

Direct Measurement of the Infrared Spectra of Humic Substances in Water by Fourier Transform Infrared Spectroscopy

The infrared spectra of aqueous solutions and suspensions of humic substances were measured by Fourier transform infrared spectroscopy and were stored in digital form. The infrared spectrum of water was recorded separately and was subsequently subtracted by means of a digital computer from the spectrum of the solution or slurry samples, to yield the spectrum of the wet organic

matter itself, free of the water absorption bands. The methods for preparing the solution and slurry samples for the infrared measurements and for digitally subtracting out the water bands are described. The results obtained in this study are compared with those obtained by the conventional KBr disk method using dried organic matter samples.

Humic substances comprise a complex mixture of organic materials resulting from the decay of plant and animal residues in the soil (Kononova, 1966). They contribute to a number of important agricultural functions; for example, depending on conditions, they serve to both mobilize and immobilize heavy metal ions in the soil (Ennis and Brogan, 1961; Szalay, 1964; Kononova, 1966). They also function as soil pH buffers (Russell and Russell, 1961; Donahue, 1965) and release nitrogenous compounds slowly as they decompose (Broadbent, 1968; Bremner, 1968).

The chemistry of humic substances is extremely complicated and not well understood. In particular no fractionation scheme has yet been devised which yields discrete humic components (Dubach and Mehta, 1963; Dubach et al., 1964; Felbeck, 1965; Curvetto et al., 1974; MacCarthy and O'Conneide, 1974). Generally, humic substances are arbitrarily classified into three groups based on operational definitions (Kononova, 1966): (a) fulvic acid, which is water soluble at all pH values; (b) humic acid, which is soluble in hydroxide solution but is insoluble in acid medium; and (c) humin, which is insoluble at all pH values. [It should be pointed out that the "insolubility" of humin may be due to its strong absorption by fine-grained mineral particles in the soil. A more precise definition may be that humin is the organic matter component of soils (or sediments) which cannot be extracted with strong alkali (0.5 N NaOH).]

Numerous studies on the infrared spectra of humic substances have been reported, but virtually all of these spectra have been measured on the thoroughly dried material pressed into KBr disks. A recent report did discuss studies carried out on humic substances in D₂O medium (MacCarthy and Mark, 1975). Comparatively few infrared studies of any chemical substance have been performed in H₂O medium and particularly in the 1600-1700-cm⁻¹ region. To our knowledge no such measurements have previously been reported for humic substances. The infrared spectrum of water has two strong, broad absorption bands centered at 3400 and 1640 cm⁻¹, and a third weaker band centered at 2120 cm⁻¹. The intense bands obscure significant regions of the humic acid spectrum when working with wet samples. However, by utilizing the multiplex advantage of a rapid-scanning Fourier transform spectrometer, data can be recorded and signal-averaged to obtain very low noise spectra of aqueous samples in a reasonable time period. Furthermore, by using the computer data-handling and storage facilities associated with recent commercially available instruments, it is possible to routinely subtract out the water bands from the spectrum of a solution or slurry sample. The resulting curve is the spectrum of the solute or suspended material. The purpose of this communication is to point out the possibility of routinely measuring the infrared spectra of humic substances in H₂O medium and to demonstrate the advantages and capabilities of such methods. In particular, it was desired to extract information concealed beneath the

intense water band centered at 1640 cm⁻¹ in the spectra of the wet samples. Other than the 3100 to 3600 cm⁻¹ area, this is the most difficult region of the infrared spectrum to work with for aqueous samples.

