Solvent Systems for the Isolation of Organic Components from Soils

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ABSTRACT

The classical methods for the isolation of soil organic matter (SOM) components use aqueous base or neutral salt solutions, and combinations of aqueous base and pyrophosphate. Organic solvents have been rarely used, largely because of difficulties in recovering solutes. This review provides the relevant chemistry of aqueous and organic chemicals relevant to extracting and fractionating the components of SOM that are bound and are not bound by the soil mineral surfaces. Uses of aqueous media to separate the SOM components on the basis of charge density differences are described. Combinations of aqueous base and urea enhance the isolations of the SOM components that have a high degree of polarity [humic and fulvic acids (HAs and FAs), polysaccharides, peptides]. However, the nature of the associations between the solute molecules has prevented the isolation of any purified SOM component. Properties are listed of organic solvents that have potential for the isolation of SOM components, and novel procedures are described for the recovery of the SOM components dissolved in organic solvents. The procedures include the uses of resins with varying degrees of polarity and the recovery of the SOM components in aqueous media. No satisfactory solvent system has been found that can isolate all of the humin materials sorbed by the inorganic colloids. However, procedures are outlined that can extract much of the material classified as humin in discussions of SOM. Because the polar components of the SOM can now be removed, it is likely that the compositions of the nonpolar humins strongly held by the mineral colloids will be resolved using procedures such as pyrolysis–mass spectrometry.

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Stevenson (1994) considered SOM to consist of all of the organic materials in soils. Hayes and Swift (1978) regarded the complete soil organic fraction to be made up of live organisms and their partly decomposed and completely transformed remains, as well as those of plants, but they considered SOM as a more specific term for the heterogeneous mixture of nonliving components resulting from the biological and chemical transformations of organic debris. The transformations are known as humification processes, and the final products as humus. This is similar to Stevenson’s (1994) definition that regarded humus as the total of organic compounds in soil which have some resistance to biodegradation but are exclusive of decaying plant and animal tissues, their partial decomposition products, and the soil biomass.

S

Soil organic matter or humus is perhaps the most complex material in nature. Most emphasis has focused on the humic substances (HSs). In the classical definitions, HSs have been regarded as a series of relatively high-molecular-weight dark-colored substances formed by secondary synthesis reactions (Stevenson, 1994), and as a “category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory” (Aiken et al., 1985). In classical definitions, unaltered biological molecular components of plants and animals, though components of humus, would not be considered to be HSs. Thus, unaltered flavonoids, tannins, terpenes, sporopollenins, and large aliphatic molecules such as algaenans, cutans, suberans (Derenne and Largeau, 2001), though components of humus, would not be regarded as HSs. There is increasing emphasis on the presence of charcoal, or char, from the burning of vegetation (Skjemstad et al., 1996; Swift, 2001). Although some charcoal compounds fall within the operational definitions of HSs, based on solubilities data, these cannot be considered to be true HSs.

The operational definitions of SOM fractions based on solubilities were first introduced by Sprengel (1837). Soil scientists define HAs as humus materials that are soluble in aqueous alkaline solutions but precipitate when the pH is adjusted to 1 (water scientists consider the precipitates at pH 2 to be HAs). There are, of course, many nonhumic components (e.g., some proteins) that are precipitated under similar conditions, and so the precipitates at pH 1 might be considered as the HA fraction. Fulvic acids remain in solution after the aqueous alkaline extracts are acidified. These materials are best referred to as the FA fraction. The FA standards of the International Humic Substances Society (IHSS) were processed by passing the acidified solutions on to XAD-8 resin [(poly)methylmethacrylate], which retain the FAs and allow polar components to pass through the resin. The FAs are recovered in 0.1 M NaOH, and H⁵⁺–exchanged by passing through IR-120 (styrenedivinylbenzene with sulfonic acid functionality, H⁵⁺–exchanged) resin, followed by freeze drying. Polar, nonhumic components, such as various peptides, saccharides, and some small organic chemicals, are not retained on XAD-8 but may be held by XAD-4 (styrenedivinylbenzene) resin.

To some extent, polar contaminants may also be removed from HAs using XAD-8 technology. Häusler and Hayes (1996) removed some of the saccharide and peptide components from HAs in a Sapric Histosol using acidified dimethylsulfoxide [DMSO + 12% (v/v) of 12 M HCl]. The acidified solution was passed on to a XAD-8 resin which retained the HAs. The DMSO was washed through with acidified water, then with distilled water, and the HAs were recovered in 0.1 M NaOH. These were then H⁵⁺–exchanged by passing through IR-120, and then freeze dried.

