EVALUATION OF A SPECTRO-PHOTOMETRIC METHOD FOR PRACTICAL AND COST EFFECTIVE QUANTIFICATION OF FULVIC ACID

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ABSTRACT

A large number of organic humic products are increasingly being applied worldwide especially in agricultural applications. Humic substances can be fractionated into three components: humic acid, fulvic acid and insoluble humin. A standard method based on acid precipitation is gaining acceptance for quantification of humic acid as the humic acid precipitates at pH < 2 and thus can be quantified by gravimetric measurements. However, the fulvic acid component remains in solution at all pH conditions and there is no practical and cost effective method available for measuring fulvic acid. This paper presents the results of our evaluation of spectrophotometric analysis of the fulvic acid content of commercial humic products. Based on the assumption that the optical properties of fulvic acids are independent of their sources, a calibration curve showing a linear relationship between varying concentrations of an IHSS standard fulvic acid and their UV/vis absorption at multiple wavelengths was established. The concentrations of fulvic acid in a variety of commercial products were then obtained by measuring the samples UV/vis absorption and applying the established calibration curve. The calculated fulvic acid concentrations were in reasonable agreement with the values obtained from carbon analysis of the test samples after corrections.

Keywords: Fulvic acid, quantitative analysis, spectrophotometry, fulvic acid solutions

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1. INTRODUCTION

It is known that humic and fulvic acids are active components of soil and aquatic organic matter and are important to agriculture [1]. The efficacy of humic and fulvic acids depends upon the optimum applied concentration [2]. Chen and Aviad [3] reported that the optimum application of humic acid for plant growth ranges from 50 to 350 ppm. Thus, accurate measurement of these components in commercial products is critical. Several methods including acid precipitation, barium chloride precipitation, and spectrophotometric measurement [4] have been established to quantify humic acid. However, Fataftah et al. [5] concluded that the humic acid contents obtained from identical samples using these different quantification methods differ significantly. They observed that barium chloride precipitation and spectrophotometric measurement method showed higher humic acid concentration than the acid precipitation method. Also, the barium chloride precipitation method can not be used to differentiate humic and fulvic acids [5]. Therefore, the acid precipitation method has been widely accepted for the separation and subsequent quantification of humic acid.

On the other hand, although the International Humic Substances Society (IHSS) had adopted a complex multiple-step method for fulvic acid extraction from natural sources using an XAD-8 resin adsorption method [4], there is no accepted method for practical and cost effective fulvic acid quantification. Our goal is therefore to evaluate and develop a spectrophotometric fulvic acid quantification protocol for industrial and routine laboratory analysis.

UV/vis and fluorescence spectroscopy of humic substances have been extensively studied [6-8]. The absorptivity of humic substances decreases with increasing wavelength and is commonly modeled with an exponentially decreasing function [9]

$$a(\lambda) = a(\lambda_r)e^{-Se(\lambda-\lambda r)} \qquad (1)$$

where λ_r is a reference wavelength and Se is a spectral slope indicating the ratio of humic acid to fulvic acid [10, 11]. A number of researchers reported similar Se values for humics in waters from different sources [12, 13] and the recommended Se value was 0.015 nm^{-1}. This suggests that either Se is not a reliable indication of the humic and fulvic acid ratio or the compositions of humic substances from different sources are similar.

It is also reported that E_4/E_6, the ratio of absorbance at 465 and 665 nm, is inversely related to the degree of aromatic condensation in humic substances [14, 15]. In practice, it is difficult to obtain

accurate absorption data at 665 nm and therefore an accurate E_4/E_6 ratio because of strong optical background in this wavelength range. The molar absorptivity ε_{280} of humic substances at 280 nm, is used to indicate the aromaticity of humic substances [8]. The parameter ε_{280} is prone to inferences of inorganic salts such as nitrate. Additionally, spectral overlap further complicates the identification and assignment of chromophores. So far, no convincing correlation is established among aromaticity, E_4/E_6, ε_{280}, and elemental composition of humic substances.

