

Research Article

Determination of Humic Acid by Spectrophotometric Analysis in the Soils

Amanollah Javanshah¹ and Asiye Saidi²

¹Pistachio research center, Rafsanjan, Iran

²Barafza keshavarze Pars Company, Rafsanjan, Special Economic Zone, Rafsanjan, Iran

Email: Javanshah@hotmail.com, Asiye_saidi@yahoo.com

ABSTRACT

Humic acid (HA) represents the organic material most widespread in nature and have positive effects on plant physiology influencing nutrient uptake and root architecture. The role of HA in the environment is extremely important. To date, the methods have been developed for the determination of HAs directly following the extraction process are time consuming. The aim of our work was to the application of UV/vis spectrophotometry method to HA determination after its extraction by Sodium pyrophosphate alkali solvent. The precision and accuracy of this method were evaluated. The performance of UV/vis spectrophotometry was compared with California Department of Food and Agriculture (CDFA) and International Organization for Standardization(ISO) 5073:2013 methods. The results for the proposed method are comparable or more applied than them. Also this methodology is easier and faster than two others.

Keywords: Humin; Humic acid; UV/vis Spectrophotometry; Precipitation

1. INTRODUCTION

Humic substances (HS) are derived from the degradation of the remains of plants, microbially-derived and animals in the environment [1-3]. These substances form a group of polyfunctional acids, which have a yellow to brown color and a molecular weight from hundreds to hundreds of thousands of Daltons [4]. The humic substances system is created by the association of various components present in the humification process, such as amino acids, lignins, pectins or carbohydrates, through intermolecular forces (donor-acceptor, ionic, hydrophilic, and hydrophobic). It is evident that the mechanisms of the formation of humic substances can be slightly different, depending on geographical, climatic, physical and biological circumstances, respectively. The role of HSs in the environment is extremely important. Because of their molecular

structure, they provide numerous benefits to crop production. They help break up clay and compacted soils, enhance water retention and slow down water evaporation from soils, increase seed germination rates and penetration, and stimulate the development of microflora populations in soils. They hold micronutrients in the soil essential for plants and their root systems and play a crucial role in maintaining the acid-base buffering ability of soils. They are also able to bind mineral particles together in soil environments, thereby contributing to the soil structure and helping to maintain the moisture content. HSs can also dissolve certain minerals, bind small organic compounds, reduce metal ions and act as mediating electron shunts in microbial and abiotic redox reactions and are capable of forming and solubilising toxic compounds in the environment [5,6].

HSs are typically classified into three main groups comprising humin, humic acid and fulvic acid. Humin is classified as a material which is insoluble in aqueous acids or bases; humic acid is a substance soluble in solutions with $\text{pH} > 2$ and fulvic acid soluble at any pH value [4].

Two common extraction procedures utilize high pH solutions to solubilise humic acid and fulvic acid: 1) extraction with a sodium hydroxide solution [7-10] and 2) extraction using a combination sodium hydroxide and sodium pyrophosphate solution [11-13]. The response of humic substances to dissolve in different extraction solutions can result from differences in the chemical structure of the molecules or the physical availability of the organic molecules associated with mineral matter. Extraction with NaOH can produce relatively high yields of organic matter, but the extraction must be repeated several times to achieve maximum recovery [14]. Extracting solutions that use sodium pyrophosphate are a good choice for soils that contain calcium as this solution dissolves metalorganic complexes that reduce the availability of organic matter to react in solution. Pyrophosphate anions complex with polyvalent cations, such as calcium, to release charged organic matter that can subsequently be dissolved in water [14-17].

Because of the heterogeneous molecular, structural and chemical nature of humic acids, sequential extraction of humic acid fractions with NaOH solution can result in significant structural differences between the first and last humic acids extracted from the same soil [18, 19]. More pronounced structural changes in humic acids occur when exposure times to alkali solutions are increased [5, 20]. In addition, when the extraction of humic acid from the soil is incomplete, the residual humin could be contaminated by carbon from the fulvic acid and humic acid that remains in the soil [21].

