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Quantification of peat derived fulvic acids by spectrophotometric method

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ABSTRACT

In the present study fulvic acids (FAs) were first isolated from fulvic water (FW) and fulvic urea (SU) and then their quantification was accomplished by spectrophotometric methods. Aqueous solutions of FAs, FW and SU were made at pH 7.0 in the visible wavelength region at 25 ^{o}C using calibration curve method. Plot of absorbance Vs concentration of FAs at fixed wavelength (424 nm) was linear up to 120 mg L^{-1}. The calculated FAs concentrations were in reasonable agreement with the gravimetric estimation made by solvent extraction.

Introduction

Humic substances (HS) are vital component of soil organic matter and in contrast to mineral soil system where the organic matter content makes up only a small fraction, the organic matter is a major part of peat [1]. Soil HS play a diverse role in stabilizing soils and sediments, binding and mobility of metal ions, water balance and retention in the soils [2, 3]. The three major components of HS, i.e., humic acids (HAs, insoluble at pH below 2), fulvic acids (soluble at all pH) and humins, have a good deal of similarity and structures but vary in molecular weights, elemental composition and functional group contents [4]. HAs have a higher molecular weight and have less oxygen containing functional groups as compared to FAs [2, 5]. The molecular weight of FAs ranges from 500-2000 and have a smaller number of total and aromatic carbons than Has, which in turn have longer chain fatty acid products and offer a higher hydrophobicity than FAs. HS are well known for their use in agriculture, environment, medicine, heavy metal adsorbents and for pollutant sequestration. Humic matter in peat is composed mostly of FAs that are principally derived from phenolic and benzene carboxylic acid structures [6]. The exact molecular structure and size of HS remains unclear and research has not yet demonstrated convincingly whether HS are cross-linked macromolecules or loosely held aggregates [7, 8]. Also there is a huge disagreement about variation in elemental composition and chemical properties of humic matter originated from peat since the material has been formed in anaerobic conditions, completely opposite to the aerobic system present in soils. In addition the HS obtained from different depths of same reservoir can also vary in acid functional group content [1, 9].

Nmerous physical and chemical methods are presently being used for characterizing structural and molecular properties of HS. These include elemental analysis, chemical and thermal degradation techniques, infrared (IR), UV-visible, nuclear magnetic resonance (NMR), electron spin resonance (ESR), surface-enhanced Raman (SER) spectroscopic techniques. However owing to the extreme heterogeneity of these groups a single definable primary or secondary structural feature cannot be determined [10].

Previously the quantification of HS had been exploited using precipitation (acid and barium chloride precipitations) and spectrophotometric measurements but, Fataftah *et a*l [11] reported that the humic acid contents obtained from identical samples using different quantification methods differ significantly. More recently Kotob *et al* [12] and Ghabbour and Davies [13] successfully proposed method for the quantification of FAs using spectrophotometric method by using linear plots of absorbance Vs FAs concentration. The purpose of the present study is to isolate and quantify the FAs from different batches of FW and SU, spectrophotometrically using the established calibration curve method.

Experimental

Materials and methods

Standard solid FA (2S103F) was obtained from IHSS. Hydrochloric acid, Sodium hydroxide, Phosphoric acid and Boric acid were obtained from Fisher Scientific. N-Butanol and acetone were purchased from Sigma Aldrich. Cellulose nitrate Membrane filter (0.22 µm) was obtained from Sartorius stedim, biotech, Germany.

Characterization of FA fractions

Techcomp–8500 UV-Vis Spectrophotometer and 1cm quartz cells were used for all absorbance measurements. ^{1}H NMR spectra were obtained on a Bruker AV-300 spectrophotometer operating at 300 MHz frequency. For CHNS Thermo Scientific FLASH 2000 Series CHNS/O Analyzer was utilized and FT-IR spectra were acquired on a Thermo scientific Nicolet-6700.

