

Synthesis of Humic and Fulvic Acids and their Characterization using Optical Spectroscopy (ATR-FTIR and UV-Visible)

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Abstract

Humic substances (HS) are complex molecules characterized by high molecular weight and high chemical heterogeneity. In order to have a deeper understanding of these molecules, there is need to synthesize humic acids (HA) and fulvic acids (FA) with determined functional groups. In this paper we report a systematic study on the synthesis of both HA and FA as a function of pH and nature of the functional groups. HA and FA with two functional groups (phenolic and carboxylic) has been synthesized through polymerization of catechol and acetic acid, calibrated by dialysis with membranes of 500, 1000 and 10000 MWCO (Molecular Weight Cut Off) and characterized using two spectroscopic measurement techniques (ATR-FTIR and UV-Visible). Our synthesis technique is inexpensive and simple to undertake. There is no saturation of the reaction medium with oxygen.

Key words: Catechol, fulvic acid, humic acid, humic substances.

Introduction

Humic substances (HS) are complex and ill-defined polydispersed mixtures of heterogeneous polyelectrolytes that are of importance in natural environment due to their role in regulating ion concentration in solution [1]. They are described as refractory dark-coloured, heterogeneous organic compounds produced as by-products of microbial metabolism. They are one of the most widely distributed organic materials on Earth [1, 2]. Formed by the random condensation of the degradation products of plants and animals, composition of humic material strongly depends on its origin, extraction procedure and purification treatment [1].

They constitute a substantial fraction of the organic matter in soils, sediments, water, and organic amendment materials such as composted urban solid waste or composted sewage sludge. HS are fractionated as Humins, Humic acids (HA) and Fulvic acids (FA). HA are defined as humic substances of high molecular weight and high chemical heterogeneity [3, 4], the fraction that is soluble in alkaline solution but insoluble at pH <2, whereas humin is the insoluble fraction and Fulvic acids (FA) is the fraction of humic substances that is soluble in water under all pH conditions. These molecules are known to be heterogeneous polyacids with polymeric structure constituted by different types of functional groups. These substances contain appreciable concentration of acidic functional groups, most of which are carboxylic and phenolic acids [1].

Characterization of the HS molecular sizes, weights, structural arrangements and functional groups is essential for an adequate understanding of their varied role in the environment. Despite intense research on HS during the past decades, the molecular structure of humic and fulvic acids is not fully understood [1]. Their study has generated lot of questions and to date there is still debate on HA structure and whether they are natural products. Indeed there is an argument that HA are man-made macromolecular species rather than natural product, that humic substances may be collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds [5, 6].

In order to have a deeper understanding of these molecules there have been efforts to synthesize HA and FA. Various synthetic pathways such as abiotic oxidation, enzymatic, oxidative and radical polymerizations have been used [1, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. Among the different starting materials that have been used include catechol (*o*-diphenol) and other types of phenols, glutamic acid, xylose, proteins or amino acids like glycine and triglycine. The aim of this paper is to present a systematic study on the synthesis of humic and fulvic acids as a function of nature of the functional groups and thereafter characterize them by ATR-FTIR and UV-Visible spectroscopy. Synthesis at pH 5.5 is carried out using catechol, C₆H₅OH and acetic acid, CH₃COOH as starting materials. Catechol is an excellent starting material for oxidative polymerization since it is the simplest molecule containing the very reactive two hydroxyl groups in ortho-position, like other polyphenols in nature. Catechol has been found to polymerize in solution, both in ethanol and in water, leading to browning of the solution [17]. Acetic acid is chosen as its one of the simplest carboxylic acid which will avail the carboxyl group.

Experimental

Synthesis

All chemicals were of analytical reagent grade and were used without any further purification. For all sample preparation and dilutions, Ultra pure water was obtained from Milli-Q academic (Millipore). The organic reagents were all stored at 4°C whenever they were not in use. The synthetic procedure was briefly as follows. Catechol (6.6g) was reacted with acetic acid (3.6g) in a 1.0L glass beaker, upon which water was added up to the 500mL mark. The pH of the reaction medium (water and catechol) was adjusted to 5.50 using 1.0M NaOH and/or 1.0M HCl and allowed to stand in the dark at 25°C (±0.1°C), under constant stirring at 3,000rpm, which provided sufficient homogeneity. The polymerization reaction was carried out for 30 days while pH was maintained at 5.50 throughout the synthesis by manual adjustment on daily basis. The colour of the medium slowly turned into black as an indicator of the polymerization reaction. At the 31st day, the reaction medium was transferred to dialysis membranes (Spectra/Por®). A series of dialysis at 500, 1,000 and 10,000 MWCO, each for 7 days was performed in order to have calibrated fractions. The fractions got were then deep frozen, lyophilized, ground and stored at 4°C to await characterization.

