., 2006; Schulten et al., 1987).

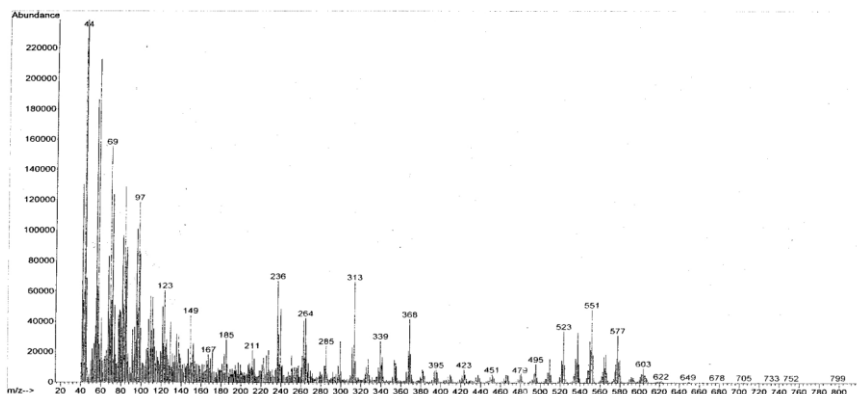


Figure 2. The spectrum mass of extracted humic acid

Kind and size of particules

The XRD pattern of this acid indicated that the obtained data were mainly aromatic nuclei and the particles had colloidal property (Figure 3). Its scanning electronic microscopy image showed that the particle sizes were about 0.2 μm (Figure.4). TG thermograms showed a weight loss in the range of 50-150 °C, which was an indicative of the dehydration reaction (Figure. 5a). The decomposition process between 200 and 500 °C occurred mainly in two steps. The DSC thermo gram of this acid exhibited three peaks (Figure 5b). The first peak, centered at 80 °C, attributed to the removal of water, while the second peak, centered at 300 °C, attributed to the burning of carbohydrates. The third peak, centered at 400 °C, may be due to the evolution of CO₂ (Pietro and Paola ,2004;Francioso et al;2004).

CHNS and Molecular weight

Fractions containing larger amounts of unsaturated groups such as aromatic moieties in the structure (Davies, 2001), showed the percentage of CHNS and C/N ratio (Table 2) in humic acid extracted from Gorgan’s forest(Gonet and Weger,1996).

Table 2. Elemental composition of extracted humic acid

| Weight of humic acid (mg) | C% | H% | N% | S% | O% | Ash% | C/N |
|---------------------------|-------|------|------|------|-------|------|-------|
| 10 | 50.90 | 5.10 | 3.39 | 0.79 | 37.79 | 2.03 | 15.01 |

The molecular weight and viscosity of humic acid in THF solvent was determined through GPC method (Lou and Lee, 2004).The result confirmed the properties of HA typical for high Molecular polyelectrolyte’s (Table. 3 and Figure 6)

Table 3 .The results of molecular weight and viscosity of humic acid

| Factor | Viscosity | Molecular Wt(g/mol) |
|--------|-----------|---------------------|
| Data | 2327 | 2153 |