Abbreviations: DMF, N,N-dimethylformamide; DMSO, dimethylsulfoxide; DOSY, diffusion ordered spectroscopy; EDA, ethylenediamine; EF, electrostatic factor; HA, humic acid; HS, humic substance; IHSS, International Humic Substances Society; NMR, nuclear magnetic resonance; SOM, soil organic matter.
Humin is defined as the HSs that are insoluble in aqueous media. There are several biological molecules that would meet the humin solubility criteria but, on the basis of the definitions outlined, do not satisfy the criteria for HSs. Thus, in general, humin is SOM components that are strongly adsorbed to the soil mineral colloids and are not extracted in aqueous acids or bases. In organic soils, or Histosols, the humin would be composed of the components that bear no morphological resemblances to their parent organic structures.

The importance of SOM to agricultural productivity has long been recognized (Hayes and Swift, 1978; Stevenson, 1994; Clapp et al., 2005). More recently, there is emphasis on its importance in terms of global warming effects. Soil management is important for considerations of the roles of soils as sources and sinks of carbon (Lal et al., 1995). Hence there is increased interest to study the chemistry of SOM in relation to its abilities to resist biological transformations.

Significant advances have been made in recent times in understanding aspects of the compositions of SOM fractions. However, the fractions are still largely operationally defined based on solubilities in aqueous basic and acidic media, and such media, as used in conventional extractions, are unlikely to isolate >50% of the SOM components. Thus, there is a need for more complete isolation, more careful fractionation, and more complete descriptions of the fractions to advance our awareness of the compositions of SOM components and of their stabilities and mechanisms of action in the soil environment.

**ASPECTS OF THE COMPOSITIONS OF THE COMPONENTS OF SOIL ORGANIC MATTER**

Solvent properties are important for considerations of the isolation of SOM components. The classical definitions assume that HSs are macromolecular and polyelectrolytes. Cameron et al. (1972) and Swift (1989) presented evidence that was convincing at the time to support the concept of humic macromolecules that assume random coil-type conformations in solution. Wershaw (1986, 1994, 1999) introduced the concept of HSs as ordered aggregates of amphiphiles (i.e., compounds with hydrophobic stretches as well as charged or polar centers) composed mainly of altered plant structures, held together by hydrophobic bonding, charge transfer, and hydrogen bonding mechanisms, and exhibiting acidic functionality. This suggests pseudomicellar arrangements (Wershaw, 1994; von Wandruszka, 1998). Piccolo et al. (2001) considered HSs to be molecular associations formed by the self assembly of supramolecular molecules arising from “the affinities of certain molecules in aqueous solutions.”

Although Engebretson and von Wandruszka (1997) did not dismiss the concept of macromolecularity, they concluded that the quenching of pyrene fluorescence by Br\(^-\) ions indicated that humic molecules could spontaneously aggregate through hydrophobic associations. They suggested that pyrene reacted with hydrophobic sites within the humic structures and was protected by the SOM from the Br\(^-\). On the basis of the procedures used in earlier work of Piccolo, and the suppression of pyrene fluorescence by Br\(^-\), Kenworthy and Hayes (1997) showed that pyrene was best protected by the most hydrophobic HA fractions. Protection was lost when dilute ethanoic acid followed by base was added to the medium and the hydrophobic associations that protected the pyrene were disrupted.

Simpson (2002) and Simpson et al. (2002) used diffusion ordered spectroscopy (DOSY) nuclear magnetic resonance (NMR) for studies of the compositions of HA and FA molecules. The DOSY technique separates structures on the basis of diffusion properties, and the spectrum directly correlates diffusion coefficients to proton chemical shift in a 2-D plot. In a pure aqueous solvent, Simpson (2002) found that all the components of the IHSS Standard peat HA had a single diffusion coefficient, and that would suggest that a single molecular structure, or a strongly associated group of molecules was being observed. However, when a trace amount of ethanoic acid was added, separate diffusivities were observed for lignin-derived, carbohydrate, peptide, and hydrocarbon-derived structures (Fig. 1). Ethanoic acid, as used by Piccolo et al. (2001), is known to disaggregate protein structures, and the separate diffusivities of the component molecules, as seen in Fig. 1, can be regarded as evidence that molecular associations, and not covalently linked macromolecular structures, compose HSs. This led Simpson et al. (2002) to depict HAs as associations of altered (oxidized) lignin structures, peptides, saccharides, waxes, long chain fatty acids, etc. At high concentrations, the aggregates displayed diffusivities consistent with those for large proteins (>66 000 Da), but...
after disaggregation with low concentrations of acids, the diffusivities observed were consistent for molecules in the range of 200 to 2500 Da. The components in the FAs were shown not to aggregate strongly, and tended to be smaller than the HAs.