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectra of aqueous solutions and solids were measured between 4000 and 400 cm⁻¹ on a Bruker Vector 22 spectrometer equipped with a KBr beam splitter and a DTGS thermal detector. The ATR accessory is a horizontal diamond crystal prism manufactured by ASI (nine internal reflections on the upper surface and an angle of incidence of 45°). The scanner velocity was 10 kHz, with the resolutions of 4 cm⁻¹ and a total accumulation time of about one minute. Reference is atmosphere for solids and pure water for aqueous solutions. ATR spectra are shown with absorbance scale corresponding to $\log(R_{\text{reference}}/R_{\text{sample}})$, where R is the internal reflectance of the device. The region between 1850 and 2500 cm⁻¹ was deleted since the diamond crystal absorbs in this region.

Despite the absorbance scale used, the ATR spectra are not strictly proportional to the absorption coefficients at every wavenumber since no further correction has been applied [18].

UV-Visible Spectroscopy

UV-Visible spectra in the region between 200-800nm were obtained using CARY/5G/UV-Vis-NIR Spectrometer equipped with a photo-multiplier detector and a resolution of 0.2nm. Reference is pure water. Samples were diluted to maintain the maximal response and all measurements were made in quartz cells.

Results and discussion

Synthesis

The change of colour from colourless, brown, yellow, green, dark green and eventually black solution was a first indication of polymerization reaction taking place, i.e. humic and fulvic acids formation. Solid formation of high molecular weight fractions is also a confirmation of polymerization. Dialysis followed by lyophilization of the solutions yielded four calibrated solid fractions of (i) <500, (ii) 500-1,000, (iii) <1,000 and (iv) >10,000 MWCO. These were then characterized using optical measurement techniques.

ATR-FTIR

The spectra have been interpreted with reference to various authors [12, 13, 15, 19, 20, 21, 22, 23]. From the ATR-FTIR spectra obtained for the four fractions (A, B, C and D) of the synthesized humic and fulvic acids (Figure 1), it is evident that a reaction took place as the spectra were totally different from those of the reactants, catechol and acetic acid (Figure 2). The absorption bands in the region between 3450-3050 cm^{-1} could be assigned to OH stretching of both phenolic and carboxylic groups. The band at 1256 cm^{-1} could be assigned to phenolic groups while the absorption bands at 1705 cm^{-1} , 1620-1590 cm^{-1} , 1407 cm^{-1} , and at 1256 cm^{-1} are assigned to carboxylic functions (Table 1). The presence of phenolic O-H bond, carboxylic acid functions and aromatic ring is observed in all the fractions (Table 1). We therefore conclude that the two functional groups (phenolic and carboxylic groups) are present in the different fractions of the synthesized humic acid.

There is a difference in band width, intensity and position of the peaks in the spectra of the four fractions of the synthesized humic and fulvic acids. A clear difference in the chemical compositions of the fulvic acid fractions A, B and C, all <1,000 MWCO and that of humic acid, fraction D >10,000 MWCO is observed. All the fulvic acids, fractions <1,000 MWCO exhibit fine, discrete peaks at the same positions which could suggest a similar chemical composition and a high molecular organization. On the contrary, humic acid, fraction D is characterized by wide absorption bands (3450-3050 cm^{-1} and 1256 cm^{-1}) and lack of absorption bands in the region 1554-1468 cm^{-1} which could be taken to indicate its heterogenic chemical environment. The spectroscopic data obtained seem to indicate that the polymer structure may be composed of a mixture of units that result in a complex polymer.