Isolation of the fulvic acids

Peat derived FAs were fractionated in water (Fulvic water) and then combined with urea to form a clear and viscous light green colored product fulvic urea (local brand name “Sulfurea”). FW and SU were provided by Life Technologies, Pvt. Ltd, Pakistan. FAs in FW and SU were isolated directly by a similar extraction process previously reported by Rebhun *et al* for the isolation of organics from effluents [14]. Briefly the acidic FW (pH~1.30) and SU (pH~1) provided by industry were extracted in n-butanol by multiple extractions. The aqueous layer was discarded after multiple extractions. Butanol layer was dried on

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a rotary at 80 ^{o}C and then treated with acetone. The off using membrane filter (0.22 µm) and the light/straw brown colored FAs were dried, weighed and used as such.

12 mg of IHSS standard sample (Pahokee Peat II 2S103F) was weighed and dissolved in deionized water in a 100 mL volumetric flask up to the mark. Serial dilutions (1/2, 1/4, 1/8, 1/16 and 1/32) of this stock solution were made and absorbance measurements were recorded in triplicate for stock and dilutions in matched 1cm quartz cells with a Techcomp–8500 UV-Vis spectrophotometer at 25 ^{o}C at pH 7.0 in the visible wavelength region (380-700 nm) utilizing water as a blank. Calibration curves between absorbance Vs concentrations of IHSS fulvic acid standard were plotted at 424, 450, 415, 400 and 380 nm and then these plots were used for the determination of unknown FAs isolated from FW and SU. Similarly solutions of FAs isolated from FW and SU were prepared in distilled water. The pH was adjusted to 7.0 by buffer system (mixed solutions of 0.2 mol/L NaOH, 0.02 mol/L of acetic acid, 0.02 mol/L of boric acid and 0.02 mol/L of phosphoric acid).

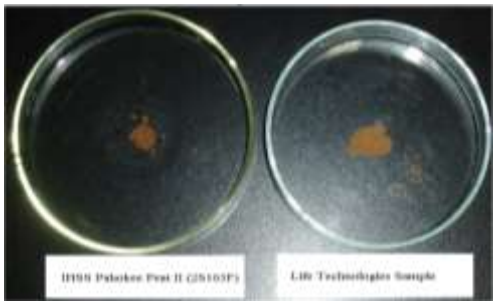

Fig. 1 Fulvic acids extracted from life technologies sample and from IHSS

Results and Discussion

Elemental analysis of CHNS/O was made on ash and water free basis. Fulvic acids utilized in the present study contained higher N content and it could be attributed to the presence of protein or peptide fragments [2]. Nitrogen being a polar element would contribute to the total polarity of fulvic acid [15]. The elemental composition was found to be, %C (52.28 ± 1.2), %H (3.69 ± 0.2), %N (2.72 ± 0.03), %S (0.42 ± 0.001), %O (40.89 ± 0.8)

Fig. 2 shows FAs spectra obtained in D_2O + 0.5 M NaOH. In general, the spectra are subdivided into three main regions. The first region, from 0.8 to 3.2 ppm, is generally assigned to protons on methyl and methylene carbons directly bonded to other carbon atoms. The resonance at 0.9 ppm is assigned to terminal methyl groups of the alkyl chains, [16] while the resonance centered at 1.3 ppm is assigned to protons of methyl groups of highly branched aliphatic structures and methylene groups of alkyl chains [17] and the signals from 1.4–1.8 ppm may belong to protons on aliphatic carbons which are two or more carbons far-away from aromatic rings or polar electronegative functional groups. The weak signals from 1.8–3.2 ppm are believed to be protons attached to aliphatic carbons (methyl or methylene groups) which are attached to electronegative functional groups (e.g. carboxyl group or some aromatic ring) [7]. The second region, from 3.2 to 4.7 ppm, may belong to protons on methyl, methylene, and methene carbons directly bonded to oxygen atoms arising mainly from sugar-like components, polyether and methoxyl groups or protons directly bonded to nitrogen atoms in the amino acid structure may have contributed to it [10]. The third region, from 6.0-8.5 ppm, is assigned to the presence of both unhindered aromatic and heteroaromatic protons with the possible contribution of unsaturated groups as well [7, 18, 19]

