Infrared Spectra of Humic Acid and Metal-Humates Precipitated from Groundwater

William A. Laride
Science, Math and Technology Department, Philippine Science High School-Western Visayas
Jaro, Iloilo City, Philippines 5000
theridewill@yahoo.com

Abstract—Brown groundwater from Santa Barbara, Iloilo, Philippines was treated with concentrated hydrochloric acid resulting in the precipitation of dark brown substances. The precipitate was analysed under an FT-IR spectrophotometer with absorption wavelengths revealing the presence of alkene, aromatic, carbonyl, carboxyl, hydroxyl, phenol and polysaccharide functional groups. Comparison of the absorption wavelengths with previous studies confirmed that the precipitate was humic acid (HA). The same water sample was also saturated with selected metal ions: Fe$^{3+}$, Cr$^{3+}$, Ni$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$, resulting in the precipitation of light to dark brown substances. When subjected to FT-IR analysis, the precipitate showed positive results for the formation of metal complexes with humic acid. The absorption wavelength revealed that the active binding sites for Fe$^{3+}$, Cr$^{3+}$, Ni$^{2+}$ and Cu$^{2+}$ metal-humate complexes are the oxygen atoms of the humic acid carbonyl and carboxylic acid functional groups. This was indicated by the reduction of the distinctive COO$^-$ symmetric stretch from 1712 cm$^{-1}$ to 1600 cm$^{-1}$ in humic acid and the appearance of COO$^-$ antisymmetric stretching at 1400 cm$^{-1}$ in the metal–humates. A similar lowering of the COO$^-$ symmetric stretch and the appearance of the COO$^-$ antisymmetric stretch were also observed for the Cd$^{2+}$ and Pb$^{2+}$ metal-humate complexes. However, it was determined that the binding sites for Cd$^{2+}$ and Pb$^{2+}$ were limited to the carboxyl functional group, because the absorption band relating to the carbonyl oxygen (C=O) was still observed in their IR spectra.

Keywords—Humic Acid; Humic Substances; Infrared Spectroscopy; Metal Humates

I. INTRODUCTION

The town of Santa Barbara is a municipality located in the central part of the province of Iloilo, Philippines. Deep wells dug in this municipality produce groundwater which varies in color from pale yellow to dark brown, depending on the location of the well. There has been no recorded toxicity resulting from drinking the colored groundwater, so many residents of the municipality have been using this readily available colored water for drinking and cooking without any pre-treatment procedures; many even believe that the colored water can provide a cure to many diseases.

According to several studies, the primary cause of coloration in water and its associated toxicity is the presence of dissolved organic matter, specifically humic acids [4, 9]. Humic acids are naturally-produced organic compounds formed by the chemical and biological transformations of animal and plant residues; they are found in significant concentrations in streams and groundwater [1]. Other natural organic substances such as fulvic acid, tannins and lignins may also contribute to water coloration [8].

Humic acids are characterized as brown amorphous substances consisting of polymeric hydroxycarboxylic acids with phenolic, alchoholic and carbonyl groups in addition to aromatic rings [1, 4, 12, 15, 18].

As natural organic ligands, humic acids and humic substances play a crucial role in speciation, transport and deposition of a variety of substances such as metal ions and lipophilic compounds. Their capacity to form complexes with metal cations

Fig. 1 Model structure of humic acid; R can be alkyl, aryl or aralkyl [18]
contributes to the physicochemical properties of trace metal species. Having many available binding sites such as carboxyl (–COOH), amine (–NH2), hydroxyl (–OH), and phenol (Ar–OH) functional groups, humic acid limits the bioavailability of metals in the natural environment [2, 11]. These humic–metal interactions are therefore representing a serious threat to the environment, living resources, ecological systems and human health [2, 19].

In this study, the substance responsible for the coloration of the Santa Barbara groundwater was precipitated using hydrochloric acid and selected cations. The precipitates were then characterized for functional group identification using infrared spectroscopy. Absorption spectra were then compared to results from previous studies.