Hayes (1985) and Clapp et al. (2005) have discussed the forces that hold molecules together. These include interparticle forces, such as multipole interactions, induction forces, dipole–induced dipole interactions, London or van der Waals forces, and hydrogen bonding interactions. Dipole–dipole interactions are strongest when the dipoles are aligned head to tail, and so the strongest H bonds are formed when the two electronegative atoms and the H are colinear. Hydrogen bonding energies can amount to 20 kJ mol⁻¹. Thus, solvents for SOM components must be able to overcome the forces holding the molecules in their associations.

PROPERTIES OF SOLVENT SYSTEMS

Whitehead and Tinsley (1964) have listed some relevant properties for solvents to be effective for the separation of HSs from soil, and Stevenson (1994) has suggested criteria for the ideal extraction methods. These concepts are summarized by Clapp et al. (2005).

Aqueous Systems

Water is the major solvent used for the isolation of SOM components. Water, A Comprehensive Treatise, edited by Franks (1975), and Water (Franks, 1983) give details of relevant structures and properties of water. On the basis of these treatises, Hayes (1985) and Clapp et al. (2005) have summarized aspects of water structure that are important for the solvation of SOM components. Theoretical calculations suggest that the most stable form of interaction between water molecules involves the linear H bond. Hydrogen bonding gives considerable structure to liquid water, and liquid water can be considered to be composed of clusters of various sizes in equilibrium with each other, and with free unstructured water molecules. When soluble ions or highly polar compounds are introduced into water, solvation occurs because of associations, through electrostatic effects, between the solvent and the solute. The ordering of the solvent around the solute gives a decrease in entropy, but this is countered by the increased entropy from the breakdown of the water cluster structure. However, when nonpolar molecules enter spaces between water clusters they associate with each other (rather than with the water molecules) in the void spaces. The clusters grow at the expense of free water and the energy and entropy decrease as free water H bonds in the clusters. The associations of nonpolar solutes gives rise to the concept of hydrophobic bonding in the presence of water (Franks, 1975, 1983).

Organic Solvent Systems

Organic molecules will dissolve a solute when the attraction forces between the solvent and solute molecules are similar. The important parameters for consideration of an organic solvent for SOM include the basicity of the system, the ability to make and to break H bonds, the relative permittivity (Kᵣ), and the dipole moment (μ) of the solvent. The boiling point, viscosity, and density are peripheral properties that do not necessarily affect the isolation processes, but these are important in considering the recovery of the solute from the solvent. Relevant values for these properties for water and the organic solvents discussed in this article are given in Table 1.

The concept of the one component solubility parameter (δ) is attributed to Hildebrand and co-workers (Hildebrand and Scott, 1951, 1962; Hildebrand et al., 1970). Solubility parameters are useful for predicting the solubility of a solute in a solvent. A good solvent for a nonelectrolyte solute will have, for example, a solubility parameter (δ) value close to that of the solute. A mixture of two solvents, one with a δ value above, and another with a value below that of the solute can provide a better solvent system for the solute than any one of the solvents alone. Hayes (1985) has outlined aspects of the concepts relevant to the present topic.

The one component solubility parameter is appropriate in specific interactions for solutions lacking polarity. To some extent, the one component parameter is replaced by multicomponent solubility parameters which give values for the different interaction forces, such as the dispersion (δ_d) and the polar forces (δ_p), and hydrogen bonding (δ_h). Table 2 gives relevant solubility parameter data for solvents discussed in the present study. A review by Barton (1975) provides the information needed to familiarize the reader with the subject.

Table 1. Boiling point (BP), viscosity (η), density (ρ), relative permittivity (K_r), electrostatic factor (EF), and base parameter (pK_HB) values for solvents used for data in Table 3.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>BP (°C)</th>
<th>η (cP)</th>
<th>ρ (g mL⁻¹)</th>
<th>K_r</th>
<th>EF</th>
<th>pK_{H_B}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>82</td>
<td>0.34</td>
<td>0.78</td>
<td>37.5</td>
<td>144.0</td>
<td>1.05</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>0.89</td>
<td>1.00</td>
<td>78.5</td>
<td>144.44</td>
<td>–</td>
</tr>
<tr>
<td>Acetone</td>
<td>56</td>
<td>0.30</td>
<td>0.78</td>
<td>20.7</td>
<td>59.62</td>
<td>1.18</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78</td>
<td>1.08</td>
<td>0.79</td>
<td>24.3</td>
<td>40.82</td>
<td>–</td>
</tr>
<tr>
<td>Pyridine</td>
<td>115</td>
<td>0.88</td>
<td>0.98</td>
<td>12.4</td>
<td>3.4</td>
<td>–</td>
</tr>
<tr>
<td>Formamide</td>
<td>210</td>
<td>3.3</td>
<td>1.13</td>
<td>109.5</td>
<td>360.0</td>
<td>–</td>
</tr>
<tr>
<td>N,N-dimethylformamide</td>
<td>153</td>
<td>0.80</td>
<td>1.13</td>
<td>36.7</td>
<td>140.2</td>
<td>2.06</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>189</td>
<td>2.00</td>
<td>1.10</td>
<td>46.6</td>
<td>209.2</td>
<td>2.53</td>
</tr>
</tbody>
</table>

† Data from Taft et al. (1969), Barton (1975), Dack (1976), and Snyder (1978).
§ –, not available.