EXPERIMENTAL SECTION

Humic Acid Samples. The humic acid was extracted from a sample of blanket-bog peat obtained at a depth of 3.5 ft at the Agricultural Institute, Peatland Experimental Station, Glenamoy, Co. Mayo, Ireland. The humic acid was extracted by the method of Ennis and Brogan (1961). Briefly, this method consists of heating the peat for 8 hr at 60° in 2% NaOH solution under a nitrogen atmosphere. The alkaline extract is separated from the fibrous material by centrifugation and decantation. It is then acidified to pH 2.0 with HCl whereupon the humic acid precipitates. Following centrifugation of the mixture the supernatant solution of fulvic acid is decanted and the humic acid is washed with distilled water and recentrifuged repeatedly until free of chloride. This organic matter had a composition of 58.5% carbon, 6.34% hydrogen, and 2.19% nitrogen (Galbraith Laboratories, Knoxville, Tenn.). A solution of sodium humate (75 mg of organic matter/ml) was prepared by titrating an aliquot of humic acid to pH 8.0. The Cu(II)-humic acid complex was prepared by washing a small quantity of humic acid in a column with excess 0.5 M CuSO₄ solution and then with excess distilled water to remove unbound Cu(II) from the system.

Introduction of Samples into Cell. All measurements were made either in a sealed CaF₂ cell (Perkin-Elmer Corp., Norwalk, Conn.) of 17.6 μm thickness or in a demountable CaF₂ cell (Barnes Engineering Co., Stamford, Conn.) having Teflon spacers (nominal thickness 15 μm). The distilled water and soluble sodium humate samples were injected directly into the sealed cell by means of a syringe. Two methods were used to introduce the slurry of humic acid into the cells. In one method a smear of the precipitated and centrifuged humic acid was applied to one plate of the cell by means of a rubber policeman. The second window was then placed on top separated by a 15-μm Teflon spacer. The two plates were then slid back and forth gently to give a sample of uniform thickness and free of air bubbles. The cell was then assembled. In the other method the humic acid slurry was shaken vigorously to give a finely dispersed viscous suspension of humic acid. This was then injected directly into the cell by means of a syringe.

Spectrometry. Spectra were recorded on a Digilab Model FTS-14 infrared Fourier transform spectrophotometer at a resolution of 4 cm⁻¹. In most cases 600 scans were signal averaged for each spectrum. In view of the relatively high transmittance of water between 3000 and 1700 cm⁻¹, both data collection and the Fourier transform were performed in double precision (32 bits per data point) in order that digitization noise does not predominate over detector noise (Griffiths, 1975). The spectrome-

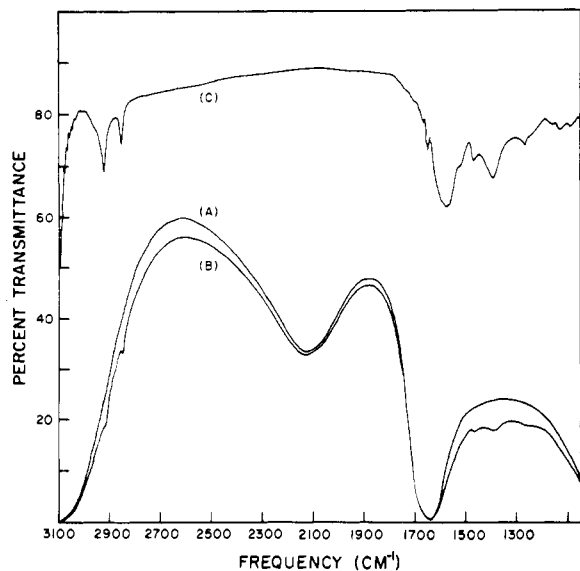


Figure 1. Infrared spectra of: (A) H₂O; (B) solution of sodium humate in H₂O; and (C) spectrum of sodium humate obtained by ratioing B with A (path length = 17.6 μ m).

ter was purged with dry air so that features due to atmospheric water vapor were not observed in ratio-recorded spectra; however, the level of CO₂ built up during the day and the CO₂ band centered at 2350 cm⁻¹ was rarely totally compensated.

