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SPECTROPHOTOMETRIC ANALYSIS OF FULVIC ACID SOLUTIONS – A SECOND LOOK

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ABSTRACT

This paper re-evaluates and modifies a previously proposed, simple method of estimating the concentration of aqueous fulvic acid solutions. Spectrophotometric measurements of aqueous solutions of three International Humic Substances Society (IHSS) standard fulvic acids (FAs) at pH 1.0 and pH 4.0 in the wavelength range 350 – 500 nm at 20°C indicate that a) the optical absorbances in this wavelength range decrease exponentially with increasing wavelength; b) the spectrum of each standard FA is independent of pH in the range 1.0 to 4.0; c) plots of absorbance vs. FA concentration at fixed wavelength are linear up to at least 135 mg FA/L; d) the absorption coefficients at each wavelength derived from the linear plots cover a small range for the three standard FAs and average to 5.3 ± 0.3, 3.4 ± 0.3, 1.9 ± 0.2 and 0.89 ± 0.10 $Lcm^{-1}g^{-1}$at 350, 370, 400 and 450 nm, respectively. The averages at 350, 370 and 400 nm are close to the actual absorption coefficients for IHSS standard FA 1S101F and are substantially lower than the absorption coefficients of IHSS standard FA 2S103F employed in the previous work; and e) substitution of the absorption coefficients of this study in the previously observed linear correlation of measured carbon concentrations and spectrophotometrically estimated FA concentrations results in a predicted average carbon content of 52% for five commercial FA samples. This estimate is similar to the average measured %C values of the standard FAs used to develop this simple analytical method, which has potential value for the certification and regulation of humic substances.

Keywords: Standard fulvic acid (FA), spectrophotometric measurements, optical absorbance, absorption coefficient, FA concentrations, commercial FA

1. INTRODUCTION

A consequence of intensive use of inorganic fertilizers, accelerated land development, and soil erosion by wind and water [1-3] is the need to replenish soil organic matter (SOM) in many parts of the world [1,4]. Humic acids (HAs), a major fraction of SOM in most soils, are insoluble in water below pH 2, whereas fulvic acids (FAs), another important class of SOM constituents, are soluble at all pH [5]. Both occur in soils mainly as a result of plant decay. HAs and FAs are much longer-lived than organic soil components such as leaf litter and corn stover. HAs markedly improve soil texture and water retention. They are stores and suppliers of plant nutrients, and HAs sequester xenobiotic substances in soils and water [5].

The world's soils can be replenished with HAs and FAs extracted from low rank coals such as lignite and Leonardite, which are practically worthless as fuels [6]. Steady growth of a humics industry in the last few decades has created a need to analyze humic products for their HA and FA contents with reliable and inexpensive methods. A comparison of extant methods of HA analysis favors HA precipitation from alkaline solution by addition of concentrated HCl followed by washing of the precipitate with water and oven drying at 110°C [7]. Experience in our laboratory shows that this *acid precipitation* method works well provided that the coal, peat or soil sample is treated in sequence with dilute HCl, water, dilute NaOH, concentrated HCl and water, and that sufficient time is taken in each step to allow the systems to reach equilibrium.

FAs occur in most soils and water at lower levels than HAs. Isolation of FAs involves the use of size exclusion and ion exchange columns and is even lengthier than HAs isolation [8]. The International Humic Substances Society (IHSS) has performed a service to science and the humics industry by making available standard and reference FAs from a variety of sources for comparison with laboratory-isolated and commercial FA samples [9,10].

FAs stimulate plant growth, especially on foliar spraying. However, there often is a maximum level of

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plant exposure to FA above which growth stimulation decreases [11] and FA may even be toxic. For this reason and for economy in foliar spraying, it is desirable to have a simple, reliable and reproducible measure of the FA contents of commercial products.