Table 2. Data for the Hildebrand (δ), total (δ_h), dispersive (δ_d), polar (δ_p), and hydrogen bonding (δ_h) parameters,† and proton donor (δ_d) and proton acceptor (δ_a) parameters,‡ of solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>δ_h</th>
<th>δ_d</th>
<th>δ_p</th>
<th>δ_h</th>
<th>δ_a</th>
<th>δ_h</th>
<th>δ_d</th>
<th>δ_p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>7.0</td>
<td>7.1</td>
<td>7.1</td>
<td>0.0</td>
<td>0.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acetone</td>
<td>9.9</td>
<td>9.8</td>
<td>7.6</td>
<td>5.1</td>
<td>3.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ethanol</td>
<td>12.7</td>
<td>13.0</td>
<td>7.7</td>
<td>4.3</td>
<td>9.5</td>
<td>6.9</td>
<td>6.9</td>
<td>6.9</td>
</tr>
<tr>
<td>Pyridine</td>
<td>10.7</td>
<td>10.7</td>
<td>9.3</td>
<td>4.3</td>
<td>2.9</td>
<td>–</td>
<td>–</td>
<td>4.9</td>
</tr>
<tr>
<td>Formamide</td>
<td>19.2</td>
<td>17.9</td>
<td>8.4</td>
<td>12.8</td>
<td>9.3</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>N,N-dimethylformamide</td>
<td>12.1</td>
<td>12.1</td>
<td>8.5</td>
<td>6.7</td>
<td>8.5</td>
<td>–</td>
<td>–</td>
<td>4.6</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>12.0</td>
<td>13.0</td>
<td>9.0</td>
<td>8.0</td>
<td>5.0</td>
<td>–</td>
<td>–</td>
<td>5.2</td>
</tr>
</tbody>
</table>

† From Barton (1975).
‡ From Snyder (1978).
§ L = large.
He pointed out that the data become empirical when multicomponent parameters are used, and thus it is important to use sets of data that are internally consistent. Keller et al. (1971) and Karger et al. (1976) further divided the hydrogen bonding parameter into the proton donor (δₗ), or acid, and the proton acceptor (δₜ), or base parameters. The data for these parameters, listed in Table 2, are from Snyder (1978) and not from the same source as those compiled by Barton (1975) and given in the same Table. Hence, values for δₜ should not be compared directly with those for δₗ and δₜ.

**SEPARATION OF SOM COMPONENTS IN AQUEOUS MEDIA**

Hydrogen⁺–exchanged acidic functional groups in SOM components give rise to H bonding between the component molecules. Thus, water is a poor solvent for structures in which extensive H bonding is involved. However, H⁺–exchanged HAs will dissolve in aqueous 5 M urea, which is a powerful hydrogen bond breaker, but not in a weaker 10% urea solution (Hayes, 1985). As the pH of H⁺–exchanged systems in aqueous media is raised, the protons dissociate and water molecules cluster around the negatively charged conjugate bases, and the structures dissolve. When the negative charges are neutralized by divalent and polyvalent cations, a pseudo cross-linking occurs in which the cations bridge charges within and between the molecules. Thus, divalent and polyvalent cation-exchanged acidic SOM components are not dissolved in water. Bremner and Lees (1949) found that bycomplexing the divalent and polyvalent metals neutralizing the charges in SOM, using dilute, neutralized sodium pyrophosphate solution (0.1 M Na₄P₂O₇), significant dissolution of the organic matter (OM) took place. Water was the solvent. In a separate study, Bremner (1950) showed that the uptake of O₂ by SOM was related to the pH of the extractant. Thus, O₂ uptake by 0.5 M NaOH was about 18 times greater than by the same system adjusted to pH 10.5, and uptake by pyrophosphate neutralized to pH 7 was negligible. Similar oxidation effects were observed for aqueous basic media by Swift and Posner (1971). Thus, neutral pyrophosphate solution has been the solvent of choice in many studies, and also mixtures of pyrophosphate and of NaOH are used, especially when the soils are not H⁺–exchanged before extraction with base. However, because weakly dissociated acidic functional groups are not ionized at neutral pH values, only the more highly oxidized or transformed humic materials are dissolved in neutral pyrophosphate. Choudhri and Stevenson (1957) showed that oxidation damage in basic media could be limited when extractions are performed in an atmosphere of N₂ gas and in the presence of stannous chloride as an antioxidant. Because of their efficiencies as extractants, aqueous NaOH or KOH have been widely used since Achard (1786) introduced aqueous KOH for the extraction of HSS from peat. The amounts extracted are significantly improved when the soils are H⁺–exchanged (using 1 M HCl) before extraction with base. Swift (1996) has outlined the IHSS procedure for extraction of HSSs from soil using aqueous NaOH in an atmosphere of dinitrogen gas.