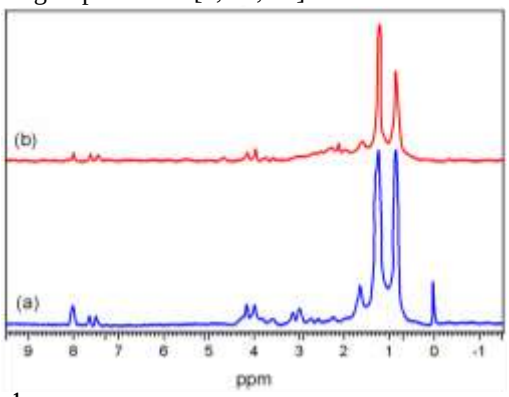

Fig. 2 ^{1}H NMR spectra of fulvic acids isolated from (a) Fulvic water and (b) Sulfurea

The FT-IR spectra of FAs and pure SU are shown in Fig. 3. The spectrum of FAs consists of a broad absorption band at around 3300 cm^{-1} which correspond to O-H stretching of phenol, hydroxyl and carboxyl groups. The Peaks at 2958, 2873 and 2928 cm^{-1} are assigned to C-H stretching vibrations of CH_2 and CH_3 groups [1, 20]. The most characteristic and sharp peak at 1720 cm^{-1} is mainly due to C=O stretching of COOH groups that are extensively present in FAs. This absorption band is less pronounced in case of HAs thus differentiating these two fractions. The Peak at 1456 cm^{-1} can be assigned to C-H-, C-H_2- and CH_3- radicals in aliphatic structures [20], while absorption at 1380 cm^{-1} can be assigned to O-H deformation, C=O vibration, asymmetric COO- vibration and aliphatic deformation. The absorption band at 1167 cm^{-1} belongs to C-O stretch of carboxylate and carbohydrates. The peak at 1036 cm^{-1} can be referred to C–O stretching of polysaccharides or polysaccharide-like substances [21]. In infrared spectrum of sulfurea, the absorption bands at 3342 and 3199 cm^{-1} correspond to N-H stretch of amino group of urea in SU, also the absorption band at 1620 cm^{-1} belong to C=O stretch of the amide [21]. The presence of this absorption band along with the –NH_2 band of suggests that the urea has been covalently bonded to the carboxylic group present in fulvic acids to form H_2N-CO-NH-CO-R (R=Remainder of Fulvic acid structure).

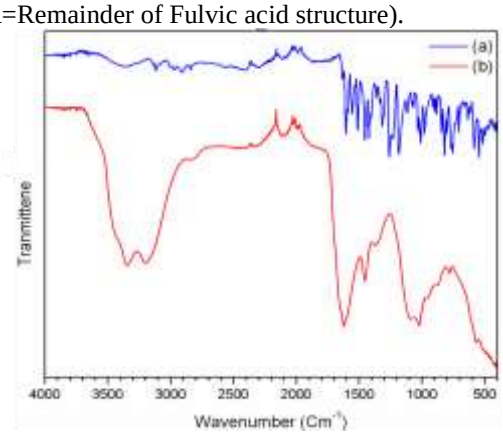

Fig. 3. FT-IR spectra of (a) Fulvic acids and (b) Sulfurea

Calibration curves were obtained after serial dilution of IHSS standard Pahokee Peat FA 2S103F and measured absorbance at wavelengths of 380, 400, 415, 424 and 450 nm as shown in Fig. 4. The wavelength 424 nm was the most suited for accurate estimation of FAs because at higher concentration of fulvic acids, 120 mg/L, we repeatedly got maximum absorbance at this wavelength (λ_{max}). In addition the selection of this reference wavelength resulted in a linear plot (Fig. 5) and it was

found that the selection of other wavelengths at 380, 400, 415 and 450 nm has pronounced effect on the linearity of the calibration curve and consequently lead to false determination of FAs present in the unknown samples especially when the calibration curve was plotted at 380 nm and below, the plot observed a fairly nonlinear behavior.