There have been many studies of FAs fluorescence [12-18] but relatively few of FA optical absorbance [18-21]. It is believed that the yellow-brown color of FA solutions is due to intramolecular charge-transfer spectral bands that extend from the ultraviolet into the visible and even into the infra-red region [12,18]. It has been concluded that the increase in absorbance of an FA solution with decreasing wavelength is close to exponential [12,18-21], a point we shall refer to later in this paper.

Surprisingly, there has been little quantitative study of FA absorption spectra with the objective of using spectrophotometry for FA analysis. The previous study [21] used linear absorption vs. FA concentration (mg/L) plots at five fixed wavelengths in the range 350 – 500 nm for solutions of an IHSS standard FA to estimate the FA content of five commercial FA samples. The authors found a linear relationship between the apparent FA content and the atomic C content of the commercial sample solutions, which adds credence to the potential value of this analytical method [21]. A caveat is that the statistical relation between FA concentration from spectrophotometry and the C content from direct analysis might depend on the choice of IHSS standard FA used to generate the calibration plots.

This paper addresses the following questions: 1) are the visible absorption spectra of different IHSS standard FAs quantitatively the same or are they different? 2) If different, which is the best available IHSS standard for the quantitative spectrophotometric analysis of FA samples and commercial FA products? The results of this work have potential for the routine analysis of FA samples and for the certification and regulation of commercial humic substance products.

2. EXPERIMENTAL

The following standard solid FAs were purchased from IHSS and used as received: Suwannee River I (IHSS catalog number 1S101F, labeled **1F** for this study), Suwannee River II (2S101F, **2F**) and Elliot Soil III (3S102F, **3F**). The XAD-8 adsorption method adopted by IHSS results in operational equivalence of these three samples [10]. All other reagents were analytical reagent grade and doubly-deionized water was used throughout.

Stock FA solutions were made up as follows. Homogenized solid **1F**, **2F** or **3F** was weighed on a microbalance and dissolved either in 0.1 M HCl (pH 1.0) or pH 4.0 acetate buffer in a calibrated volumetric flask. Serial dilution of each stock solution with 0.1 M HCl or pH 4.0 buffer was made in triplicate for each desired FA concentration. The FA concentration range investigated was [FA] = 0 – 135 mg/L.

The absorbance of each stock and diluted stock solution of each desired FA concentration at the respective pH was measured in triplicate in matched 10 mm quartz cells with a Perkin-Elmer Model Lambda 20 spectrophotometer at 20^{o}C and at the same wavelengths employed in the previous study: 350, 370, 400, 450 and 500 nm [21]. The absorption data at each fixed wavelength were averaged. Water was used as a blank. Calibration plots of absorbance vs. [FA] were made to assess linearity and provide absorption coefficients ε ($Lcm^{-1}g^{-1}$) from the slope.

3. RESULTS

3.1 Analytical Properties of IHSS Standard Fulvic Acids

Table 1a shows elemental analytical data from Huffman Laboratories, Wheat Ridge, CO [10] for the FA standards employed in this paper and for the IHSS Pahokee Peat II fulvic acid standard 2S103F employed in ref. [21], from hereon labeled **4F**. Standards **1F**, **2F** and **4F** have lower ash contents (measured by high-temperature combustion of dried samples in air) than standard **3F**, which also has the lowest carbon content on a dry, ash-free basis. We note that **4F** has a lower atomic H/C ratio than the other three standard FAs, consistent with a higher aromaticity. On the other hand, the atomic O/C ratios of **1F** – **4F** are similar. Solution-state ^{13}C NMR data [10,22] in Table 1b and acidic functional group data [10,23] in Table 1c [10] indicate that **1F** and **2F** have quite similar compositions.

3.2 Spectral Characteristics of IHSS Standard Fulvic Acids

The experimental results of this study, consisting of the absorbances of FA solutions prepared from three IHSS standard FAs **1F** – **3F**, measured in triplicate and averaged at wavelengths 350, 370, 400, 450 and 500 nm and at pH 1.0 and pH 4.0 are collected in the Supplementary Materials of this paper.

