Factors Affecting the Solubility and Possible Fractionation of Organic Colloids Extracted from Soil and Leonardite with an Acetone–H₂O–HCl Solvent

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Organic acid colloids were separated from a Loveland soil and from leonardite with an acetone–H₂O–HCl solvent. The colloids were recovered from the solvent by dialysis and evaporation of the dialyzate. The solubility of these colloids was studied in numerous organic solvents and solvent mixtures. The leonardite colloids were dissolved by moderately H-bonded solvents with solubility parameters of 10.8 to 12.1. The soil colloids showed similar trends, but were less soluble than the leonardite colloids. However, colloids from both sources were readily soluble in mixtures of solvent–water–HCl. These findings suggest that characteristics used in describing high polymer solutions of resins, commercial polymers, and lignins can be applied to organic acid colloids from soils and leonardite.

Investigators of soil organic matter chemistry have, in recent years, pictured the structure of organic matter as consisting of heterogenous polymers or complexes. Stevenson (1966) described humic substances as a series of brown-to-black, high molecular weight polymers formed by a secondary synthesis reaction. Hobson and Page (1932) and Waksman and Iyer (1932) suggested lignin-protein complexes, and Mattson and Koutler-Anderson (1943) suggested nitrogenous complexes formed by the reaction of oxidized lignin with ammonia. Flaig (1964), in a review of his numerous investigations, suggested that humic substances result from transformations of lignin, the essential reactions of the lignin decomposition products being decomposition of side chains, demethylation, oxidation to quinones, and finally, polymerization and condensation with nitrogen-containing compounds. Swaby and Ladd (1962), and Ladd and Butler (1963) hypothesized a three-dimensional copolymer for humic acid consisting of amino acids, phenols, and quinone units without ordered sequence. Numerous workers also have suggested that some of the organic nitrogen in soil may be in complexes formed by the reactions of carbohydrates and their derivatives with amino compounds to form brown substances called melanoidins. The browning reactions, along with other postulated polymerizaton reactions and evidence supporting their possible occurrence in soils, have been reviewed in considerable detail by Bremner (1965).

Although investigators have suggested that some of the soil organic matter may exist as heterogenous polymers, there has been no general attempt to apply high polymer solution chemistry (Hildebrand and Scott, 1964; Small, 1953; Burrell and Immergut, 1966) to soil organic colloids. The objective of this study was to take characteristics used to describe high polymer solutions and determine if they apply to the solubility of some organic colloids from soil and leonardite. Such characteristics have been used to evaluate lignin solubility (Schuerch, 1952; Brauns and Brauns, 1960).

THEORY

Hildebrand and Scott (1964), Small (1953), and Burrell and Immergut (1966) have discussed the process of dissolving a polymer by a solvent and how this process is related to the familiar free energy equation,

$$\Delta F_m = \Delta H_m - T\Delta S_m \tag{1}$$

Two substances mix when the free energy of mixing (ΔF_m) is negative. Generally, the dissolution of a polymer is connected with a large increase in the entropy of mixing term (ΔS_m)—that is, an increase in randomness of molecules; thus, the sign of the free energy of mixing, Equation 1, is determined by the heat of mixing term (ΔH_m). Hildebrand and Scott (1964) have shown that the solubility of many nonpolar solutes and nonpolar solvents can be accounted for satisfactorily if the heat of mixing term is estimated from the equation:

$$\Delta H_m = V_m \phi_1 \phi_2 \left[(\Delta E_1/V_1)^{1/2} - (\Delta E_2/V_2)^{1/2} \right]^2 \tag{2}$$

where

ΔH_m = heat of mixing
V_m = total volume of mixture
ΔE = energy of vaporization of component 1 or 2
V = molar volume of component 1 or 2
ϕ = volume fraction of component 1 or 2

Hildebrand has assigned the symbol δ to the quantity $(\Delta E/V)^{1/2}$ in Equation 2, and has termed δ the solubility parameter. The most direct method of calculating solubility parameters for solvents is from the heat of vaporization according to the formula:

$$\delta = \sqrt{\frac{\Delta E}{V}} = \sqrt{\frac{\Delta H - RT}{\dfrac{M}{d}}} \tag{3}$$

where ΔE = latent or internal heat of vaporization, ΔH = total or external heat of vaporization, $RT = 600$ calories at 25° C., V molar volume, M = molecular weight, and d = density (grams/cc.). Hildebrand and Scott (1964) have a complete chapter devoted to an evaluation of solubility parameters, and Burrell and Immergut (1966) have calculated and listed the solubility parameters for numerous solvents.