II. METHODOLOGY

Water samples were collected from private deep wells located along Montinola Street, Poblacion, Santa Barbara, Iloilo, Philippines. The deep well located in this area was selected as a sampling site, since wells in this area produce very dark groundwater [18].

A. Precipitation of Humic Acid

For the precipitation of humic acid, a 1.0 L water sample was acidified with concentrated hydrochloric acid (Riedel-de Haen A.R.) to pH < 2.0. The mixture was stirred thoroughly for 15 minutes and left overnight to allow the precipitate to settle completely. The supernatant was siphoned off, and the remaining mixture was centrifuged at 1000rpm for 10 minutes. The precipitate was then filtered (Whatman Filter Paper No. 1, 11 μm-pore size) and washed five times with 0.010 M HCl solution to completely protonate the humic acid and remove any soluble organic compounds that adsorbed to the precipitate. The precipitate was then oven dried at 45°C.

B. Precipitation of Metal-humate Salts

The following nitrate salts were used for the precipitation of metal humates: Ni(NO3)2•6H2O (Merck), Pb(NO3)2 (Univar APS Finechem), Cu(NO3)2•3H2O (FlukaChemica), Cd(NO3)2•4H2O (Merck), Cr(NO3)3•9H2O (FlukaChemica), and Fe(NO3)3•9H2O (Mallinckrodt).

In order to precipitate the metal humate, a measured amount of nitrate salt, equivalent to a 2.0 M concentration of the cation was slowly added into the 1.0 L water sample with constant stirring. The disappearance of the original brown color of the water sample indicated the complete precipitation of the metal humate. The mixture was left overnight to allow the precipitate to settle completely before the supernatant was siphoned off. The remaining concentrated mixture was centrifuged at 1000rpm for 10 minutes, filtered (Whatman Filter Paper No. 1, 11 μm-pore size), and then washed five times with deionized water. The precipitate was then oven dried at 45oC.

Infrared absorbance of the precipitate was measured following the potassium bromide (KBr) pellet method using the Avatar 330 FT-IR Thermo Nicolet spectrophotometer. Approximately 1.0 to 2.0 mg of the dried precipitate was mixed with 200 to 250 mg of high purity standard grade KBr powder. The mixture was then finely pulverized and placed in a 13mm diameter pellet-forming die. A force was applied for several seconds until a transparent pellet was formed. FT-IR absorbance of the KBr sample was measured over an interval of 4000 to 225 cm⁻¹. Pure KBr powder was used as background to correct for infrared light scattering losses in the pellet and for moisture adsorbed on the KBr.

III. RESULTS AND DISCUSSION

A. FT-IR Spectrum of Humic Acid

As shown in Fig. 2, the FT-IR absorbance of the humic acid precipitate indicated major absorptions at 3395cm⁻¹ (broad), 2869cm⁻¹ (weak), 1712cm⁻¹ (medium), 1618cm⁻¹ (medium), 1507cm⁻¹ (weak), 1239cm⁻¹ (medium), 1091cm⁻¹ (weak), 609cm⁻¹ (weak) and 588cm⁻¹ (weak).