Table 1 (a) Elemental analytical data for IHSS FA standards[a]

IHSS sample[b]	moisture	ash	C	H	H/C	O	O/C	N	S	P
1S101F W, **1F**	8.8	0.46	52.44	4.31	0.99	42.20	0.60	0.72	0.44	<0.01
2S101F W, **2F**	16.9	0.58	52.34	4.36	1.00	42.98	0.61	0.67	0.46	0.004
3S102F S, **3F**	na	2.64	49.79	4.27	1.03	44.34	0.67	3.25	1.23	0.11
2S103F P, **4F**	9.3	0.90	51.31	3.53	0.82	43.32	0.63	2.34	0.76	<0.01

(b) ^{13}C NMR data

	C=O	COOH	Aromatic	Acetal	Hetero-aliphatic	Aliphatic
1S101F W, **1F**	7	20	24	5	11	33
2S101F W, **2F**	5	17	22	6	16	35

(c) Acid groups by titration

	COOH	PhOH
1S101F W, **1F**	11.44	2.91
2S101F W, **2F**	11.17	2.84

[a] Source: IHSS, ref. [10]; All elemental data shown are % w/w on a dry, ash-free basis; [b] Letters W-P indicate the sample origin: W (water), S (soil), P (peat).

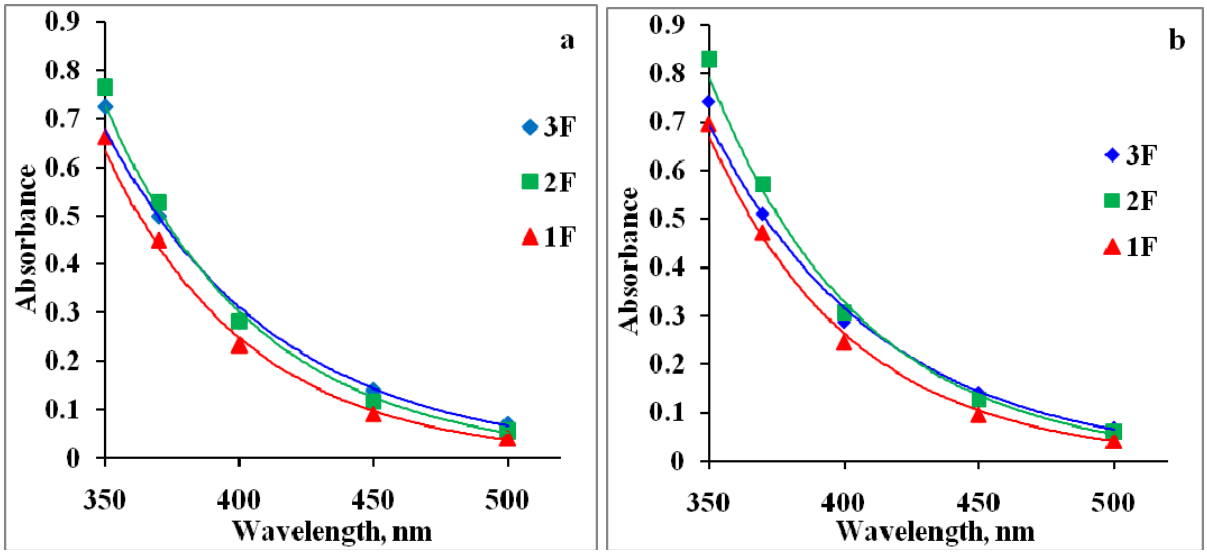

Figure 1 UV-visible spectra of IHSS standard fulvic acids **1F**, **2F** and **3F** at (a) pH 1.0 and (b) pH 4.0. All the data are at 20°C. The experimental absorbance data have been fitted with exponential curves.