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A nonpolar solute and a nonpolar solvent are miscible when their solubility parameters approach one another in value. However, for polar solutes and polar solvents the solubility parameter, or value of ΔH_m, may not be the only important factor in deciding the sign of the free energy ΔF_m in Equation 1. Strong intermolecular forces, such as the hydrogen bond, involve permanent orientation of molecules, thus affecting the randomness of the molecules and size of the entropy-of-mixing term of Equation 1. Small (1953) has indicated that for polymers containing functional groups such as hydroxyl, carboxide, or amide, either in the main chain or as pendant groups, hydrogen bonding usually controls the solubility of such polymers. Hydrogen bonding thus becomes as important as solubility parameters in determining the solubility of many polymers. The capacity to donate or share an electron pair with a bonded proton has been measured by Gordy (1941) and correlated linearly with basic strength. Ethers and ketones are stronger hydrogen bonders than esters, and amides, amines, and dimethyl sulfoxide are stronger hydrogen bonders than ethers. Burrell and Immergut (1966) have listed the H-bonding tendency for numerous solvents and have grouped organic solvents into p (poorly H-bonded: hydrocarbons containing halogen, nitrate, and cyano groups), m (moderately H-bonded: esters, ethers, ketones), and s (strongly H-bonded: alcohols, amines, amides, and acids). This is the same designation as used in Table I.

MATERIALS AND METHODS

Organic acid colloids were obtained by solvent extraction of a Loveland fine sandy loam (Loveland, Colo.) and leonardite (obtained from Knife River Coal Mining Co., Box 177, Bismarck, N.D.). Leonardite is a coallike substance, similar in structure and composition to lignite coal, and is believed to be derived from lignite coal by natural oxidation. The major difference between leonardite and lignite is the higher oxygen content of leonardite, which is entirely due to a larger number of carboxylic acid groups in the leonardite (Fowkes and Frost, 1960). The colloidal substances from leonardite have many properties (milliequivalent of acidity, fluorescence, and IR spectra) similar to organic colloids from soils. Carboxyl groups were determined by methods of Schnitzer and Gupta (1965). The leonardite colloid had 5.16-meq. COOH groups per gram, whereas the colloids from the Loveland fine sandy loam contained 4.00 meq. per gram. Similar fluorescence spectra (Porter and Stewart, 1965) were obtained for both sources of colloids. The major portion of the IR spectrum for both sources of colloids is featureless; however, the colloids have similar sharp bands that indicate hydroxyl and carboxyl or carbonyl functions.

The leonardite colloids are easily obtained and provide an excellent model for working out experimental procedures for less readily available soil organic colloids. A modified slurry-sedimentation procedure of Young and Frost (1963) was used to separate the organic acid colloids from the soil and leonardite. The material to be extracted was shaken as a slurry with an acetone-H_2O-HCl solvent (4 parts acetone-1 part water-0.12N with respect to HCl) in a 9-liter, 45-cm.-high glass bottle. The slurry (1 gram of leonardite or 10 grams of soil per 100 ml. of solvent)

Table I. Solubility Parameter, Water and Ethanol Solubility, and H-Bonding Group for Various Organic Solvents