As mentioned, classical solubility procedures for HAs and FAs are operationally defined and the fractions will contain codissolved or coprecipitated biological molecules that are not regarded as HA and FA. Hayes et al. (1996) and Hayes (1996) used aqueous solvent systems to fractionate SOM components at different pH values. The soil was exhaustively extracted with 0.1 M pyrophosphate (pH 7) until the extracts had negligible color, then exhaustively extracted sequentially with 0.1 M pyrophosphate (pH 10.6), and finally with 0.1 M NaOH + 0.1 M pyrophosphate (pH 12.6). The objective was to isolate organic fractions, and HSSs in particular, on the basis of charge density differences. Humic and FAs were isolated by the IHSS procedure (Swift, 1996), and by a modification that used XAD-8 and XAD-4 resins in sequence (Malcolm and MacCarthy, 1992). In the latter procedure, the aqueous extracts were diluted to a concentration <15 ppm, the pH of the solution was adjusted to 2, and then pumped slowly (~40 mL min⁻¹) on to XAD-8 resin in tandem with XAD-4 resin. The components held by the resin were then desalted with distilled water, back eluted in 0.1 M NaOH, H⁺–exchanged, and freeze dried. Associations of HA molecules can slowly take place in the dilute solutions to give precipitates. The composition of these precipitates were significantly different from those which remained in solution. Also, some insoluble materials may be recovered during the desalting process. These and the precipitates referred to can further be fractionated by dissolving, diluting as mentioned above, adjusting the pH to 2.5, and repeating the resin treatment process. Should any further precipitates be formed, the recovery and dissolution and resin application procedures can be repeated at pH 3. Ethanol extracts (Soxhlet) of the resins following recoveries in the aqueous media gave what are termed the neutral components.

Compositional differences between the samples are emphasized in the cross polarization magic angle spinning ¹³C NMR spectra in Fig. 2 for the HAs, FAs, and XAD-4 acids isolated from a silty clay grassland soil (Hayes et al., 1996; Hayes, 1996). These spectra show distinct compositional differences between the isolates at the different pH values. Note the progressive decrease in carboxyl contents in all fractions (180 ppm resonance), as would be expected, in the extracts as the pH is raised. The spectra for the HA (1–3) were obtained for samples processed by the IHSS procedure, and those for HA 4 and the FAs (5–7), and XAD-4 acids (8–10) were for samples isolated by the XAD-8 and XAD-4 resin in tandem process outlined above. Spectrum 11 was isolated using the Soxhlet with ethanol as the solvent.

Spectrum 1 (HAs isolated at pH 7) indicates relatively high aromaticity (120–140 ppm), and the O-aromatic resonance (145–150 ppm) and the sharp methoxyl peak at 56 ppm indicate significant contributions from altered lignin to the components of the mixture. There is strong evidence for the presence aliphatic methylene C, and for the C of ether and carbohydrate functional groups (60–90 ppm). The contribution of carbohydrate to the 60–90 ppm resonance is confirmed by the shoulder.
at 105 ppm (anomeric C). Note the low aromaticity for the HAs isolated at pH 10.6 (Spectrum 2), and the lack of convincing evidence for lignin-type residues, the distinct anomeric C peak, the strong resonance at 60–90 ppm, and the clear evidence for aliphatic functionality.

Spectrum 3, for the HAs isolated at pH 12.6 is significantly different from spectra 1 and 2, and indicates strong contributions from hydrocarbon structures, and the ratio of the resonances at 120–140 and 140–150 ppm, as well as the resonance at 56 ppm would suggest that aromaticity is largely contributed by partially altered lignin structures. The clear resonance centered around 105 ppm would suggest that much of the 60- to 90-ppm resonance is for carbohydrates.

Spectrum 4 (material that precipitated from the dilute solution at pH 2 for samples isolated by the resin in tandem procedure) is very different from Spectrum 3. Aromaticity is significantly lower, and there is little evidence for lignin content. The evidence for anomeric C weak; hence the strong resonance at 60 to 90 ppm is from functionalities (alcohol and ether) other than carbohydrate. The rounded shape of the resonance peak at 50 to 60 ppm is more suggestive of amine (peptide) than methoxyl functionalities.

The resonances for the FAs (spectra 5, 6, and 7) isolated at the different pH values are again significantly different from each other, and from the corresponding spectra for the HAs. Note the decreases in carboxylic and aromatic resonances and the increase in lignin-type and carbohydrate signals as the pH values of the extractants increase.