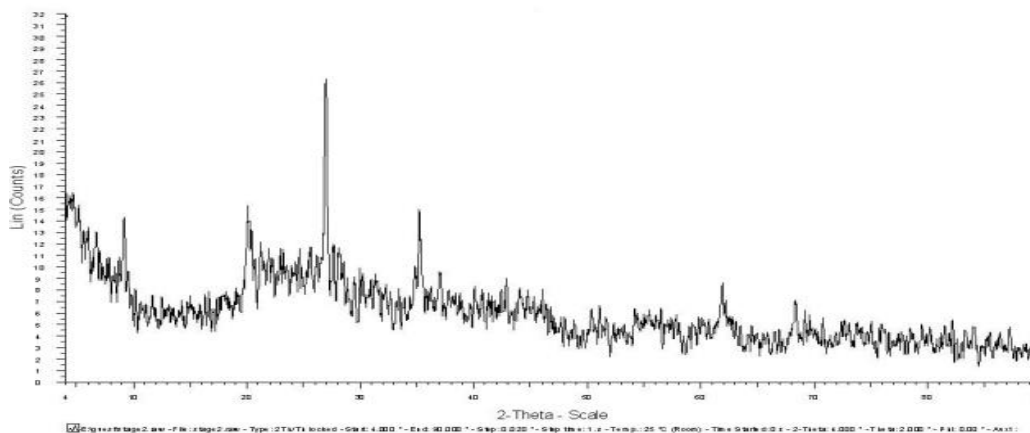


Figure 3. The XRD pattern of extracted humic acid



Figure 4 .scanning electronic microscopy image of extracted humic acid

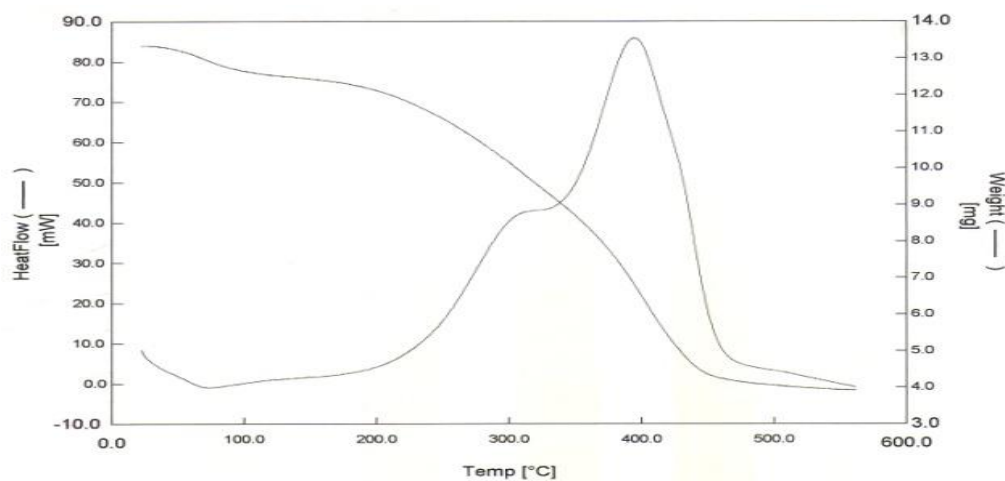


Figure (5a). Thermogram TG of humic acid extracted -5b) Thermogram DSC(reference Al₂O₃)

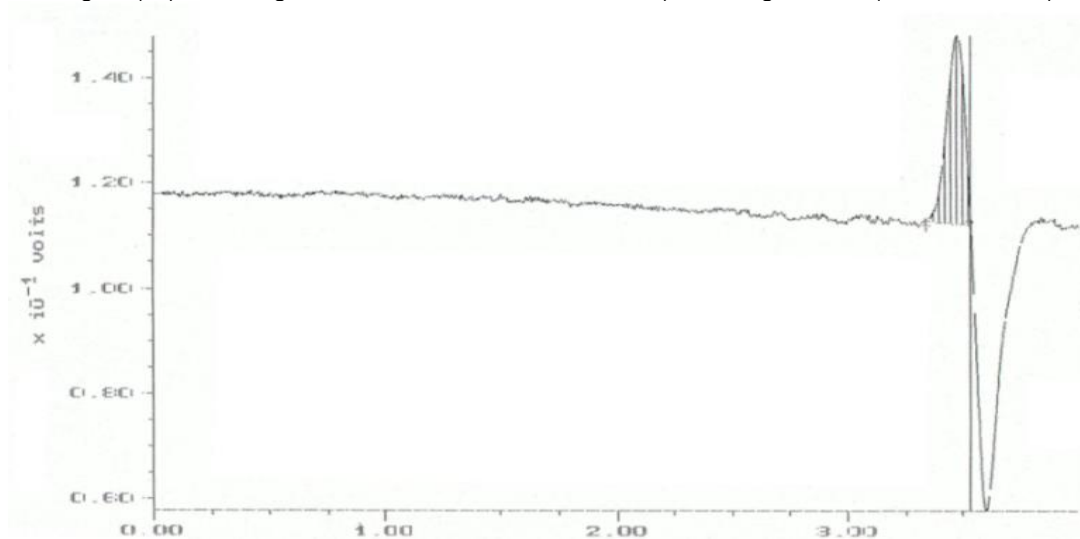


Figure 6.Gel permeation chromatography of Extracted humic acid in THF solvent

Table 4. Cationic exchange capacity for each functional group.

| Functional group | COOH | OH | total |
|--------------------|-----------------|-----------------|-----------------|
| CEC (mmol/g dry's) | 3.10 ± 0.30 | 2.10 ± 0.25 | 5.20 ± 0.40 |

Potentiometer titration

The total cationic exchange capacity and capacities of each functional group were determined by potentiometer titration method using 0.20 M NaOH in aqueous media (Table 4, Figure.7). Potentiometer titration curve with logarithmic scale was used for the calculation of acidic constants (pKa) of benzoic carboxyl and phenol, which were 4.60 ± 0.25 and 8.54 ± 0.35 respectively (Sardashti and .Mohammadian.moghadam,2007;Sardashti and Rumeau,1999).

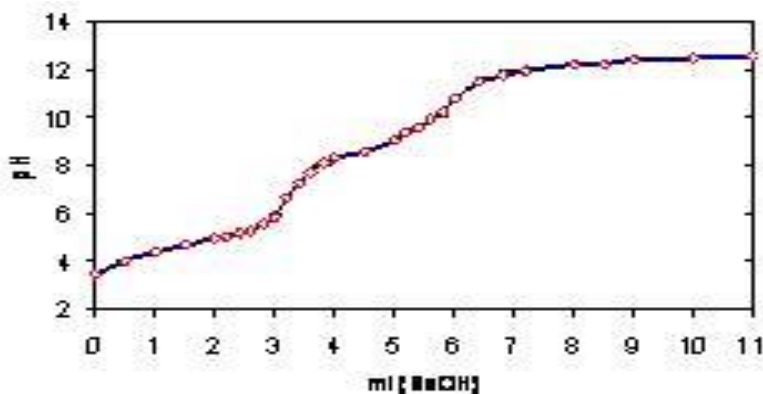


Figure 7.potentiometer titration of extracted humic acid using 0.2M NaOH ($\mu=0.02M$) in aqueous media.

CONCLUSION

As in soil, the useful effects of humic acid in aquatic systems can be largely ascribed to its metal-binding and cation-exchange properties. Another useful function of humic acid is its ability to be employed for disposal of radioactive wastes. In conclusion, the humic acid in soil seems to be of great ecological and geochemical significance, but it still needs to be investigated thoroughly. The results of present research indicate Iran, are in better agreement with Stevenson's model than the other models that the properties of humic acid extracted from forest soil of northern Iran, are in better agreement with Stevenson's model than the other models (Stevenson, 1994).

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