Solvent	Solubility[a] Parameter, δ	Water[b] Solubility, G./100 Ml.	Ethanol[b] Solubility, G./100 Ml.	H-Bonding Group
Hexane	7.3	0.01	50	p
Diethyl ether	7.4	7.50	∞	m
Cyclohexane	8.2	i	∞	p
Methyl isobutyl ketone	8.4	2.00	∞	m
Carbon tetrachloride	8.6	0.08	∞	p
Toluene	8.9	0.05	∞	p
Ethyl acetate	9.1	8.60	∞	m
Tetrahydrofuran	9.1	v.s.	∞	m
Benzene	9.2	0.08	∞	p
Chloroform	9.3	1.00	∞	p
Methyl ethyl ketone	9.3	35.3	∞	m
Methylene chloride	9.7	2.00	∞	p
Ethylene bromide	9.7	1.20	∞	p
Acetone	9.9	∞	∞	m
Dioxane	10.0	∞	∞	m
Isoamyl alcohol	10.0	2.67	∞	s
Aniline	10.3	3.5	∞	s
Cyclopentanone	10.4	s.s.	∞	m
Pyridine	10.7	∞	∞	s
N,N-Dimethylacetamide	10.8	∞	∞	m
N-Methyl-2-pyrrolidone	11.0	c	c	m
Furfural	11.2	8.30	∞	m
Ethylene glycol monomethyl ether	11.4	∞	v.s.	m
Isopropanol	11.5	∞	∞	s
Acetonitrile	11.9	∞	∞	p
Dimethyl sulfoxide	12.0	∞	∞	m
N,N-Dimethyl formamide	12.1	∞	∞	m
Butyrolactone	12.6	∞	∞	m
Nitromethane	12.7	9.10	∞	p
Ethyl alcohol	12.7	∞		s
Ethylene glycol	14.6	∞	∞	s

[a] Solubility parameters and H-bonding group (p = poorly H-bonded, m = moderately H-bonded, and s = strongly H-bonded) were obtained from Burrell and Immergut (1966).
[b] Water and alcohol solubilities were obtained from Hodgman et al. (1953).
[c] Values for this compound were not found.

was allowed to settle, and the dark brown solution was siphoned off and filtered through Whatman No. 42 filter paper. The process was repeated several times until the dark brown color of the extract began to diminish. The extracts were dialyzed against demineralized water using Visking dialysis membranes. The acetone, acid, and some low molecular weight soil organics were removed by this procedure. Kemper and Evans (1963) reported an average pore radius of 18.9 A. for this Visking membrane. This suggests that the organic colloids retained by the membrane had diameters greater than 36 A. The dialyzates were evaporated to dryness at 40° C.

The organic solvents selected gave a range of solubility parameters from 7.3 to 14.6 with varying H-bonding capacities (Table I). In addition, the solvents were mixed in various combinations with water, ethanol, and HCl, when miscible. These mixtures consisted of 4 parts solvent to 1 part water or ethanol by volume, and when HCl was used, enough was added to produce 0.12N solution. These mixtures were included in the tests because various investigators (Schuerch, 1952; Whitehead and Tinsley,

1964; Duback and Mehta, 1963; Fowkes and Frost, 1960; Polansky and Kinney, 1947; Thompson *et al.*, 1964) have noted that humic acid and lignin substances are extractable with organic solvents (acetone, dimethyl formamide, and dioxane), especially in combination with water.

Small samples (10 mg.) of the organic colloids were shaken for 30 minutes at room temperatures with 10 ml. of the individual or mixed solvents. The solutions were filtered through Whatman glass filter paper (GF/A, 2.4 cm. in diameter) on a Tracerlab E-8B stainless steel filter apparatus. The glass filter papers were prewashed with the desired solvent and dried to constant weight prior to use. After filtration of the solution, the filter paper plus residue were dried to a constant weight, and the milligrams of colloid soluble in 10 ml. of solvent were calculated.

RESULTS AND DISCUSSION

Extraction of Organic Colloids from Soil. Organic colloids may be extracted quickly and easily from soils using the aqueous HCl mixtures of any of the following solvents: tetrahydrofuran, acetone, dioxane, cyclopentanone, *N,N*-dimethylacetamide, *N*-methyl-2-pyrrolidone, ethylene glycol monomethyl ether, dimethyl sulfoxide, *N,N*-dimethyl formamide, and butyrolactone. The HCl facilitates the separation of the organic colloids from mineral portions of the soil, probably by breaking polyvalent ion salt bridges. After the breaking of ionic forces, the organic solvents probably compete with the soil organic colloids for adsorption sites on the secondary silicate minerals through H-bond formation and van der Waals' forces. Considerable difficulty was encountered in trying to evaluate the amount of colloidal C removed from soils by these various solvents because of solvent adsorption by silicate minerals. Carter *et al.* (1965) have shown that ethylene glycol monomethyl ether is retained by silicate minerals and that there is a relationship between surface-area measurements by ethylene glycol and ethylene glycol monomethyl ether. The solvents previously mentioned as being effective in the extraction of organic colloids from soil are also retained by silicate minerals. Studies initiated to determine the adsorption characteristics of silicate minerals for the various solvents will be reported later in another paper.