The wavelength of absorptions are interpreted as follows: (a) the strong and broad absorption band at 3396 cm⁻¹ band corresponds to the hydrogen-bonded hydroxyl groups; (b) the weak absorption band at 2869 cm⁻¹ is attributed to the asymmetric and symmetric stretching vibrations of aliphatic C–H bonds in methyl and methylene units; (c) the band at 1712 cm⁻¹ is attributed to C=O stretching of the carboxyl and carbonyl groups; (d) the 1618 cm⁻¹ band is attributed to the aromatic C=C double bands conjugated with C=O and COO⁻; (e) the absorption at 1507 cm⁻¹ corresponds to the amide band due to peptide linkages; (f) the absorption band at 1239 cm⁻¹ is attributed to the C–O stretching vibration and O–H bending deformations, due mainly to carboxyl groups; (g) the weak band at 1035 cm⁻¹ is attributed to alcoholic and polysaccharide C–O stretch, O–H deformation and Si–OH bend in silicate impurities [15].
These interpretations strongly suggest that the precipitate obtained from the water sample is humic acid, corroborated by the results of previous studies. In a study involving the characterization of humic acid isolated from polymeric and organic materials in a polluted river, absorption spectra were similarly measured at 3450, 2950, 1720-1650, 1540, 1450-1370 and 1300-1000 cm\(^{-1}\) [5]. Infrared spectra of humic acid isolated from soil and water sources reported by the International Humic Acid Substances Society (IHSS) in 2008 also produced similar results. Similar wavelengths of absorptions were also reported for trace humic acid isolated from tap water [3].

**TABLE 1 SUMMARY OF MAJOR WAVELENGTH OF IR ABSORPTION FOR HUMIC ACID AND METALHUMATES**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wavelength of IR Absorption (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>3396(b)*, 2869(w), 1712(m), 1618(m), 1507(w), 1239(m), 1091(w), 609(w), 524(w)</td>
</tr>
<tr>
<td>Fe(^{3+})Humate</td>
<td>3325(b), 1604(m), 1389(m), 1040(w), 687(w)</td>
</tr>
<tr>
<td>Cr(^{3+})Humate</td>
<td>3394(b), 1609(m), 1408(m), 1128(m), 1063(m), 837(w), 500(w)</td>
</tr>
<tr>
<td>Ni(^{2+})Humate</td>
<td>3392(b), 1601(s), 1394(s), 1040(w), 677(w)</td>
</tr>
<tr>
<td>Cu(^{2+})Humate</td>
<td>3401(b), 1600(w), 1412(m), 1100(w), 832(m)</td>
</tr>
<tr>
<td>Cd(^{2+})Humate</td>
<td>3435(b), 1735(w), 1615(w), 1408(m), 1165(w), 1070(w), 859(s), 721(m)</td>
</tr>
<tr>
<td>Pb(^{2+})Humate</td>
<td>3428(b), 1729(m), 1626(m), 1387(s), 1049(m), 836(m), 676(m), 560(w)</td>
</tr>
</tbody>
</table>

*b* = broad, *m* = medium, *s* = strong, *w* = weak

**B. FT-IR Spectra of Metal-humates**

Table 1 displays a summary of the absorption wavelengths of the metal-humate precipitates. In general, the IR spectra of the metal humates are characterized by their broad absorptions at 3400 cm\(^{-1}\), which is due to the formation of aqua-complexes of metals with humic acid, and the additional interfering absorption of the O-H in water molecules. The absorptions at 1100–1040 cm\(^{-1}\) are attributed to the inorganic orthophosphates, P-O linkage, P=O with hydrogen bonded –OH in phosphate group, alcoholic and polysaccharide C-O stretch, O-H deformation and Si-OH impurities [12, 15]. Another absorption band also appeared at 830 – 850 cm\(^{-1}\) which, according to some authors, is due to the out-of-plane bend of the aromatic rings or the out-of-plane bending of the amine group [10].

Absorption spectra for the metalhumates of Fe\(^{3+}\), Cr\(^{3+}\), Ni\(^{2+}\)and Cu\(^{2+}\) are presented in Figs. 3 and 4. Their IR spectra generally exhibit lower wavelengths of absorptions (1600 – 1609 cm\(^{-1}\)) for the COO\(^{-}\) symmetric stretching and strong absorptions for the COO\(^{-}\)antisymmetric stretching from 1389 – 1412 cm\(^{-1}\).