Fractionation and characterization of HAs have been in recent years using chromatographic [22], spectroscopic [23] and electrophoretic techniques such as isoelectric focusing in polyacryl amide gel [24] and capillary isotachopheresis [25]. However, few methods have been developed for the determination of these compounds, so that the quantification of the dissolved organic carbon (DOC)

after the isolation of the HAs from the original sample is the most widely applied method for this purpose. To date, only two new methods have been developed for the determination of HAs directly following the extraction process. The first of these is based on high-performance liquid chromatography (HPLC) with fluorimetric detection [22], while the second uses capillary zone electrophoresis (CZE) with UV detection for the determination of the HA content in soil samples, after a single extraction-precipitation solubilization process and without requiring further purification steps [26]. But both methods are usually time and money consuming and require special equipments. Therefore, it is very important to create a sensitive, low cost, and an easily performed assay to rapidly detect HA.

This paper describes the application of UV/vis spectrophotometry to HA determination and comparing it with precipitation and two relevant standard methods. Although absorption spectra of HAs are usually featureless the use of absorbance ratios at 465 to 665 nm has been proposed by to characterize the degree of humification.

2. Experimental

2.1. Materials and Instruments

The Technical-grade humic acid was obtained from Sigma-Aldrich. Sodium pyrophosphate alkali solvent:

(15g sodium pyrophosphate and 7 g sodium hydroxide were dissolved in 1000ml distilled water).

The UV/Vis absorbance of samples was measured in a Cary 50 spectrophotometer (Varian, USA). The instrument was equipped with a quartz cell with a path length of 1 cm. thermostatic water bath (Raymand, Iran) was used.

2.2. Preparation of the HA standard solutions

Individual stock solution of HA (10mgL⁻¹) was prepared in the sodium pyrophosphate alkali solvent. From this solution, several standard working solutions containing 0.002-0.012 % of HA were prepared in pyrophosphate solvent too. Humic substances absorbance in UV-VIS spectral range was measured in the mixture of sodium pyrophosphate alkali solvent. For preparation calibration series each standard working solution was transferred into a spectrophotometric cell and was recorded its absorbance in two wavelengths 465

and 665 nm. As **Figure 1** shows, the correlation coefficients of calibration curve was 0.999.

2.3. Analysis of real samples

Six samples were obtained following extraction from the Zarand Coal Processing Industries (Zarand, Iran) using a simplified extraction procedure detailed below. HA was extracted by applying a simplified method adapted from the literature [27]. Soil samples, ranging from 0.1 to 0.3 g depending on the HA content, were weighted and transfer into a 250 ml-conical flask, then 150 ml of sodium pyrophosphate alkali solvent were added and the flask were put on a boiling water bath for 150 minits, shake the flask every half a hour. After suitable dilution for each sample, its absorbance was recorded in two wavelengths 465 and 665 nm.

3. Result and Discussion

3.1. Extraction optimization

In this section of the work, several variables such as extraction solvent volume, extraction temperature and time were optimized. To examine the effect of extraction solvent volume, solutions containing different volumes of the sodium pyrophosphate alkali solvent were subjected to the 0.2g sample. When the volume of extraction solvent was increased, the extraction HA concentration increased. The optimum volume of 150 mL was selected for the extraction solvent (**Figure**

of the extraction temperature is necessary. This parameter was optimized for 60 min at temperatures ranging from 25 to 100 °C on a boiling water bath. It was observed that, the extraction efficiency for the majority of the compounds increases by increasing the temperature to 95°C (**Figure 2b**). The effect of extraction time on the extraction efficiency was studied by monitoring the HA concentration as a function of time. Therefore, the sample was exposed to extraction solvent at 95°C, while the extraction time was varied from 30 to 180 min. As **Figure 2c** shows, an extraction time of 150 min was sufficient. Thus, for 0.2g sample the volume of extraction solvent, extraction temperature and time were set at 150 mL, 95°C and 150 min respectively for all subsequent experiments.