UV-Visible spectroscopy

UV-Visible spectra obtained were similar to those of other humic substances [2, 24]. The UV-Visible absorbance of the four fractions of the synthesized HA and FA generally decreased as the wavelength increased, though the absorption spectra for the fractions <500 and >10,000 MWCO were broad and featureless [25, 26, 27]. In our synthesized molecules the bands of the FA fractions <500, 500-1,000, <1,000 and HA fraction >10,000 MWCO are characterized by a shoulder, intense, very intense and wide bands respectively as shown in Figure 3. A strong absorption band observed at $\approx 275\text{nm}$ for the synthesized humic substances confirmed polymerization reaction. Absorbance in this UV region is normally attributed to π - π^* electron transitions in phenolic arenes, aniline derivatives, polyenes and polycyclic aromatic hydrocarbons with two or more rings [28, 29, 30, 31]. This absorption band which we attribute to conjugated aromatic rings was more distinct in the spectra of samples with low molecular weight cut-off.

Absorbance ratios E_2/E_3 and E_4/E_6 measured at (250/365nm) and (465/665nm) respectively were calculated for each HA and FA fractions [4]. The values are shown in (Table 2). According to prior studies, E_4/E_6 ratio is inversely proportional to the degree of condensation or the molecular weight, such that a high ratio would reflect a low degree of aromatic condensation and the presence of relatively large proportions of aliphatic structures [1, 32, 33, 34, 35]. This ratio is independent of HA concentration and in general < 8.5 [3, 28, 36, 37, 38, 39, 40, 41]. The E_4/E_6 ratio of our synthesized HA and FA was between 3.29 and 4.36. E_4/E_6 ratio (4.28) of the HA fraction >10,000 MWCO compares well with that of the synthesized humic acid (4.29) reported by Chen et al [26]. The FA fraction 500-1,000 MWCO had the highest E_4/E_6 ratio suggesting that most of its chromophores are concentrated; i.e., carboxylic and ketonic C=O and aromatic C=C [35]. Based on these results, it may be interpreted that FA fractions <500 and <1,000 MWCO have higher aromaticity as compared to FA fraction 500-1,000 and HA fraction >10,000 MWCO.

The E_2/E_3 ratio was between 2.63 and 5.06 and were comparing well with other reported values of both natural and synthesized humic substances [12].

Conclusions and Perspectives

HA and FA were produced as evidenced by the spectroscopic (ATR-FTIR and UV-Visible) measurements, through polymerization reaction of catechol and acetic acid. The synthesis technique used is new and advantageous as it's cheap and easy to undertake. Polymerization reaction is carried out at ambient conditions and duration of synthesis is relatively short. The reaction medium is not saturated with oxygen. The reactants are inexpensive and easily available.

Following the spectroscopic data, it can be concluded that all fulvic acids <1,000 MWCO are more or less the same in their chemical composition whereas humic acid, fraction >10,000 MWCO is probably characterized by a heterogenic chemical environment. Characterization of the synthesized humic and fulvic acids indicated the presence of the two functional groups (phenolic and carboxylic) and aromatic condensation. Our synthesized molecules are complex and polydispersed, characterized by high molecular weights which make them similar to the natural humic substances. Synthesis of humic and fulvic acids at ambient conditions without saturating with oxygen allowed for the study of humic and fulvic acids evolution with time. It will be the subject of a forthcoming paper. A study on the interactions between the well-characterized HS and toxic metals can now go a long way in providing valuable information of what actually happens between humic and fulvic acids and toxic metals in the environment.

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Table 1: ATR-FTIR major peaks for fractions A, B, C and D and suggested assignment, where; sh, m, w, and i represents a shoulder, medium peak, wide band, and intense peak respectively.

Wavenumber (cm^{-1})	A	B	C	D	Assignment
3450-3050	w	w	w	w	νOH (H-bonded)
2925 & 2852		m			νCH (aliphatic)
1705				m	$\nu\text{C=O}$ (H-bonded COOH)
1620-1590	sh	sh	sh	i	$\nu\text{C=O}$ (COO ⁻) $\nu\text{C=C}$ (aromatic)
1554		i	i		Stretchings of aromatic ring
1515			i		
1468		sh	i		
1407	i	i	i	sh	δCH_3 , $\nu_{\text{as}}\text{COO}^-$
1370		i	i	sh	δOH , νCO (phenol)
1256	i	i	i	w	δOH , νCO (COOH)
1232	i	i	sh		
1194	sh	sh	i		

Table 2: Absorbance ratios of the four fractions of the synthesized HA and FA.