The isolation of organic or humic substances from soils is a most important problem if one is to study their structure and properties. In such an endeavor, hopefully, the organic substances separated from soil will not be altered and will not have reacted with the extractant. Whether any of the above-mentioned extracts meets such a requirement has not been evaluated. The author has used the acetone–H_2O–HCl procedure at room temperature assuming that organic colloids separated by this procedure are not drastically altered. The acetone appears easily removed at low temperatures and by dialysis. A study initiated using radioactive acetone should provide more positive proof that this assumption is correct. Young and Frost (1963) have stated, "Acetone does not react with, nor is it adsorbed on humic acid." The data that allowed them to reach this conclusion were not presented in their paper. The percentage of carbon removed from various

soils extracted with the acetone–H_2O–HCl solvent was determined by extracting 5 grams of $CO_3{}^{-2}$-free soil with 25 ml. of solvent and determining carbon in the soil before and after extraction by combustion procedures (Stewart *et al.*, 1964). The results of the carbon analysis (Table II) indicate that an acetone–H_2O–HCl solvent extraction will remove from 10 to 23% of the total carbon from soils. Several successive extractions improved the amount of carbon extracted, but it would probably require hydrofluoric acid pretreatment or an ultrasonic vibration treatment to appreciably alter the amount of C extracted. Such procedures still need to be evaluated. Higher yields of organic colloids can be obtained from soils using alkali hydroxides and alkali salts of pyrophosphoric acid (Choudhri and Stevenson, 1957). Substances isolated in these alkaline solutions are subject to auto-oxidation (Choudhri and Stevenson, 1957) and possible hydrolytic fragmentation. As mentioned above, the acetone–H_2O–HCl solvent was used by the author in an attempt to obtain unaltered material. The solubilities in organic solvents of materials isolated by alkaline extracts still need to be evaluated.

Solubility. In Table I, the solvents used are listed in order of increasing solubility parameters. The H-bonding capacity is scattered at random throughout the table. The solvent sequence of Table I is used in the solubility table for organic colloids (Table III). The organic colloids from leonardite (Table III) were fairly soluble in *N,N*-dimethylacetamide, *N*-methyl-2-pyrrolidone, ethylene glycol monomethyl ether, dimethyl sulfoxide, and *N,N*-dimethyl formamide. These moderately H-bonded solvents represent a solubility parameter range of 10.8 to 12.1. However, adding water or alcohol to the solvent greatly increases the solubility of the leonardite colloids in some solvents that have lower solubility parameters. Tetrahydrofuran, solubility parameter 9.1, is one of these solvents that is noticeably influenced by water and ethanol. Usually water has a more pronounced effect than ethanol. Other solvents that are noticeably influenced by water content are acetone, dioxane, cyclopentanone, and butyrolactone. The exact effect of the water or ethanol is not known, but the hydrogen-bonding capacity of the mixture is probably different from that of the individual solvent. Cowie and Toporowski (1961) have presented evidence that suggests that dimethyl sulfoxide–water mixture belongs to a class of solutions possessing numerous solute-water bonds which are stronger

Table II. Percentage Carbon Removed from Various $CO_3{}^{-2}$-Free Soils by Extraction with an Acetone–H_2O–HCl Solvent (Mean of Two Replicates)

Soil	Before Extraction, % C	After Solvent Extraction, % C	% of Total Carbon Removed
Loveland fine sandy loam	1.009	0.907	10.1
Fort Collins acid loam	1.697	1.460	14.0
Pierre clay	1.932	1.706	11.7
Apishapa sandy clay loam	1.416	1.175	17.0
Mountain soil (Bennett Creek)	5.57	4.26	23.5

Ambient Temperatures, 21-27° C.