The XAD-4 acids can be considered as components of the FA fraction that were separated from the FAs by the XAD-8 treatment. These are not by definition HSs. Note the very low aromaticity in all these fractions (spectra 8, 9, and 10), and how aromaticity decreases as the pH of the extractant is raised. Aliphatic hydrocarbon resonances are also low, and the shoulder in the 50- to 90-ppm resonance could indicate peptide structures, and the strong anomeric C resonances indicate that carbohydrates are the major contributors to the 60- to 90-ppm resonance.

Spectrum 11 is designated as hydrophilic neutrals, or the fraction removed after the XAD-4 resin had been eluted in 0.1 M NaOH and then extracted in ethanol using a Soxhlet procedure. It is clearly compositionally different from the other fractions isolated. Note the strong aromatic resonance, the anomeric C, and the strong 60- to 90-ppm resonance. The resonance at 50 to 60 ppm would suggest peptide structures.

In the classical definitions, humic components that are not extracted in aqueous base are considered to be humin. The author and his colleagues recently performed exhaustive extractions at pH 7, 10.6, and 12.6, and then subjected the residual materials to repeated extractions with 0.1 M NaOH + 6 M urea. The NMR spectrum of the material in the urea extract from a Mollisol was very similar to that extracted at pH 12.6 (Fig. 3). However, the extracts in the basic urea medium were different from those in base in the cases of Irish Brown Podzolic soils. It is likely that the urea extract in the case of the Mollisol was protected from the base by, for example, hydrogen bonding processes or by steric constraints. It would appear that the humin materials (Fig. 4) that are not extracted in the base + urea system are very different from those in the HA (Fig. 3) and the FA components. Note the large resonance at 0 to 40 ppm (Fig. 4), indicating that hydrocarbon structures were major contributors to the composition, and that would explain the nonpolar nature of the material. There is clear evidence for resonance in the 60- to 80-ppm region, and there is evidence for a weak contribution from carboxyl groups.

The exhaustive extraction procedures described above to extract SOM components at different pH values can...
CP/TOSS; \( v = 5 \text{ kHz} \)

Fig. 3. Cross-polarization–total sideband suppression (CP/TOSS) \(^{13}\text{C}\) nuclear magnetic resonance spectra of humic acids (HAs) isolated in 0.1 \( M \) NaOH at pH 12, and in 0.1 \( M \) NaOH + 6 \( M \) urea after prior exhaustive extractions at pH 7 and at 10.6. CON = amide, COO = carboxyl, Arom-C = aromatic carbon; OC = oxygen alkyl carbon; alpha. = aliphatic carbon.

have agronomic significance. Recently Ve et al. (2004a, 2004b) have shown highly significant differences in N mineralization from HA fractions in rice soils. Nitrogen mineralization was significantly greater from the mobile HA than from the more highly humified HA components. The work described here can provide extensive fractionations that could allow the pinpointing of components with desirable (or otherwise) agronomic significance.

ISOlATION OF SOM COMPONENTS IN ORGANIC SOLVENTS

Basicity per se is not a determining factor in solubilization unless the solute swells in the solvent. Thus, basic organic solvents such as ethylenediamine (EDA, or diaminoethane) are ineffective solvents in the absence of water (Hayes et al., 1975; Clapp et al., 2005). The pH of these aqueous organic solutions has the controlling effect in the dissolution process. Solubilization of \( \text{H}^{+} \)-exchanged HA in 2.5 \( M \) EDA (pH 12.5) matched that in 0.5 \( M \) NaOH, but the solubilization could be attributed in both cases to the dissociation at the high pH of the acid groups in the HAs. Repulsion between the charges gave fully expanded conformations and the conjugated bases of the acidic functional groups were soluble in water. Anhydrous EDA was shown to be a poor solvent for the HA (Hayes et al., 1975). However, EDA, or any primary amine cannot be recommended for the extraction of SOM components. The C and N contents of EDA-soluble substances have been shown to be greater than for those isolated in NaOH and neutral salt solutions (Hayes et al., 1975; Hayes, 1985, Clapp et al., 2005). Such primary amines react with carbonyl groups to form Schiff base structures. Also, the amines react with C atoms \( \alpha \) to the keto groups in quinone structures, and the functional groups that are formed resist cleavage by acid washing (see Clapp et al., 2005, p. 33).

Hayes (1985) tested the abilities of water and of a range of organic solvents to dissolve the \( \text{H}^{+} \)-exchanged IHSS Standard HA isolated from a Florida (Belle Glade) Sapric Histosol. The HAs were mixed with solvents (0.2% w/v), some of which are listed in Table 3, and swelling was allowed to take place overnight. After centrifugation, each supernatant solution was diluted with the extractant until an absorbance reading (against the solvent) at 400 nm could be obtained. The absorbance values in Table 1 are the product of the readings obtained and the dilution factors used.