Figure 1 shows that the absorption spectra of samples **1F** – **3F** are similar at pH 1 and pH 4. The spectra of **4F** are also similar in the pH range 2 – 7.2 [21]. These observations have two consequences. First, spectrophotometric measurements of FA solute-ions can be made in the pH range 1 – 7.2 without the need to control pH [21]. Second, pH-independence of the spectra is consistent with *intra*molecular charge-

transfer as their origin [12,18] because *inter*molecular charge-transfer would be expected to be affected as neutral acidic FA groups RCOOH become anions after proton dissociation at higher pH.

A form of Eq (1) that describes the exponential character of FA near-UV-visible absorption spectra has been noted [21]. Here, A is the absorbance at some wavelength λ, A_o is the absorbance at theoretical infinite wavelength and S is a slope parameter characteristic of the sample [18,20,24]. Eq (2) follows from Eq (1) and predicts that a plot of ln A vs. λ will be linear with a slope equal to -S. A smaller value of the slope parameter indicates that the spectrum extends further into the visible region.

$$A = A_o \exp(-S\lambda) \quad (1)$$

$$\ln A = \ln A_o - S\lambda \quad (2)$$

Figure 2 and Figure S1 of the Supplementary Materials show linear plots of Eq (2) for all samples **1F** – **3F**, consistent with intramolecular charge transfer in a continuum of energy states as the origin of the yellow color of FAs [12,18]. Values of S from the slopes of the plots in Figures 2 and S1 are collected in Table 2.

3.3 Calibration Curves

Figure 3 shows linear plots of absorbance at 370 nm as a function of the FA concentration in the range 0 - 135 mg/L at pH 1.0 and 4.0. The absorbance-fulvic acid calibration plots at the other reference wavelengths are collected in Figure S2 of the Supplementary Materials, and respective absorption coefficients ($Lcm^{-1}g^{-1}$) are given in Table 3. The linearity of the plots also is consistent with intramolecular charge-transfer as the origin of the yellow color of FA solutions [12,18].

4. DISCUSSION

4.1 General Observations

The spectrophotometric results of this study are consistent with previous work. First, as demonstrated in Figure 1 and the Supplementary Materials, we have found that the absorption spectra of IHSS standard FAs **1F** – **3F** at wavelengths in the range 350 - 500 nm are practically independent of pH in the range 1 – 4. Little variation of the spectra of IHSS FA standard **4F** was observed in the pH range 2 – 7.2 over this same wavelength range [21]. Second, as demonstrated in Figure 2 and the Supplementary Materials, plots of ln (absorbance) vs. wavelength are close to linear over the 350 – 500 nm wavelength range.

Table 2 Slope parameters S (Eq. (2)) of aqueous FA near UV-vis spectra in solution at 20ºC

Concentration	S ± sd[a], nm^{-1}			
mg/L	pH 1	Average	pH 4	Average
1F				
15	0.0189	0.0188 ± 0.0001	0.0179	0.0187 ± 0.0004
45	0.0188		0.019	
75	0.0187		0.0188	
105	0.0188		0.0189	
135	0.0188		0.0187	
2F				
15	0.0172	0.0175 ± 0.0002	0.0161	0.0171 ± 0.001
45	0.0175		0.0168	
75	0.0176		0.0172	
105	0.0177		0.0177	
135	0.0176		0.0176	
3F				
15	0.0182	0.0163 ± 0.001	0.0183	0.0164 ± 0.001
45	0.0163		0.0162	
75	0.0159		0.0159	
105	0.0157		0.0158	
135	0.0156		0.0158	

[a] Standard deviation of the mean

The slope parameters for FA standards **1F** – **3F** in Table 2 are of the same order of magnitude but slightly larger than the value S = 0.015 nm^{-1} for Suwanee River FA in Table 1 of reference [18].