	Leonardite Solubility, Mg./10 Ml.						Loveland Solubility, Mg./10 Ml.					
Solvent	Solvent	Solvent-H₂O	Solvent-EtOH	Solvent-HCl	Solvent-H₂O-HCl	Solvent-EtOH-HCl	Solvent	Solvent-H₂O	Solvent-EtOH	Solvent-HCl	Solvent-H₂O-HCl	Solvent-EtOH-HCl
Hexane	0.8	a	a	a	a	1.4	1.0	a	1.3	a	a	a
Diethyl ether	1.4	a	4.0	a	a	3.2	1.1	a	1.5	2.7	a	4.6
Cyclohexane	2.0	a	a	a	a	a	1.0	a	a	a	a	a
Methyl isobutyl ketone	0.4	a	2.7	3.9	a	8.6	1.1	a	5.7	2.7	a	9.9
Carbon tetrachloride	0.4	a	0.6	a	a	4.6	1.0	a	1.5	a	a	4.5
Toluene	0.4	a	2.4	a	a	3.5	2.5	a	2.0	a	a	5.3
Ethyl acetate	0.2	a	1.3	2.4	a	5.1	2.2	a	2.9	3.4	a	5.9
Tetrahydrofuran	1.5	>10.0	4.5	7.2	9.4	9.5	2.7	3.2	3.7	7.4	8.8	8.7
Benzene	0.8	a	1.3	a	a	4.2	0.9	a	1.1	0.9	a	4.8
Chloroform	1.7	a	2.1	a	a	4.4	0.8	2.0	2.0	a	a	5.7
Methyl ethyl ketone	1.7	a	3.3	3.7	a	0.8	4.9	a	1.1	4.5	a	7.8
Methylene chloride	0.2	0.0	4.5	a	a	6.5	0.5	a	1.0	0	a	6.4
Ethylene bromide	a	a	2.3	a	a	a	0.3	a	2.2	a	a	a
Acetone	1.0	7.8	3.4	4.5	>10.0	7.7	1.6	2.5	1.8	5.3	8.8	8.7
Dioxane	1.6	8.7	3.9	3.5	8.7	9.4	1.5	1.5	1.0	4.9	9.0	8.9
Isoamylalcohol	1.4	a	1.0	4.9	a	2.4	1.4	a	1.9	4.9	a	3.8
Aniline	0.5	a	1.3	0.7	a	5.1	0	a	0.9	1.2	a	2.5
Cyclopentanone	0.9	8.7	5.5	a	9.0	10.0	1.7	3.3	1.5	a	8.4	8.8
Pyridine	2.1	8.5	0.3	3.8	8.2	3.9	2.7	1.3	2.1	3.1	1.5	2.3
N,N-Dimethylacetamide	9.9	>10.0	>10.0	>10.0	>10.0	>10.0	3.4	6.5	1.5	6.7	8.2	8.2
N-Methyl-2-pyrrolidone	>10.0	>10.0	9.1	9.9	>10.0	9.2	1.5	0.6	1.5	6.7	8.2	8.2
Furfural	5.9	a	8.2	forms condensation products			2.0	a	a	forms condensation products		
Ethylene glycol monomethyl ether	8.8	7.8	6.0	6.5	6.5	9.4	7.8	8.9	1.4	8.6	9.6	9.2
Isopropanol	1.3	2.8	0.8	3.5	4.5	4.8	0.9	1.4	1.5	4.0	1.2	4.6
Acetonitrile	0.3	4.3	1.9	1.4	5.2	8.9	1.9	1.2	0.7	3.1	6.2	6.3
Dimethyl sulfoxide	9.8	9.8	>10.0	9.9	9.4	9.9	3.1	2.3	1.6	9.0	8.0	8.2
N,N-Dimethyl formamide	9.9	>10.0	9.2	>10.0	>10.0	>10.0	3.0	2.1	1.4	8.9	8.4	2.4
Butyrolacetone	1.6	9.7	6.7	9.6	>10.0	>10.0	2.1	4.3	3.7	8.4	8.5	8.3
Nitromethane	3.0	0.2	1.4	1.1	0.8	5.1	0.4	1.1	1.4	1.5	1.0	5.6
Ethyl alcohol	1.1	2.4	a	5.0	3.8	a	2.6	2.2	1.8	5.3	8.8	8.7
Ethylene glycol	3.9	1.9	5.0	7.5	4.4	5.8	0	0	0.4	0.5	3.8	6.8

ᵃ Not determined usually because of lack of miscibility of solvents.

than hydrogen bonds between water molecules. Perhaps the other moderately H-bonded solvents also form solute-water bonds which alter the H-bonding capacity of the solvents.

