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ORIGINAL ARTICLE

Characterization of different humic materials by various analytical techniques

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Abstract In a previous paper, we studied the characterization of the three humic materials, humic acid, fulvic acid and humin, by simple analytical techniques such as elemental analysis and potentiometric titration. In this work, the same humic fractions were investigated by the developed analytical methods CP/MAS ^{13}C NMR, IR and visible spectroscopy's; to evaluate the harmony of the different analytical methods used for characterization of humic materials and to recognize and evaluate the properties of humic acid, fulvic acid and humin separated from the same source. The results of these developed analytical methods were compared with that of simple analytical techniques. It was found that the low cost simple analytical techniques are suitable for description of humic materials, where the results are in fine agreement with each other. The E_3/E_5 ratio was found to designate a more or less similar indication like E_4/E_6 ratio.

1. Introduction

The hasten growth of nuclear energy will necessarily increase the quantity of radioactive waste that must be controlled carefully. A large variety of radioactive waste and irradiated fuels of different intensities and half-lives is produced in nuclear power generation. These radionuclides are hazardous to human health and must be isolated from the environment using very safe disposal systems. Many of these radionuclides have varying degrees of affinity for humic substances. They are capable of reacting with humic compounds forming organo-metal complexes of different stability and solubility. These organo-metal reactions determine the final fate of the metallic cations (including radionuclides) in soils, sediments, and water (Rashid, 1985). The presence of these substances facilitates the mobilization, transport, segregation, and deposition of trace metals in soil, sediments, sedimentary rocks, and biogenic deposits of various types (Stevenson, 1994). They play a key role in the chemical weathering of rocks and minerals, and they function as carriers of metal cations in natural waters; a portion of the trace metals found in soils and sediments, as well as coal and other biogenic deposits occurs in organically bound forms. Several authors have indicated the high affinity of humic substances for actinide and lanthanide metal ions, and their strong influence on the distribution of the metal ions

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in a terrestrial and aquifer system (Buffle, 1988; Kim, 1986; Stevenson, 1985; Helal et al., 1998, 2005; Helal, 2001; Helal and Aly, 1998). Information on the complexing properties of humic substances with metal ions is important not only from the ecotoxicological viewpoint, but also for the interpretation of the radionuclides distribution in soil. The humic substances are thought to have a special role in the environmental mobility of metals (Weber, 1988). In natural water systems where humic acid is present, the complexation with humic acid plays a significant role in the geochemical behavior and migration of uranium and other actinide ions in the geosphere (Kim, 1993; Saito et al., 2002; Denecke et al., 1997). Hence, the impact of humic acid on the stability and solubility of U(VI) solid phases and species predominantly formed is of particular interest (Kolokassidou and Pashalidis, 2009).

Knowledge of the basic structures of humic materials is required for a full understanding of the role and function of these constituents in the environment. However, because of the multiplicity of component molecules of which they are composed, together with the numerous types of linkages that bind them together, accurate structural formulas are unachievable. Various techniques have been used to investigate the compositions and structural characteristics, including nuclear magnetic resonance spectrometry, gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, spectroscopic analysis, chromatography and others (elemental analysis, metal complexation, etc.). Despite the application of these powerful techniques, structural identification of humic substances at molecular level has not been successful (Frimmel and Contam, 1998; Leenheer and Croue, 2003), and cannot be expected in the near future (Frimmel and Contam, 1998). Each fraction (humic acid, fulvic acid, etc.) must be regarded as consisting of a series of molecules of different sizes, few having the same structural configuration or array of reactive functional groups. NMR and IR can be used as complementary methods for the characterization of humic materials. Solid state ^{13}C direct polarization/magic angle spinning (DP/MAS) ^{13}C NMR has been used for studying organic molecules for more than two decades. It is the most popular technique in studying humic materials structures (Wilson, 1987). However, CP/MAS spectra are not quantitative (Wilson, 1987; Mao et al., 2002). The use of NMR spectroscopy to study humic substances has gained prominence over the last few years. FTIR spectroscopy is a powerful tool that can be used in the identification of complex compounds. FTIR spectra serve as a qualitative tool for monitoring chemical groups and bands of humic acids. FTIR spectroscopy gives more insight into structural changes of humic acid evolution.