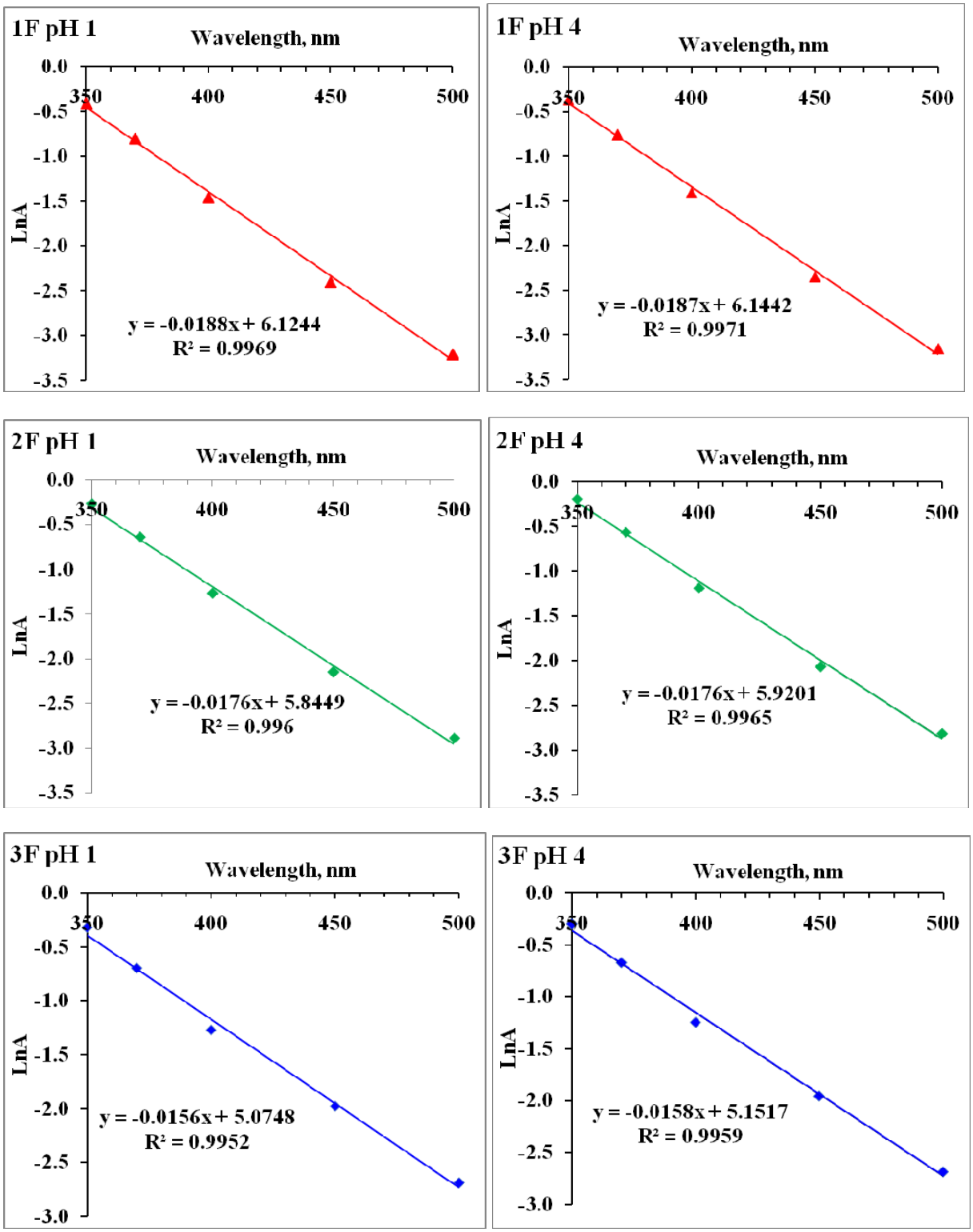

Figure 2 Plots of Eq. (2) for IHSS standard fulvic acids **1F**, **2F** and **3F** (all 135 mg/L) at (a) pH 1.0 and (b) pH 4.0. All the data are at 20°C.