It is understandable that even if the elemental compositions of two humic substances are similar, but their optical properties could be different due to 1) different functional groups derived from the same elemental composition, and 2) different structures, configurations and corresponding intra- and inter-molecular interactions of different functional moieties [16]. It is equally possible that various individual chromophores display significantly different optical properties; however, the sum of intra- and inter-molecular interactions leads to similar overall optical behavior of humic substances obtained from different sources.

Most of the previous studies focus on spectral details and little correlation between optical parameters and bulk properties such as concentration are reported. In this study, instead of focusing on subtle spectral differences between different humic substances, we aim to establish a quantitative correlation between UV/vis absorption and the concentrations of fulvic acid isolated from different sources based on the assumption that the overall optical properties of humic substances are very similar regardless of their origins. In this study, the optical absorption of fulvic acids at multiple wavelengths was measured to eliminate possible interferences of most inorganic salts and contaminants that may absorb at some, but not all, wavelengths.

2. EXPERIMENTAL

Standard fulvic acid (2S103F) was obtained from IHSS. Reagent grade potassium hydroxide (KOH), nitric acid (HNO_3) and hydrochloric acid (HCl) were purchased from Aldrich and VWR. Potassium hydrogenphosphate (K_2HPO_4), potassium dihydrogen-phosphate (KH_2PO_4), and 85% o-phosphoric acid (H_3PO_4) were purchased from Fisher Scientific. A Millipore system provided ultrapure water. A Shimadzu 160U spectrophotometer and 1 cm plastic cuvettes were used for all absorbance measurements.

1000 mg/L fulvic acid stock standard solutions were prepared in 20 mM pH 2 and pH 7 phosphate buffers. Five commercially available liquid and solid humic substance samples were obtained from different countries with different origins. For isolation of fulvic acid, the pH of these samples was adjusted to less than 2 to precipitate humic acid. After removal of humic acid precipitates and insoluble solids, the supernatants were adjusted to pH 2 and 7 followed by dilution and absorbance measurement at 350 nm, 370 nm, 400 nm, 450 nm, and 500 nm. The carbon contents of the same samples were measured by Huffman Laboratories, Inc., Golden, CO.

3. RESULTS

3.1. Calibration Curves

Calibration curves were obtained by serial dilution of pH 2 and pH 7 fulvic acid standard stock solutions followed by absorbance measurement at the respective wavelengths. The calibrated concentrations ranged from 5 mg/L to 180 mg/L.
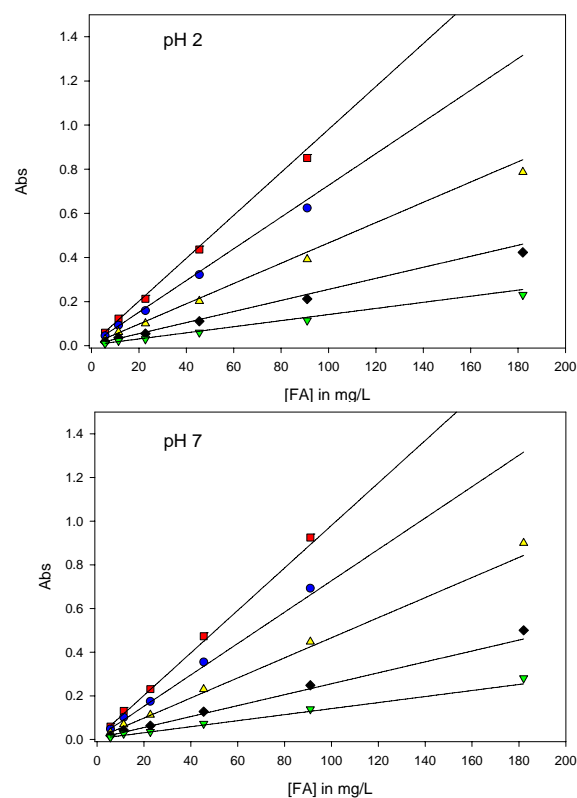
Figure 1 Calibration curves of IHSS fulvic acid at different wavelengths (350 nm ■; 370 nm ●; 400 nm ▲; 450 nm ◆; and 500 nm ▼) in pH 2 and pH 7 aqueous solutions.