For a suitable study of humic materials, good characterization must be achieved for the different fractions to ascertain their purity and to facilitate the comparison between the fractions obtained from different sources. There are several techniques of characterization, ranging from simple methods such as elemental analysis and potentiometric titration, to powerful techniques such as nuclear magnetic resonance spectrometry. In a previous paper (Helal, 2007), we studied the characterization of the three humic materials, humic acid, fulvic acid and humin, by simple analytical techniques. In this paper, the same humic fractions were investigated by CP/MAS ^{13}C NMR, IR and visible spectroscopy's to evaluate the concord of the different analytical procedures used for characterization of humic materials and to recognize and evaluate the properties of humic acid, fulvic acid and humin separated from the same source.

2. Experimental

2.1. Chemicals and materials

The used chemical compounds were of AR purity grade. All experiments in this work were carried out at a temperature of 25 ± 1 °C and atmospheric pressure, and in all steps, the vessels were cleaned with water, organic detergents and finally several times by distilled water, then dried.

2.2. Isolation and purification of humic materials

The three humic materials humic acid, fulvic acid and humin were isolated from the agricultural land soil of Ibri, Oman. The soil at the research location is a highly productive sandy loam. The soil samples accumulated from the meadow were air- dried, crushed, sectored and sieved, through 2 mm mesh sieve, prior to extraction. In a previous paper (Helal, 2007), we have described the method of isolation and purification of the investigated humic fractions.

2.3. Characterization of humic materials

After separation and purification, the obtained humic fractions were characterized by CP/MAS ^{13}C NMR and IR spectroscopy's, in addition to the E_3/E_5 ratio.

The ^{13}C NMR spectra of the humic fractions were determined using the cross polarization technique with magic angle spinning (CP/MAS) on an IBM/BRUKER WP200 SY NMR spectrometer. The ^{13}C NMR spectroscopy was kindly performed by Dr. Y. El-Arafawey, Ibri College of Education.

The IR spectroscopic measurements were made with a Perkin Elmer 1650 FTIR spectrometer using KBr. Sub-samples of humic fractions were further dried at 105 °C for 72 h. A 1.5-mg portion of each sub-sample was ground up with 300 mg KBr and compressed under vacuum at an elevated pressure for 10 min into a small disc of about 10 mm diameter and 1–2 mm thick. Since KBr does not absorb light in the IR region, 4000–500 cm^{-1}, a complete spectrum of each humic sample could be obtained.

The E_3/E_5 ratio of humic acid and fulvic acid samples were measured in the visible (400–800 nm) region using UV–visible recording spectrophotometer 160A obtained from Shimadzu, Japan, with matched glass cells. Two mg of each humic acid or fulvic acid sample were dissolved in 10 ml 0.05 N $NaHCO_3$ and the absorbance at 350 and 550 nm were measured using the spectrophotometer. The value of the absorbance at 350 nm divided by absorbance at 550 nm is the E_3/E_5 ratio.

3. Results and discussion

3.1. CP/MAS ^{13}C NMR spectroscopy

The CP/MAS ^{13}C NMR spectra of the three humic fractions are shown in Fig. 1. It displays quite distinct peaks in the aliphatic (0–50 ppm), aromatic (108–165 ppm) and carboxyl (165–190 ppm) regions. In addition, it has peaks in the region

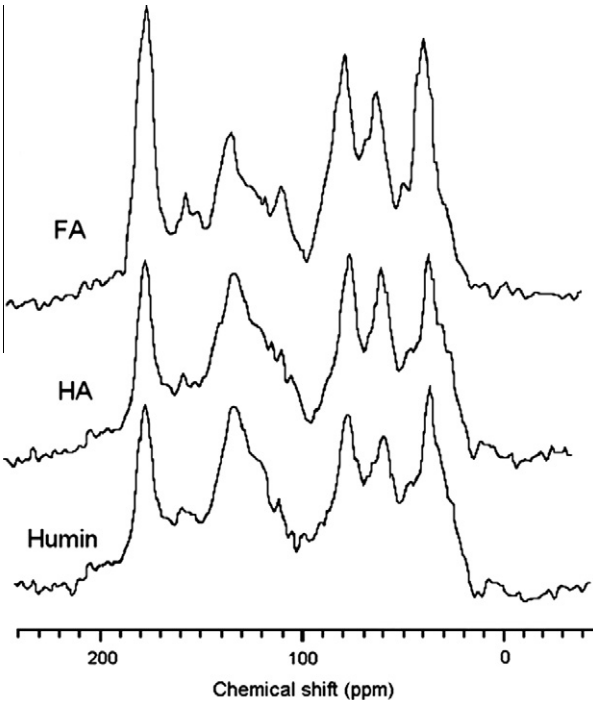

Figure 1 The CP/MAS ^{13}C NMR spectra of humin, humic acid and FA.