Several authors have suggested that humic acid forms complexes with metals through the carboxylic acid functional group. This interaction can be concluded from the reduction of the absorption band of the COO\(^{-}\) symmetric stretching to lower wavelength and the appearance of the COO\(^{-}\)antisymmetric stretching [2, 12, 15, 19]. Other authors have also explained the occurrence of metals forming complexes with carboxylic acid, resulting in a lowering of the COO\(^{-}\) absorption band due to the resonance character of the parent carboxylic acid and the formation of electrovalent bonds between the metal and carboxyl functional groups [10].

Fig. 2 FT-IR spectra of humic acid
The disappearance of the absorption at 1712 cm\(^{-1}\) originally present in the humic acid spectra also suggests that most of the carboxylic acid functional groups were deprotonated to COO\(^-\) during complex formation, perhaps demonstrating that all carboxylic acid functional groups participated in the complexation process. These results further strengthen the theory that the complexation of metals with humic acid is primarily achieved through the carboxyl functional groups.

The absorption wavelengths of the Cd\(^{2+}\) and Pb\(^{2+}\) humates also showed a lowering of the absorption band of the COO\(^-\) symmetric stretching (1615 – 1626 cm\(^{-1}\)) and the appearance of the COO\(^-\) antisymmetric stretching (1386 – 1408 cm\(^{-1}\)), as shown in Fig. 5. These reductions of the COO\(^-\) symmetric absorption band and appearances of the COO\(^-\) antisymmetric stretching indicate that Pb\(^{2+}\) and Cd\(^{2+}\) cations also formed complexes with humic acid.

Both the Cd\(^{2+}\) and Pb\(^{2+}\) metalhumates still showed weak absorption bands at 1735 cm\(^{-1}\) and 1728 cm\(^{-1}\), respectively. These absorptions, characteristic of the carbonyl (C=O) functional group, imply that the carbonyl functional group of humic acid may not have participated in complex formation with Cd\(^{2+}\) and Pb\(^{2+}\) cations; thus, it can be concluded that the carbonyl oxygen atoms in humic acid were not involved in the coordination with Cd\(^{2+}\) and Pb\(^{2+}\). Furthermore, the reductions of the COO\(^-\) symmetric absorption band and appearances of the COO\(^-\) antisymmetric stretching demonstrated that Cd\(^{2+}\) and Pb\(^{2+}\) were bound through the carboxyl and alcohol functional groups.
IV. CONCLUSIONS

The results of this study confirmed the presence of humic acid, which primarily caused the coloration in the groundwater of Santa Barbara, Iloilo, Philippines. Infrared spectroscopy also demonstrated that Fe$^{3+}$, Cr$^{3+}$, Ni$^{2+}$ and Cu$^{2+}$ ions form complexes with humic acid through the carbonyl and carboxylic acid functional groups. For Cd$^{2+}$ and Pb$^{2+}$ ions, complexation with humic acid is achieved through the carboxyl and perhaps the alcohol moieties.

ACKNOWLEDGMENT

The author would like to thank the University of the Philippines-Visayas, Miag-ao, Iloilo for allowing the researcher to conduct the FT-IR analysis of the samples and the Philippine Science High School-Western Visayas Research laboratory.

REFERENCES


William A. Laride was born in Ormoc City, Leyte (Philippines) on May 2, 1982. He earned his Master’s degree in Chemistry from the University of the Philippines in the Visayas, Miag-ao, Iloilo in 2010. He finished his Bachelor’s degree major in Physics and Chemistry from the University of San Carlos, Cebu City in 2003.

He is currently the Science, Math and Technology (SMT) Department Head of the Philippine Science High School Western Visayas in Iloilo City, Philippines where he teaches Physics, Chemistry, Science Research and Earth Science subjects.

He is one of the authors of iLEARN SCIENCE: An Activity-based Book used in selected public and private high schools in the Philippines which was published in 2014 by Innovative Educational Materials, Inc. His major interests of study are on chemical hydrology and environmental studies.