The data in Table 3 show that water is a poor solvent for \( \text{H}^{+} \)-exchanged HAs. By definition, HAs are insoluble in water, although traces of \( \text{H}^{+} \)-exchanged HAs can become separated from the hydrogen bonded matrix and will become dissolved. Both water and the \( \text{H}^{+} \)-exchanged HAs have strong hydrogen bonds, and the intermolecular hydrogen bonding interactions between the water molecules are stronger than similar bonds that might form with the HAs. Hence, sufficient \( \text{H}_2\text{O} \)-HA associations will not occur for solvation of the humic molecules to take place. The solvation effects will be different when the acidic functional groups in the HAs become dissociated as the pH is raised, as mentioned above. Similarly, monovalent metal cation-exchanged HAs will dissolve in water because both the cations (and especially Na\(^+\) and Cs\(^+\) ions with high hydration energies) and the conjugate bases of the acidic functional groups will solvate in water. Compared with the data obtained for dissolution by 0.5 \( M \) NaOH, the data in Table 3 show that acetonitrile, ethanol, and pyridine are poor solvents for HAs. On the other hand, formamide (a polar protic solvent), and \( N,N \)-dimethylformamide (DMF) and DMSO can be considered to be good solvents. Acetonitrile, DMSO, and DMF are dipolar aprotic.

Table 3. Absorbance values for solutions obtained from mixtures (0.2% w/v) of \( \text{H}^{+} \)-exchanged humic acids with aqueous and organic solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Absorbance at 400 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 ( M ) NaOH</td>
<td>24.0</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>21.0</td>
</tr>
<tr>
<td>Formamide</td>
<td>19.0</td>
</tr>
<tr>
<td>( N,N )-dimethylformamide</td>
<td>18.0</td>
</tr>
<tr>
<td>Pyridine</td>
<td>5.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0</td>
</tr>
<tr>
<td>Water</td>
<td>1.0</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>0.0</td>
</tr>
</tbody>
</table>
so solvents defined by Parker (1962) to have relative $K_r$ values $>$15 and incapable of donating H atoms to form strong hydrogen bonds.

On the basis of the data in Table 1 it is reasonable to predict from the data in Table 3 that organic liquid solvents with electrosstatic factor (EF) values $>$140 and with $pK_{HB}$ values $>$2 should be good solvents for HSs. The EF is the product of $K_r$ and $\mu$, the dipole moment, and the $pK_{HB}$ is a measure of the ability of the solvent to be an acceptor in hydrogen bonding. Acetonitrile lacks the abilities to make and to break hydrogen bonds (low $pK_{HB}$ value), although its $K_r$ and EF values are in the ranges that would apply for the good solvents. It should be emphasized that swelling in organic solvents is slow, as opposed to that in aqueous base, and several hours of contact between the organic solvent and solute are required for swelling and solvation.

Water, of course, has an abundance of the desirable properties, but as pointed out, its strength of intermolecular hydrogen bonding inhibits its abilities to form the necessary hydrogen bonds with the $H^+$-exchanged HAs. The $K_r$ and EF values for ethanol are well below the ranges for the good solvents for $H^+$-exchanged HSs, and the $K_r$ value for pyridine indicates that it is not a good solvent for $H^+$-HAs, as shown in Table 1. In the absence of water dry HAs do not swell extensively in pyridine. In the presence of water, however, and at pH values $>$9 (the $pK_b$ for pyridine is 8.96), pyridine molecules predominate in solution, and theory suggests that it will solvate functional groups in HSs. Water is a stronger hydrogen bond acceptor than that in aqueous base, and pyridine will not break the hydrogen bonds that are responsible for the solvation of $H^+$-HAs. Data from Hayes (1985) stress the importance of establishing the optimum amounts of concentrated HCl to be added to DMSO for extractions of OM from mineral soils.

Piccolo (1988), and Piccolo et al. (1998) have compared the yields and compositions of extracts from a Sapric Histosol soil using aqueous NaOH, pyrophosphate, and dipolar aprotic solvents (acetone, DMF, and DMSO) aqueous HCl systems. The yields from the dipolar aprotic systems were significantly less than those from the base and pyrophosphate, and the NMR spectra showed significant differences in the compositions of the various isolates (Piccolo et al., 1998). The dipolar aprotic solvents isolated more of the hydrophobic components of the SOM, although the NMR spectra indicated considerable similarities between the spectra of the base and DMSO extracts (Piccolo et al., 1998).

