Quantitative Spectroscopic Determination of Humic Substances via Correlation of Optical Density to E_4/E_6 Ratio

A Summary

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Humic Substances (HS) are heterogeneous molecules that are increasingly being purchased and applied for agricultural, nutraceutical, and pharmacological purposes. For humic acids, the two tests done by state/provincial standards boards are the gravimetric humic acid precipitation test (pH 1-2) and the colorimetric test. In the gravimetric test, non-precipitating fulvic compounds are not included in the numbers, so a commercial sample that is high in fulvic acids would test out at a lower number (or zero) than samples high in humic acids.

In the colorimetric test (at 460nm) for humic acids, humic substances with a higher optical density (larger molecular weight and degree of condensation) will result in a higher reported result than that from lighter humic substances. However, it is the less absorptive low molecular weight humic substances (LMWHS) that have a stronger bioactive effect on microbes, plants and animals compared to larger humic molecules.

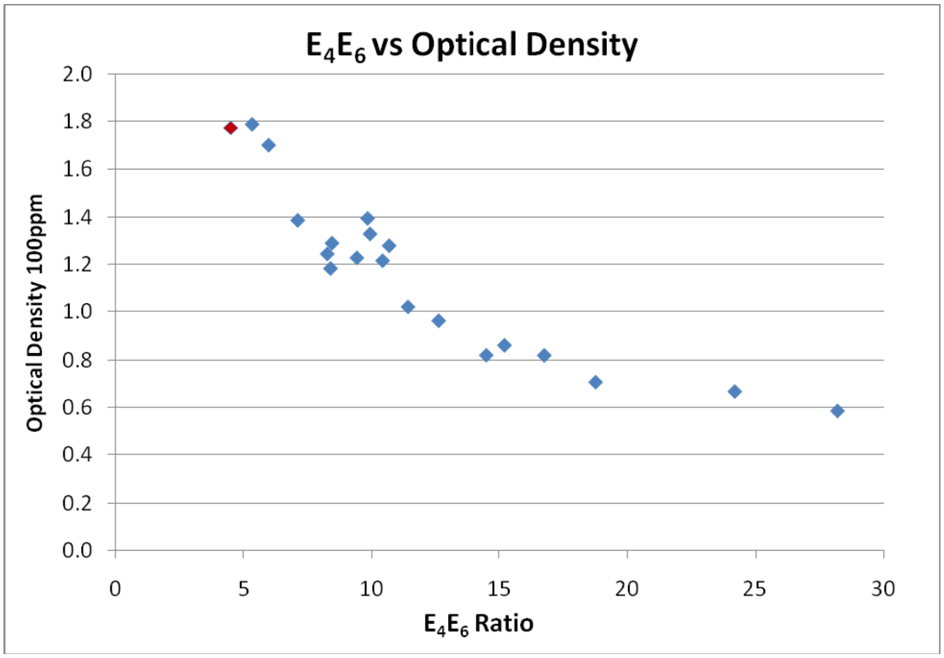
Almost all state/provincial regulatory agencies do not recognise low molecular weight humic substances (LMWHS), such as fulvic acid, as saleable compound because there is no commonly accepted quantitative test for it. LMWHS can be registered for sale in a few states as a “black box” material, if claims are supported by in-state scientific trials for each plant type, and in each climatic and soil region.

It is well known that humic acids have a higher optical density than fulvic acids. The research literature indicates that the optical density of humic substances (HS) increases with molecular size, because their degree of condensation per unit mass increases. The question for the spectroscopic quantitative analysis of HS is how do we determine the optical density of a sample?

To answer that, let’s look at another measure used by researchers to differentiate humic acids from fulvic acids: the E_4/E_6 ratio. The E_4/E_6 ratio is calculated by dividing the absorbance of a sample at 400nm by that at 600nm. Sometimes the 460/640 or 465/665 wavelengths are used. Researchers found that the E_4/E_6 ratio increases as the average molecular weight of humic substances decreases. It is easy to determine, and is substantially higher for fulvic acids (~8+) than humic acids (3 -5).

Thus as optical density increases, the E_4/E_6 ratio decreases. If this inverse relationship between optical density and E_4/E_6 ratio is continuous and well correlated, then the E_4/E_6 ratio of the material can be used to determine the approximate optical density of the sample. From that, the quantity of humic substances in the sample can be determined.

By extracting humic substances from oxidized lignite under varying extraction ratios, extraction pH, and subsequent acidification levels, this author was able to demonstrate that the properties of optical density and E_4/E_6 ratio do vary continuously. Additionally, using these 19 extracts, this author was able to show that a correlation between optical density and the E_4/E_6 ratio exists ($R^2 = 0.92$).



Once the optical density is determined, then the quantity of humic substances can be assayed by absorbance and comparison to standards varying in E_4/E_6 ratios for each type of source material (lignites, peat, organic soils, etc.). This adaptation of visible light spectroscopy to humic substances is a rapid and inexpensive method that can quantify humic substances and provide an additional measure related to their average molecular weight. If this relationship between the E_4/E_6 ratio and optical density is confirmed independently, then perhaps it can be presented as a more accurate way to determine total soluble humic content and relative molecular size.

Other measures, such as the Cation Exchange Capacity, CHO analysis etc. cannot be used because many vendors of humic substances “load up” their material with additives for many reasons, including eligibility for registration as a plant nutrient, nutraceutical or soil amendment. These include vitamins, nutrient elements/fertilizers, organic acids, amino acids, enzymes, and extracts from compost, manures, rock dusts, blue-green algae and kelp.

Together with the pH and soluble salts content of commercially available humic substances, a consumer could make an informed decision based on comparing the analysis between labels. The following is an example of a possible label:

Humic Substances Analysis

Percent Humic Substances	= 3.0%
E_4/E_6 Ratio	= 12 (higher is better for bioactivity)
Salinity	= 17 mS/cm (lower is better)
pH	= 5.5 (4.0 – 9.0 is preferred)