Table 1 Linear regression equations obtained from standard calibration[a]

pH		350 nm	370 nm	400 nm	450 nm	500 nm
2.0	Equation	A= 0.0093FA + 0.0116	A= 0.0068FA + 0.0128	A= 0.0043FA + 0.0088	A= 0.0023FA + 0.0068	A= 0.0012FA + 0.0043
	R^2	0.9998	0.9997	0.9997	0.9995	0.9982
7.2	Equation	A= 0.0101FA + 0.0035	A= 0.0075FA + 0.0061	A= 0.0049FA + 0.0026	A= 0.0027FA + 0.0025	A= 0.0015FA + 0.0017
	R^2	0.9999	0.9999	0.9998	0.9996	0.9990

[a] A: absorbance; FA: fulvic acid concentration in mg/L

Table 2 The UV/vis absorbance at pH 2.0 and corresponding fulvic acid concentrations

Sample	Dilution	350 nm	370 nm	400 nm	450 nm	500 nm	$[FA]_{optical}$ mg/L	$[FA]_{carbon}$ mg/L
1	1/32	1.80	1.21	0.65	0.35	0.12	5328	6400
2	1/2	2.49	2.49	1.43	0.63	0.37	598	760
3	1/15	1.05	0.81	0.53	0.20	0.10	1750	1900
4	1/15	0.55	0.36	0.25	0.13	-	860	1200
5	3/40	1.97	1.49	0.89	0.34	0.15	2670	3500

$[FA]_{optical}$ values were calculated from absorbance and calibration curves at respective wavelengths. $[FA]_{carbon}$ was the carbon content obtained from carbon analysis.

As shown in Figure 1 and Table 1, linear relationships were obtained between absorption and concentration of both pH 2 and pH 7 fulvic acid solutions at all five tested wavelengths.

It is important to note that although the absorbances of pH 7 solutions are slightly higher than those of pH 2 samples, the calibration for IHSS fulvic acid is similar for both pHs at all measured wavelengths.
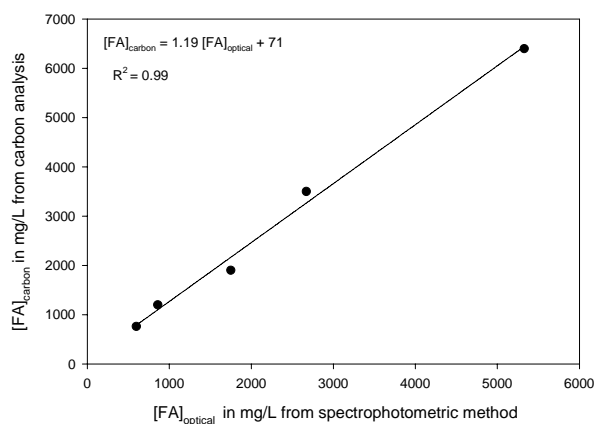
Figure 2 The correlation between fulvic acid concentrations obtained from the spectrophotometric method at pH 2.0 and carbon analysis.

After appropriate dilution, serial solutions with decreasing fulvic acid concentrations were prepared for each of the five commercial fulvic acid samples. Since the calibration for IHSS fulvic acid is similar for both pH 2 and 7 at all measured wavelengths, we only present the absorbance at 350 nm, 370 nm, 400 nm, 450 nm and 500 nm for each solution at pH 2 (Table 2).

The concentration of each fulvic acid sample was calculated by averaging the concentrations obtained from five serial solutions at multiple wavelengths according to the respective dilution factors. To further analyze the fulvic acid contents of each fulvic acid sample, each sample was analyzed for their carbon contents and back calculated to obtain the fulvic acid content.