The organic colloids from the Loveland fine sandy loam (Table III) are considerably less soluble in the individual solvents than the leonardite colloids, although some of the same solvents that dissolved leonardite colloids show a tendency to dissolve the soil organic colloids. The soil organic colloids are similar in solubility to the leonardite colloids in the solvent mixtures that contained HCl. The presence of HCl in the solvent mixtures enhanced the solubility of the soil colloids. The HCl probably assures free carboxylic acid groups in the colloids rather than insoluble salts. The soil colloids contained 20.5% ash, whereas the leonardite colloids contained 10.8% ash.

The increased amount of ash in the soil colloids probably accounts for the major differences in solubility between the two sources of organic colloids. The soil colloids were treated with hydrofluoric acid mixture—the same procedure as used by Stewart and Porter (1963) for fixed NH₄-N—to reduce the ash content. The HF-treated soil organic colloids were readily soluble in N,N-dimethyl formamide (9.3 mg. per 10 ml.), dimethyl sulfoxide (9.5 mg. per 10 ml.), and 2-methylpyrrolidone (>10 mg. per 10 ml.). The solubility of the HF-treated soil organic colloids was similar to the solubility of the leonardite colloids in these solvents.

Fractionation. Two general procedures, precipitation and extraction, are used to separate high polymers into a series of fractions differing in average molecular weight. In the precipitation method, various amounts of precipi-
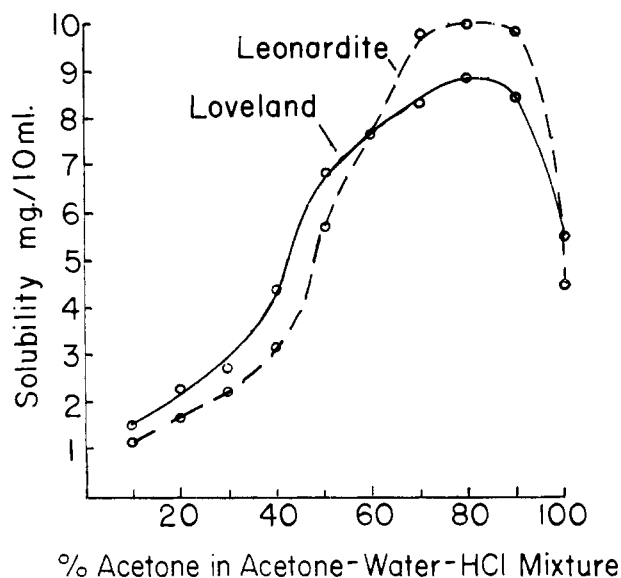
Figure 1. The solubility of organic colloids from soil and leonardite in water–HCl mixtures containing varying amounts of acetone

tant (usually nonsolvent) are added in a series of steps; the highest molecular weight materials are the first to separate followed by successive fractions of lower average molecular weight. In the extraction procedure, the polymer is successively extracted with a blend or mixture of solvents of increasing solvent power for the polymer. In this case, the lowest molecular weight fractions are extracted first, and the larger polymers are extracted last. By mixing various proportions of acetone and water, a blend of solvents that showed varying degrees of dissolving power was obtained. The ability of the various solvent mixtures to dissolve the organic colloids increased as acetone content increased, reaching a maximum at 8 parts acetone to 2 parts water (Figure 1). This was the same solvent–water ratio as was used to extract the original colloids. Figure 1 suggests that a series extraction of the colloids, starting with the solvent mixture containing the smallest proportion of acetone, might yield a series of fractions differing in molecular size. To test this hypothesis, equipment to measure molecular weight of the extracted colloids would be necessary. Such equipment is not now available to the author.

The various organic solvents also may be useful in fractionation of the colloids according to molecular size on the new lipophilic Sephadex Gel LH-20, which swells in water and polar organic solvents. The author now is attempting to work out the procedure for this type of separation.

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