Table 3 Absorption coefficients ($Lcm^{-1}g^{-1}$) of IHSS standard FAs at pH 1 – 4 and 20ºC

Sample	Wavelength, nm				
	350	370	400	450	500
1F	5.1	3.4	1.7	0.73	0.33
2F	5.9	4.1	2.2	0.93	0.43
3F	5.4	3.7	2.1	1.0	0.54
4F[a]	9.3	6.8	4.3	2.3	1.2
4F[b]	10.1	7.5	4.9	2.7	1.5

[a] At pH 2.0, data from ref. [21]; [b] at pH 7.2, data from ref. [21]

This may be due to (a) the different wavelength range examined and (b) non-linear least-squares spectral curve fitting in refs. [18,24] rather than linear least squares fitting through Eq. (2) used here. Third, plots of absorbance vs. [FA] up to 180 mg/L were reported to be independent of pH in the range 2 – 7.2 [21]. Our plots (Figures 3 and S2) are linear and practically independent of pH in the range 1 – 4 up to at least 135 mg FA/L. All these results point to intramolecular charge transfer as responsible for the yellow color of fulvic acid solutions [18]. Linearity of the plots in Figures 3 and S2 argues against FA aggregation even at the highest experimental FA concentration.

4.2 Potential Analytical Applications

No two fulvic acid solutions are exactly alike unless they are sampled from a large volume of a thoroughly mixed fulvic acid solution. If made up from different solid FAs, the results for the solutions may resemble Figure 1 in that the spectra are not quite the same but the spectrum of a given FA is pH-independent. Then we may ask if the spectrum of the fulvic acid solution is exponential, as demonstrated in Figure 2. If so, we ask if a calibration plot like that in Figure 3 is linear in the range 0 – 180 mg/L. If we find a pH-independent, exponential spectrum and linear calibration plots like Figure 3 in the range 350 – 500 nm then the chances are good that the sample is a FA, especially if backed up by data such as those in Tables 1 and 2.

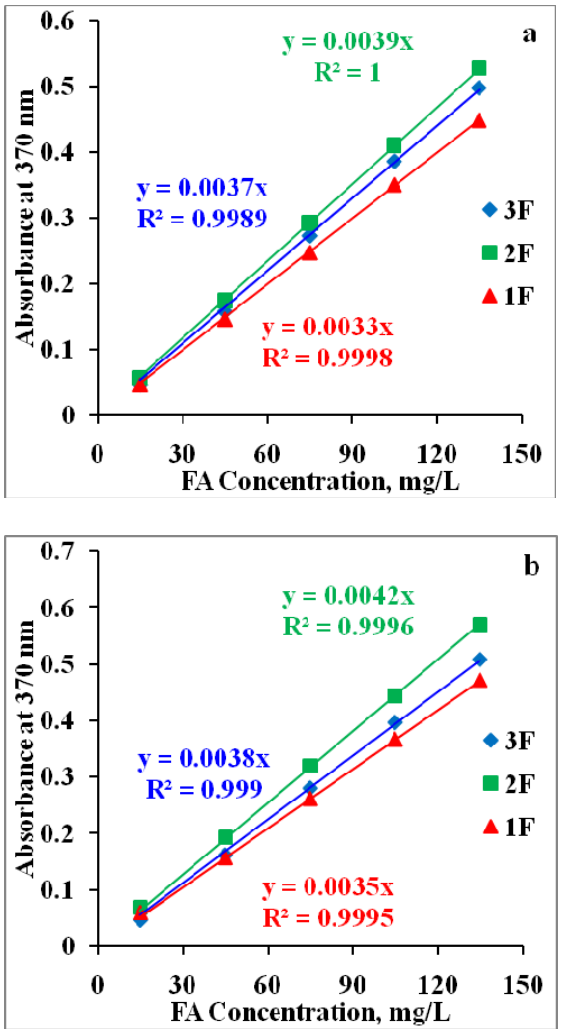

Figure 3 Plots of absorbance vs. FA concentration for IHSS standard fulvic acids **1F**, **2F** and **3F** at 370 nm and (a) pH 1.0 and (b) pH 4.0. The slope of each plot is the absorption coefficient ε ($Lcm^{-1}g^{-1}$). All the data are at 20ºC.