No.	Sample	E ₂ /E ₃	E ₄ /E ₆
1.	FA (<500 MWCO)	2.63	3.45
2.	FA (500-1,000 MWCO)	3.54	4.36
3.	FA (<1,000 MWCO)	5.06	3.29
4.	HA (>10,000 MWCO)	2.74	4.28

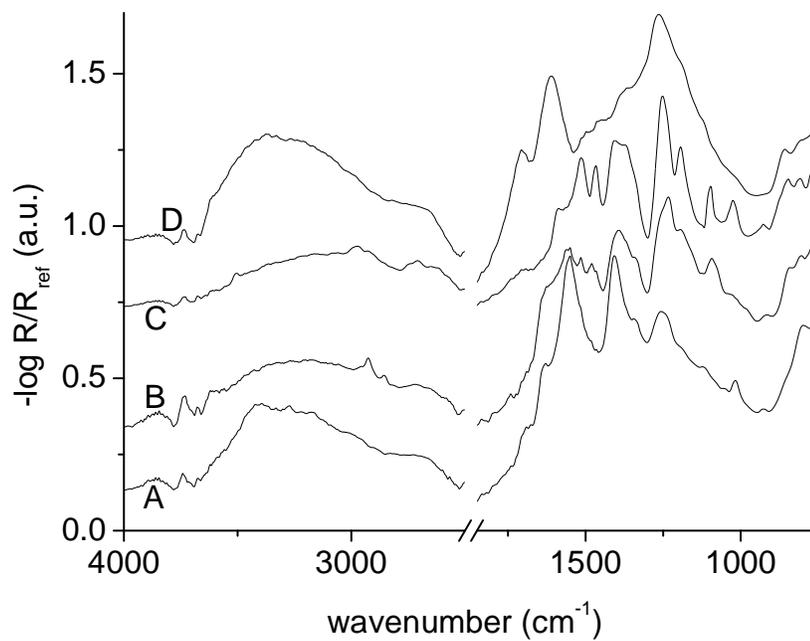
Figure 1: ATR-FTIR spectra of the four fractions of the synthesized Humic and Fulvic acids where; A, B, C and D are FA <500, FA (500-1,000), FA <1,000 and HA >10,000 MWCO respectively.

Figure 2: ATR-FTIR spectra of the reactants, catechol and acetic acid.