Two methods of recording spectra were employed depending on the nature of the sample. For clear solutions of sodium humate contained in the 17.6 μ m sealed cell, the optical thickness of water in the solution was found to be identical with that of pure water held in the same cell. In this case, the single-beam spectrum of pure water was recorded and stored, after which the single-beam spectrum of the solution was measured using the same cell. A simple ratio of these two spectra yielded a transmittance spectrum of the solute in which the absorption features due to water were usually well compensated. When slurries of humic acid were studied in a sealed cell, the above technique could not be used since the absorbance of water in the slurries was invariably found to be different from that of the solvent alone; this effect was due to the displacement of water from the cell by the undissolved humic acid. Furthermore, when a demountable cell is used, the optical path length is not precisely reproducible. In these cases digital methods had to be used to compensate for the water bands.

In order to employ the digital compensation methods described here, it was first necessary to obtain a quantity which was directly proportional to solute (or slurry) concentration. Accordingly, ratio-recorded solution spectra were measured (referenced to the unattenuated instrumental background), converted into a linear absorbance format, and stored in the disc memory of the FTS-14 spectrometer; an absorbance spectrum of water was also measured under the same conditions and stored. The absorbance spectrum of water could then be multiplied by a factor chosen by a process of trial and error so that when the scaled spectrum of water is subtracted from the sample spectrum, no absorption features (positive or negative going) assignable to water could be observed in the spectrum. In view of the spectral structure due to humic acid in the region around 1640 cm⁻¹, the 2120-cm⁻¹ water band and the 3100-cm⁻¹ region of the spectrum were used for this purpose. The final step involved converting the resulting spectrum back to the linear transmittance format.

RESULTS AND DISCUSSION

Figure 1A shows the transmittance spectrum of a

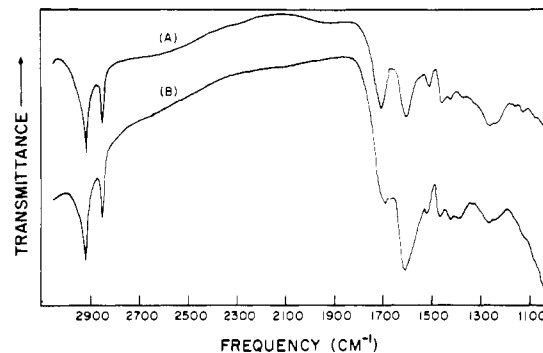


Figure 2. Infrared spectra of (A) humic acid in H₂O and (B) Cu²⁺-humate in H₂O.

17.6- μ m path length of water (vs. air) over the range 3100 to 1050 cm⁻¹. Curve B of Figure 1 shows the spectrum of an aqueous solution of sodium humate of the same path length. Curve C of Figure 1 shows the effect of ratioing 1A and 1B; it is apparent that in this case, where the sample was a clear solution held in a sealed cell, there was no need to apply the scaling routine described above. It is interesting to compare spectrum C with spectrum B (Figure 1). The aliphatic C-H stretching bands (2850 and 2920 cm⁻¹) which occur as slight humps on the shoulders of the broad O-H stretching band of water in spectrum B become well-defined bands in spectrum C. In addition, whereas spectrum B and spectrum A are virtually superimposed at ca. 1640 cm⁻¹ (the maximum of the HOH bending mode) the ratioed spectrum C reveals structure concealed beneath the water band. The bands in the 1050 to 1550 cm⁻¹ region of the humic acid spectrum which can be seen superimposed on the water background in curve B are quite distinct in the ratioed spectrum C.