The amounts of water in the dipolar aprotic media strongly influence their abilities to dissolve SOM components. Hayes (1985) has described how water (5% of the total weight) in a urea (10%) DMSO solution was an excellent solvent for $H^+$-HAs and for SOM. However, dissolution was depressed as increasing increments of water were added. The amounts of water in the dipolar aprotic solvent systems used in the work of Piccolo (1988) and of Piccolo et al. (1998) could explain the relatively low yields obtained. The yields from DMSO and the good solvents described by Hayes (1985) were significantly greater than those for solvent systems containing excess water (and no mineral acid additives) as used by Hayes et al. (1975).

Dimethylsulfoxide–$H_2$O interactions are stronger than the associations between water molecules, and there are strong interactions also between DMSO and carboxyl and phenolic hydroxyl groups. In Fig. 5, a hypothetical representation is given, based on a model by Martin and Hauthal (1975), of the types of associations that can occur when humic and other SOM components are dissolved in DMSO. It shows the links between the sulfoxide functional group and carboxyl and phenolic hydroxyls in the humic structures. Dimethylsulfoxide can be considered to have hydrophobic (represented by the methyl groups) and hydrophilic faces. Thus, the hydrophobic face can associate with hydrophobic components, and that would explain the increased C contents.
in dipolar aprotic solvent extracts compared with those in a base (Hayes et al., 1975; Piccolo, 1988).

Dimethylsulfoxide-concentrated H2SO4 mixtures could provide powerful solvent systems for HSs, as used by Simpson (A.J. Simpson, 2005, personal communication) for NMR studies of HA. The recovery of solutes from such systems can provide problems. In the work of Piccolo, referred to above, the dipolar aprotic solvent systems were dialysed. Disintegration of the dialysis membranes might be expected from concentrated acid-dipolar aprotic solvent systems. A more appropriate recovery procedure would be to pass the extract sequentially to the XAD-8 and XAD-4 resins, and to elute the solvent in dilute acid, then with water, back elute in base, and separate the HAs and FAs in the back eluate, as outlined in this text. Clapp and Hayes (1999) used that approach to successfully isolate a humic fraction that remained associated with a Mollisol soil clay fraction after exhaustive extractions had been performed with basic and pyrophosphate preparations.

CONCLUSIONS AND RELEVANCE OF EXTRACTION PROCEDURES FOR APPLICATIONS IN SOIL AND WATER ENVIRONMENTS

There are two general approaches to study SOM. One approach attempts to study SOM in its natural state. The second approach seeks to isolate SOM from its soil mineral constituents and to fractionate the isolates into relatively discreet components for compositional studies. Adherents for studying intact or natural SOM argue that fractionation alters its organic components, and reactivity in the field. This author does not agree with that approach. Attempts to study the complex mixtures in SOM can be compared with studies of biological structures and functions in the human body. For example, much is known about the chemistry of the human body parts that has been usefully applied in the medical profession.

Alterations of component molecules during the extraction and separation of SOM fractions can be minimized. For example, negative oxidation during extractions in base can be significantly decreased by \( \text{H}^- \) — exchanging the OM, and using 0.1 \( M \) base in an atmosphere of \( \text{N}_2 \). Also, use of aqueous solvents with increasing pH values will minimize oxidation. Another approach is to avoid pH values >10, and use DMSO-acid or DMSO-urea solvent systems after the SOM has been exhaustively extracted at pH 10. The data in Fig. 2 show that the components isolated at increasing but discrete pH values are significantly different. It can be seen from the data in Fig. 3 and 4 that HA and FA can be removed more effectively by using combinations of aqueous base and urea. The residual humin fraction is a major part of SOM strongly sorbed by the mineral colloids, and has so far defied dissolution in aqueous or organic solvents. However, based on the NMR spectrum shown in Fig. 4, the compositions of the hydrophobic components of humin may be relatively simple to resolve by using procedures such as pyrolysis mass spectrometry.

On the basis of the experience of the author, more organic components might have been isolated by Piccolo (1988) and by Piccolo et al. (1998), had less water been used in the solvent systems. However, the NMR spectra of the isolates in the acetone system from three mineral soils (Spaccini et al., 2000) were largely composed of aliphatic hydrocarbon constituents, and were very different from the components isolated in base or pyrophosphate solutions. This indicates that combinations of solvent systems, both aqueous and organic, singly or in combination, are useful for studying SOM. Applying this to isolate the components of SOM that have special growth promoting or inhibition effects on plants could help resolve the debate regarding whether or not dissolved OM components can influence plant growth by hormones (Clapp et al., 2001) or by enhanced uptake of nutrients (especially Fe; Chen and De Nobili, 2004). Isolation and some fractionation of SOM components was essential, for example, to provide the fractions used by Ve et al. (2004a, 2004b), and by Schmidt-Rohr et al. (2004) to determine the availability of N for rice grown in lowland soils.

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