As shown in Figure 2, a linear correlation was obtained between the fulvic acid concentrations measured spectrophotometrically and the carbon analysis with an average correction factor of 0.83 carbon analysis to spectrophotometric values.

4. DISCUSSION

Fulvic acid calibration using IHSS standard fulvic acid and correlation between fulvic acid concentrations obtained from spectrophotometric and carbon analysis methods both indicate that the spectrophoto-

metric method can be applied as a simple alternative method for the quantification of fulvic acid. Our results also suggest that although the structure, onfiguration, functional moieties, molar mass, and intra- and inter-molecular interactions may be significantly different, the overall optical behavior of fulvic acids from different sources is similar. Additionally, due to the absence or minimum absorption of most inorganic salts in the 350 nm to 500 nm range, no removal of inorganic salts is required.

Since similar linear calibration curves were obtained at pH 2 and 7, fulvic acid can be quantified directly after removal of insoluble solids and precipitation of humic acid at pH of 2 without changing the pH of the tested samples.

Based on these experimental results, a cost effective and simple fulvic acid isolation and quantification method is proposed as shown in Scheme 1.

For solid samples, alkaline extraction of humic and fulvic acid is first performed to separate humic substances from insoluble solids. Adjust the pH of the filtrate to 2 or below and humic acid will be precipitated. The spectrophotometric method can be applied to quantify fulvic acid contents in the supernatant. Alkaline extraction is not necessary for liquid samples. Future efforts are required for the analysis of samples from different locations and manufacturers by different laboratories to confirm and validate the above described approach.
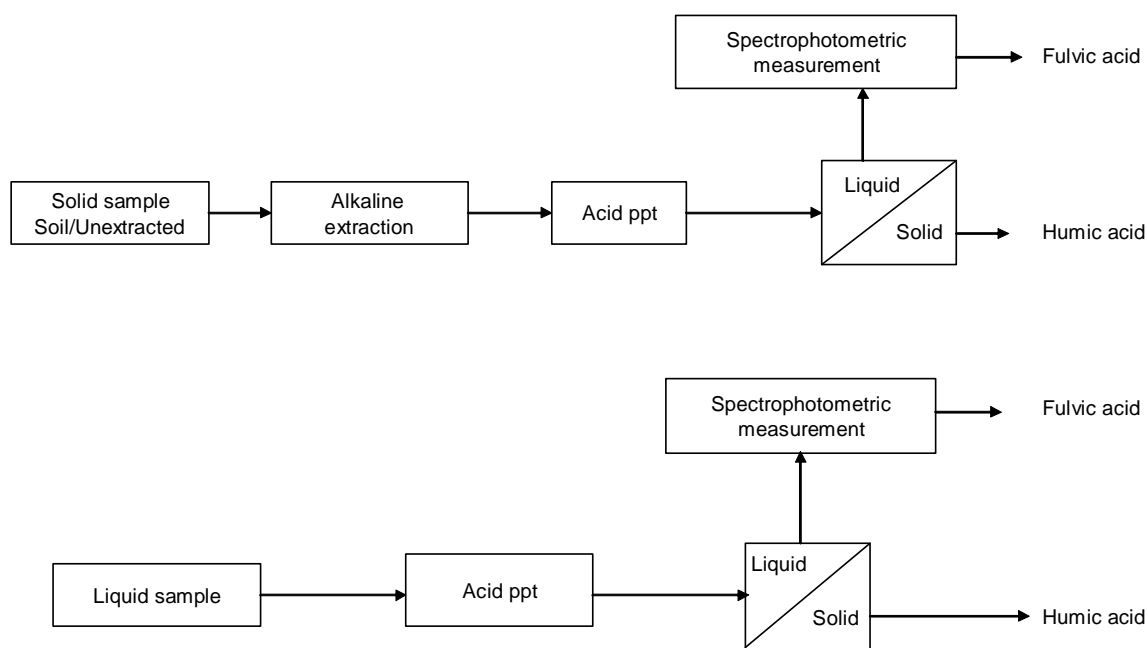
Scheme 1 Suggested fulvic acid quantification protocol

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