50–96 ppm which assigned for O or N substituted carbons in, e.g., carbohydrate, ether, alcohol and amine structures. The spectra of humic acid and humin are more or less similar, and the three spectra are typical of those for soil derived humic materials (Minai and Y., 1989). Fulvic acid shows a higher fraction of purely aliphatic resonance than humic acid and humin. The aromatic section within the spectra of humic acid and humin exhibit strong resonances at 132 ppm and a relatively narrow phenolic resonance, while in fulvic acid the aromatic resonance is weaker and the phenolic resonance is relatively stronger. The carboxylic content of humic acid and humin is much lower than that of FA. As found also in the IR spectra (Fig. 2), it is clear from the figure that the spectrum of fulvic acid is quite different as it shows a stronger peaks for carboxyl carbons (165–190 ppm), phenolic carbons (150–160 ppm) and aliphatic carbons (0–50 ppm), and weaker peak for aromatic carbons (108–165 ppm). Therefore, the ^{13}C NMR confirms the findings of the other analyses (Helal, 2007) and shows the low degree of aromaticity humic acid, compared to the others.

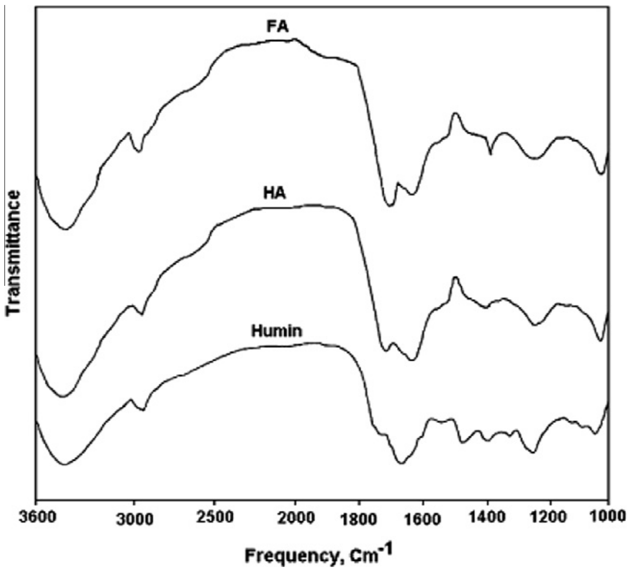

Figure 2 The IR spectra of humin, humic acid and FA.

3.2. *Infrared spectroscopy*

The IR spectra of the three humic fractions are shown in Fig. 2. They have a diversity of bands more or less typical to those distinguishing the humic materials (Stevenson, 1994; Aiken et al., 1985). Major absorption bands are in the regions of 3400–3300 cm^{-1} (H-bonded OH groups), 2940–2900 cm^{-1} (aliphatic C–H stretching), 1750–1720 cm^{-1} (C=O stretching of COOH), 1620 cm^{-1} (aromatic C=C, COO^{-}, H-bonded C=O), 1280–1230 cm^{-1} (C–O stretching and OH deformation of COOH) and 1040 cm^{-1} (C–O stretching of polysaccharide or Si–O of silicate impurities). The spectra evidently show predominance of OH, COOH and COO^{-} groups which are the most characteristic features of soil humic materials. It is clear from the spectra that fulvic acid is characterized by stronger absorption near 1720 cm^{-1} which implies the high carboxylate capacity, and that of humic acid is stronger than that of humin. This is exactly the case in the results of potentiometric titration (Helal, 2007). The spectrum of fulvic acid is also characterized by the absorption at 1400–1390 (OH deformation and C–O stretching of phenolic OH). The results of IR spectra indicate that fulvic acid is more aliphatic, and humic acid and humin are more aromatic. It is obvious that the IR results are in good agreement with the other characterization findings.