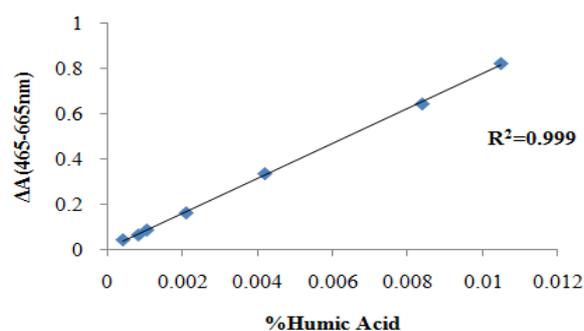
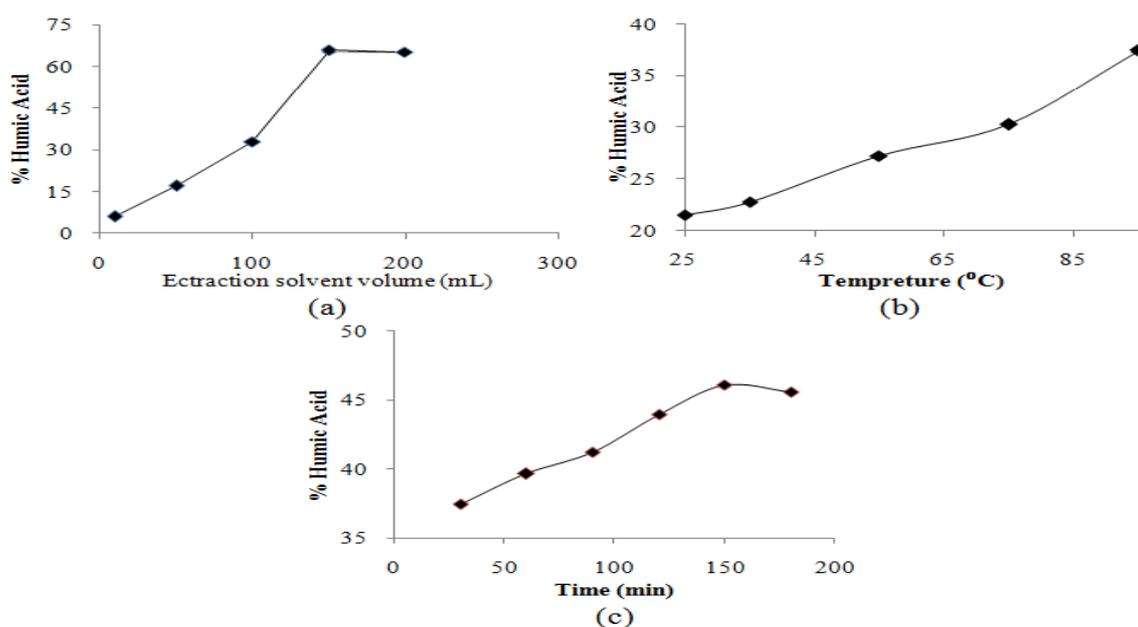


Figure 1. Calibration curve of standard working solutions containing 0.001-0.012 % of HA.



2a). The extraction temperature is an important variable in HA extraction. Therefore, optimization

Figure 2. Effect of extraction parameters on the HA extraction efficiency: (a) extraction solvent volume, (b)

extraction temperature and (c) extraction time. 3.2. Method validation

For precision validation of the method five replicate analyses were done for all samples. The standard deviations varied between 0.8 and 1.5. A comparison of the proposed method with two other methods was done.

First is based on California Department of Food and Agriculture (CDFA) method. In this procedure HAs were dissolved by treatment with 0.5N sodium hydroxide and then precipitated with hydrochloric acid. ISO 5073:2013 is the second method. It specifies volumetric methods for the determination of total humic acids and free humic acid of brown coals and lignites. In **Table 1**, the result of this method has been compared with the values reported by two other methods.

It is seen that the results for the present method are comparable or very better than others. In CDFA method, sodium hydroxide is used as extracting solvent. This compound cannot extract HAs that are metalorganic complexes but sodium pyrophosphate is a good choice for this soil type. Pyrophosphate anions complex with polyvalent cations, such as calcium, to release charged organic matter that can subsequently be dissolved in water.

4. CONCLUSION

A spectrofluorimetric method of analysis was developed for the determination of humic acid. The extraction of humic acid was done by the sodium pyrophosphate alkali solvent.

Quantitative results obtained using this method was compared to those obtained using the CDFA and standards methods.

The proposed method can provide successfully comparable concentration values relative to the routine method of humic acid analysis. Also this methodology is easier and faster than two others, which is normally used to monitor this sort of samples.

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Table 1. Comparison of the HA extraction efficiency from the proposed method with CDFA and ISO 5073:2013.

Sample number	UV/vis spectrophotometry	CDFA method	ISO 5073:2013 method
1	15.65	3.59	15.62
2	13.94	3.61	13.1
3	10.63	3.66	10.53
4	14.83	3.18	12.22
5	12.74	3.37	13.01
6	11.4	3.08	11.55

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