The main difference between the spectrum of sodium humate (Figure 1C; pH 8.0) and that of humic acid (Figure 2A) is the occurrence of the band centered at ca. 1700 cm⁻¹ in the latter. This band is assigned to the carbonyl stretching mode of the un-ionized carboxyl group. Upon neutralization this band shifts to ca. 1570 cm⁻¹ and becomes superimposed on other bands in that region. This assignment is substantiated by the occurrence of a band at 1395 cm⁻¹ which is found in the spectrum of sodium humate but not in that of humic acid itself and is also attributable to carboxylate groups. Identical changes in the infrared spectrum of dried humic acid after neutralization have been reported by other workers using the KBr disk technique (Farmer and Morrison, 1960; Wagner and Stevenson, 1965; Theng et al., 1967; Stevenson and Goh, 1971). These changes have also been observed for humic acid samples studied in D₂O medium (MacCarthy and Mark, 1975). It is interesting that the ca. 1700 cm⁻¹ band has virtually disappeared completely by pH 8.0 indicating that this band is due almost totally to carboxyl groups with little contribution from aldehyde and ketone groups. A similar result has been reported by Wagner and Stevenson (1965) and by Theng et al. (1967) using the KBr disk method. The bands at 1610 and 1510 cm⁻¹ (Figure 2A) (pH invariant), observed also by the KBr disk technique, have previously been assigned to the carbon-carbon stretching vibrations of aromatic systems (Stevenson and Goh, 1971; Cleary-Stynes, 1968; Juo and Barber, 1969). Bands at ca. 1600 and 1510 cm⁻¹ in the spectrum of fulvic acid have recently been assigned to the bending vibrations of amines and amine hydrochlorides (MacCarthy and O'Connell, 1974). The band at 1460 cm⁻¹ is present in both humic acid and sodium humate whereas the band at 1420 cm⁻¹ which occurs in humic acid is absent from the humate. The 1420-cm⁻¹ band may arise from O-H bending vibrations of carboxylic acids, which in simple com-

pounds occur at ca. 1400 cm^{-1} (Meloan, 1963). The 1460-cm^{-1} band may be the aliphatic C-H bending vibration as previously reported at ca. 1450 cm^{-1} (Schnitzer, 1965) and ca. 1440 cm^{-1} (Juo and Barber, 1969). There are apparently two overlapping bands with maxima at 1260 and 1220 cm^{-1} in humic acid. The former is also present in sodium humate whereas the 1220-cm^{-1} band has diminished considerably in intensity on neutralization. Other workers (Schnitzer, 1965; Juo and Barber, 1969; Stevenson and Goh, 1971) have observed, using the KBr disk method, a similar disappearance of the 1220-cm^{-1} band of humic acid upon neutralization and attribute this band to the C-O stretching vibration and the O-H bending deformations of carboxyl groups. Similar effects have been observed with a peat fulvic acid (MacCarthy and O'Conneide, 1974).

In the spectrum of the Cu(II)-humic acid complex (Figure 2B) the intensity of the 1710-cm^{-1} band has decreased considerably compared to that of the humic acid. This effect suggests that carboxyl groups participate in the complexation reaction in the aqueous phase.

These preliminary results show that it is possible to readily measure the infrared spectra of humic acid and sodium humate in water using a Fourier transform spectrometer. In most spectral regions the spectra are of at least the same quality as those recorded by the KBr disk method on the thoroughly dried material. In fact, the definition of bands found in the spectra reported here is superior to many of those reported earlier which had been measured using the KBr disk method; in this respect it is well known that the spectra of samples dispersed in KBr disks may be strongly dependent on the preparation of the sample, and in particular on the particle size to which the sample is ground (Price, 1972; Smith and Potts, 1972). Compared to the KBr disk method, this technique has the advantages that very little sample preparation is necessary, spectra of solutions are reproducible, the long drying step is avoided, and the organic matter is studied in its native wet state rather than in the dried form. However, great care has to be taken over the accurate compensation of water bands, and at frequencies close to the 1640 cm^{-1} water band an increase in the noise level of the spectrum does have to be tolerated in view of the low transmittance (<1%) at the band maximum. The advantages of studying the humic material in the wet state have recently been pointed out (MacCarthy and Mark, 1975). In comparison to the method of measuring the spectra in D_2O , the use of H_2O in this study has the advantage that no elaborate precautions to eliminate atmospheric moisture are necessary. The spectral range covered in these experiments was limited by the CaF_2 transparency cutoff at ca. 1000 cm^{-1} , and silver bromide windows will be used in future experiments to avoid this limitation.

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