3.3. E_3/E_5 *ratio*

The ratio between absorbance at 350 and 550 nm (E_3/E_5 ratio) was determined, according to Dick and Burba (1999), from the optical density of the humic and fulvic acid solutions. Chen et al. (1977), demonstrated that the E_4/E_6 ratio is related to the molecular weight and the oxygen content of the humic substances. In addition, other ratios like E_3/E_5 can be used similarly (Dick and Burba, 1999). The E_3/E_5 ratio varies inversely with the mean molecular size. High E_3/E_5 ratios suggest that the humic materials have a small molecular size. The absorbance ratio of the humic substance is generally a traditional parameter to estimate its degree of humification and/or its molecular size (Lguiratia et al., 2005). Usually, the E_4/E_6 ratio is expected to decrease with increasing molecular weight and content of condensed aromatic rings. In addition, the lowest value of the E_3/E_5 ratio of humic acid as compared with fulvic acid suggests that humic acid has a higher average molecular size. The E_3/E_5 ratios are found 8.01 and 6.00 for fulvic acid and HA, respectively. As previously found for the E_4/E_6 ratios (Helal, 2007), the E_3/E_5 ratios are frankly comparative to both the values of COO^{-} and %O (Table 1). The higher value of E_3/E_5 ratio of fulvic acid is investigative of a weakly developed aromatic nucleus, a lesser molecular weight, a stronger acidic and a extra aliphatic structure (Rashid, 1985). Comparing the deduced results of E_3/E_5 ratio with that of elemental

Table 1 Characterization results of humic fractions (Helal, 2007).

Sample	Yield (%)	Elemental composition (%)					Atomic H/C ratio	Atomic O/C ratio	Atomic C/N ratio	COO^- (meq OH/g)	pK_a	E_4/E_6 ratio	E_3/E_5* ratio
		C	H	O	N	S							
FA	0.32	51.5	5.03	39.41	2.11	1.95	1.17	0.57	28.5	5.0	5.17	7.0	8.01
HA	0.75	56.2	4.48	34.90	2.60	1.82	0.96	0.47	25.2	4.4	5.50	4.5	6.00
Humin	1.08	57.6	4.57	33.74	2.34	1.75	0.95	0.35	25.7	4.2	5.65	–	–

* Present work.

composition and potentiometric titration indicate that they are in good harmony with each other. Therefore, the E_3/E_5 ratio is an appropriate procedure for characterization of humic and fulvic acids.

The previous and present characterization results confirm that the humic acid is mature. This is also supported by the O/C ratio. The high abundance of aromatic structures of humic acid shown by the peak at 1650 on IR spectrum most likely reflects the advanced level of decomposition of the aliphatic moieties of humic acids, owing to oxidation (Chen et al., 1977). Ait Baddi et al. (2004) reported that the fractions excluded from Sephadexs gel chromatography during elution of mature humic acids from composted olive mill waste, were characterized by high FTIR peaks at 2925 and 1650 cm^{-1}, which represented humic acids of high molecular weight. Based on IR spectral analysis of humic acid, its molecular weight would also be high.

Table 1 indicates the characterization results of the three humic fractions by the simple analytical methods (Helal, 2007). The results of E_3/E_5 ratios are added to the table, for comparison. As indicated in the previous paper (Helal, 2007), the H/C ratios of humic acid and humin are higher than that of FA. This reduction shows that humic acid and humin are more compacted (less protonated) than FA. This implies a well-constructed carbon lattice and high molecular weight of humic acid and humin. The decline of H/C ratio from fulvic acid to humin sustained the amplified aromaticity of humic acid and humin (Ding et al., 2002), and illustrate that aliphatic C may be more rich in fulvic acid. This means that the humification route is more superior in humic acid and humin. With additional humification, humic acid and humin may become greatly aromatic with development of polycondensed rings. This corresponds to the decline of aromatic carbon and enrichment of aliphatic carbon as observed in the CP/MAS ^{13}C and IR spectra of fulvic acid (Figs. 1 and 2). It is clear that the previous obtained relationships are in good agreement with findings obtained in the present spectroscopic results. Moreover, as was observed from the titration results (Helal, 2007), both humic acid and humin dissociates nearly similarly at all pH, while fulvic acid behaves in a different way at higher pH (8–10) which is the area of dissociation of phenolic OH. The phenolic section in fulvic acid appears to be specific of fulvic acid, this is also clear in the spectra of both IR and NMR.

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