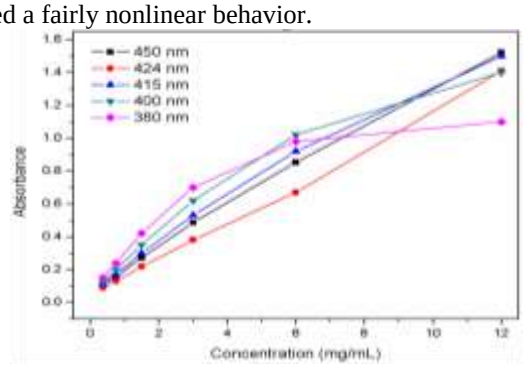

Fig. 4. Plot of absorbance Vs FA concentration for IHSS standard fulvic acid at different wavelengths

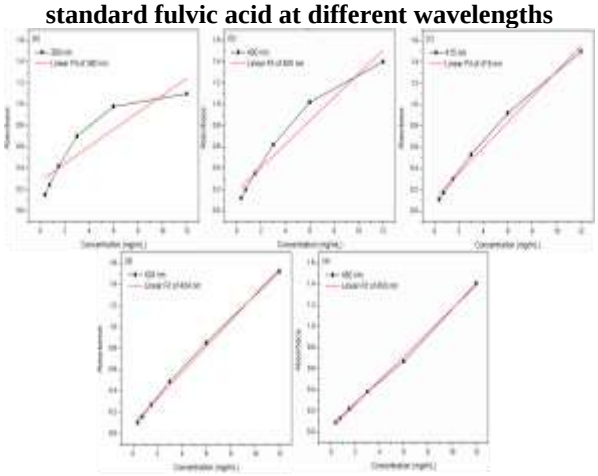

Fig. 5. Calibration Curves of IHSS standard FA at different reference wavelengths

Conclusions

The IHSS fulvic acid standard Pahokee Peat (FA 2S103F) chosen for comparative purpose in the present study have a good deal of similarity in visual, elemental analysis and spectroscopic properties with the FAs isolated from FW and FU in this study. Although the fulvic acids from different sources differ in elemental composition and functional group types and contents, but they observe a good similarity in optical properties. By exploiting the spectroscopic properties, the concentration of fulvic acids can be estimated by calibration curve method but the selection of the appropriate wavelength may have superior impact on the estimation. Unlike previously reported absorption measurements at 350 or 370 nm for Suwannee River, we obtained reliable results by choosing absorption wavelength at 424 nm for the Pahokee Peat (FA 2S103F). Moreover the variations in pH from 1.30-7.0 have no profound effect on the quantification of FAs.

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Table 1. UV/visible absorbance at pH 7.0 for IHSS standard fulvic acid

Dilutions (mg/100mL)	424 nm	450 nm	415 nm	400 nm	380 nm
0.375 (1/32)	0.1023	0.09	0.11	0.12	0.15
0.75 (1/16)	0.1579	0.13	0.17	0.20	0.24
1.5 (1/8)	0.2718	0.22	0.30	0.35	0.42
3.0 (1/4)	0.4864	0.38	0.53	0.62	0.70
6.0 (1/2)	0.8527	0.67	0.92	1.02	0.98
12.0 (Stock)	1.5209	1.41	1.50	1.40	1.10

Table 2. Estimation of unknown concentrations of FAs from FW and SU using calibration curve (at λ = 424 nm)

Serial No.	Sample code	Absorbance	Unknown concentration (mg/L)
1.	Batch A (FW)	1.51	117
2.	Batch B (FW)	0.65	46.5
3.	Batch C (FW)	1.22	92.9
4.	Batch A (SU)	1.48	114.8
5.	Batch B (SU)	0.50	34.7
6.	Batch C (SU)	0.83	60.7

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