On the basis that a sample is a fulvic acid solution, what absorption coefficient ε ($Lcm^{-1}g^{-1}$) should be used to estimate its concentration from the Beer-Lambert law? The absorption coefficients of the standard FAs at a given wavelength in Table 3 are different. However, the values for FA standards **1F** – **3F** at 350, 370, 400 and 450 nm average to 5.3 ± 0.3, 3.4 ± 0.3, 1.9 ± 0.2, and 0.89 ± 0.10 $Lcm^{-1}g^{-1}$, respectively. These averages are similar to the actual absorption coefficients of FA standard **1F.** These data set apart samples **1F** – **3F** from IHSS standard FA **4F**, which was isolated from Pahokee peat and has

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absorption coefficients that are 1.8 to 2.8 times larger than the above averages for standards **1F** – **3F,** depending on the wavelength of interest (Table 3).

In previous work, appropriately diluted commercial FA solutions were spectrophotometrically analyzed using the absorption coefficients of standard FA **4F** [21]. Choice of an absorption coefficient that is larger than the average for other FA standards will underestimate the FA concentration of a sample solution. Taking the average 2.3 of the 1.8- to 2.8-fold greater value of the absorption coefficients of **4F** compared to **1F** – **3F** at the same wavelengths into account gives Eq. (3) for the five commercial FA samples investigated previously [21]. Here, $[FA]_{carbon}$ is the measured concentration of elemental carbon in a sample (mg/L) and $[FA]_{optical}$ is the FA concentration (mg/L) indicated by spectrophotometric analysis. The intercept 31 of Eq. (3) is the result of reasonably assuming a linear correlation of the data in Figure 2 of reference [21].

$$[FA]_{carbon} = 0.52[FA]_{optical} + 31 \qquad (3)$$

This intercept theoretically is zero and has a much larger uncertainty than the slope. Setting the intercept at zero gives $[FA]_{carbon}/[FA]_{optical} = 0.52$. In other words, the linear correlation of $[FA]_{carbon}$ with $[FA]_{optical}$ for commercial FA sample solutions [21] predicts that the "average" FA molecule contains 52% carbon. For comparison, the average carbon content of standard FAs **1F** – **4F** in Table 1a is 51.5% [10].

5. CONCLUSIONS

Absorbance measurements at 350 or 370 nm are a practical, rapid means of estimating the concentration of a fulvic acid solution that has the characteristic pH-independent, exponential spectrum of a standard FA in the 350 – 500 nm wavelength range. Measurements at 350 nm instead of 370 nm offer a 1.4 fold advantage in sensitivity with an estimated error in the FA content of ± 6%. Given the length and complexity of FA sample isolation and the likelihood of at least some sample loss on the chromatography columns involved [8-10], the simple spectrophotometric approach described here and in ref. [21] merits application for the analysis of fulvic acid solutions. The results of this work have potential use for the routine analysis of FA samples and for the certification and regulation of commercial FA products.

6. SUPPLEMENTARY MATERIALS

Figure S1: Plots of Eq. (2) for IHSS standard fulvic acids **1F**, **2F** and **3F** at (a) pH 1.0 and (b) pH 4.0.
Figure S2: Plots of absorbance vs. FA concentration for IHSS standard fulvic acids **1F**, **2F** and **3F** at (a) pH 1.0 and (b) pH 4.0. The slope of each plot is the absorption coefficient ε.

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