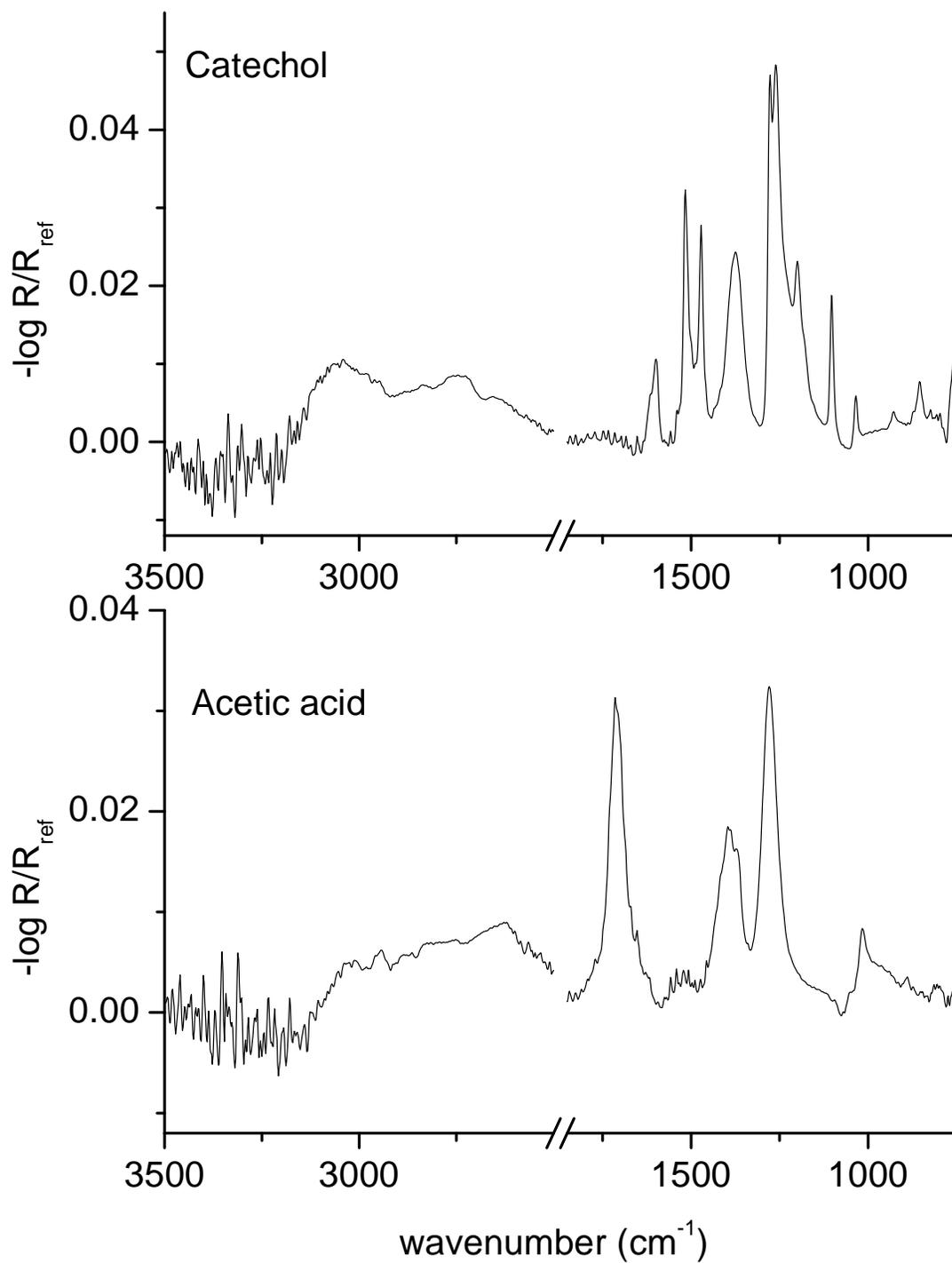
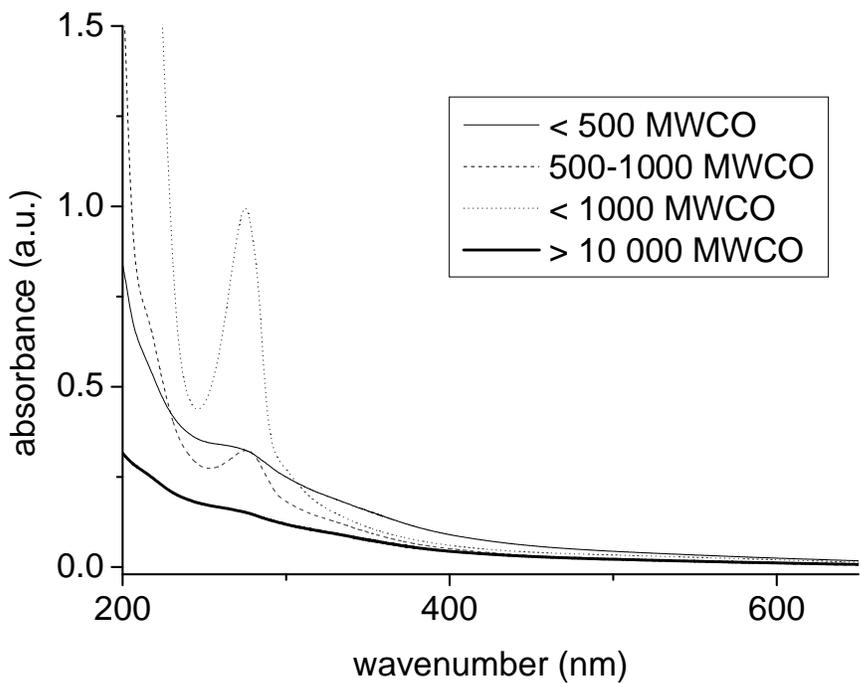


Figure 3: UV-Visible spectra of the fractions of synthesized HA and FA.



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