CHAPTER - I

INTRODUCTION

The physical, chemical as well as the biological properties of soils depend largely on the interaction of clay minerals, metal ions and hydrous metal oxides with the soil organic matter. Humic substances or humus, as the soil organic matter is usually termed, constitute a minor fraction of a soil. Van Bemmelen (1) in 1888 first recognised soil humic matter as a colloidal substance and since this time the literature on the colloidal properties of humic matter has formed an interesting part of the history of colloid chemistry. These naturally occurring large polymers are mostly composed of a large number of atoms bonded together by chemical valency, rather than colloidal aggregates of smaller molecules. The polyelectrolytic properties of these weakly acidic compounds have also been confirmed (2,3,4,5,6,7,8).

Soil humus is reported to be the products of biological activity. The polymeric materials e.g., polysaccharides, lignins and proteins present in plants and animals are first degraded to simple compounds. These smaller units are utilised by soil microorganisms in their metabolic path ways or undergo enzymatic or chemical reactions to form new polymers. Some of these polymers are relatively resistant to further decompositions or form resistant complexes with metal ions and clays and thus constitute an important part of soil humus (9,10,11). Experimentally it has been found that about 50-55% of the total humic acid molecules as has been identified is made of amino acids, hexosamines, polycyclic aromatics and oxygen containing functional groups. The remaining unknown fractions have been assumed to consist of easily oxidisable heterocyclic components having higher degree of unsaturation sufficient to cause the dark colour characteristics for all types of humic substances (12). It has been suggested from chemical degradation and spectroscopic studies that humic acids contain or readily give rise to a complex aromatic core, responsible for E.S.R. signals, to which are attached a number of chemical units e.g., (a) polysachharides (b) proteins (c) simple phenols (d) metals.

According to Oden (13) humic substances may be broadly divided into humic acids and fulvic acids. The humic acid constitutes that fraction of the soil organic matter which is soluble in dilute alkali but is precipitated with mineral acids. The fulvic acids are soluble in both alkali and acid. In the humic acid groups a small portion becomes soluble in ethanol or in ether, it is termed hymatomelanic acid. Again, there is another fraction which is insoluble in acid and alkali known as humin.

Although metals are assumed to be present as salts of -COOH, anion formation by chelation with phenolic groups is an attractive alternative, particularly in view of the ion exchange and sequestration properties of humic substances. The analogy of the humus like substances obtained by various chemical considerations of different quinones with amino acids also proves the foregoing assumptions for the chemical structure of natural humic substances (14,15,16,17). The different physical studies e.g., I.R., E.S.R. etc. and chemical properties, like metal retention and complex forming tendencies of synthetic model humic substances indicate that these substances are condensation products of different quinones and amino acids, brought about in soil by some complex physico chemical methods.

The formation and nature of humus in soil depend completely upon environmental conditions i.e., (i) upon the organic residues of plants and animals (ii) by the type of application of fertilisers and green manures and (iii) crop rotation as also (iv) upon climatic condition. These factors influence and produce a variety of types of humus materials. In ever green forests, surface soil humus does not interact with metals and clays and so gives rise to humus materials.

The humus content of soil varies considerably from 0.2% to about 20%. Humus is not distributed evenly through the soil depth. The concentration of humus decreases with increase in depth. It has got highest concentration in the upper 20-30 cm of the soil. In the brown forest soil as well as for red and yellow soil, the existence of humus is practically nil below the upper 15 cm. Podzolic soil has a zone at the junction of the surface layer and the mineral soil layer. A well marked humus podzol may have an accumulation of humic matter in the B_1 - horizon. In this type of soil the total organic matter may be upto 5-6% by weight and 75-80% of this can be extracted as humic acid.

Humic substances being in intimate contact with other soil constituents, do not exist as free compounds in normal soils in any significant amount. They can not be usually extracted from soil by water but they are made available with the help of chemical reagents which can displace polyvalent cations. Generally acid, alkali and complexing agents are used for the extraction.

Isolation from soil

The method to be used in the extraction procedure of humic substances from soil should have (a) universal applicability (b) Completeness of extraction (c) selective extractive property.

The solvent systems usually applied for humic extractions fall in the following groups (i) acidic solvents (ii) basic solvents (iii) chelating agents and (iv) basic solvents with reducing property. In order to make a complete extraction, a preliminary treatment of the soil with 2N HCl is essential to decompose any bicarbonates and carbonates present. During this process a partial hydrolysis of organic compounds may occur. A number of workers (18,19) reported the removal of a part of organic matter from soil by treatment with HCl or HF acids or by a suitable combination of both.

The most commonly used solvent for humic matter extraction is dilute aqueous NaOH at room temperature. Its principal advantage is that it extracts the highest percentage of the total soil humic matter than any other solvent currently available. Some questions have been raised concerning possible alteration induced in the extracted humic matter by this solvent. Bremner (20) has demonstrated that alkali soil suspensions absorb molecular oxygen from the atmosphere. Several studies have been made in an attempt to ascertain whether such alteration occurs or not. To select an alternative solvent a number of solvents like $Na_4P_2O_7$, $(NH_4)_2CO_3$, Na_2CO_3 and their different combinations have been tried by many workers. These weaker basic solvents have been found to be less effective in extraction of humic matter from soil. Moreover ammoniacal solutions react with lignin and other phenolic components and probably with carbohydrate components (21). A large number of complex forming reagents such as Na_2-EDTA, oximes, acetyl acetone have been tested by Martin and Reeve (22) who have found the removal of large amount of organic matter from podzolic soils by these reagents. One of the main drawback of using $Na_4P_2O_7$ is that it extracts the metal complexes from the soil.

In order to select an ideal solvent for the maximum extraction, minimum ash and minimum chemical change, Bremner (23) compared 0.5 (N) NaOH, 0.1 M $Na_4P_2O_7$ and 0.5 (N) Na_2CO_3/$NaHCO_3$ solutions but failed to reach any definite conclusion and opined that the three criteria used as objectives in this study may be mutually exclusive.

Kim, Schnitzer and Skinner (24) have failed to observe any modification caused by alkali extraction with 0.5 (N) NaOH of the organic matter in Podzol B_h horizon. Levesque and Schnitzer (25) have recently studied the effectiveness of extraction of organic matter with Dowex-1 resin and have compared this with NaOH extraction.

On the basis of their observations they have concluded that NaOH is the more suitable reagent for obtaining purified organic matter as the end products.

Purification and Fractionation

Purification of humic substances from different contaminations e.g., salts, clays, sesquioxides etc. can be achieved by an appropriate combination of several processes viz. dialysis, reprecipitation, centrifugation, redissolving and finally, repeated treatment with H-resin. Practically ash free humic acids can thus be obtained (26,27).

Fractionation of humic substances have been done by a number of techniques. One possible method of fractionation is fractional precipitation from solutions at different pH's used by Springer (27) and Schlichting (28). Recently, fractionation by gel filtration on columns of Sephadex G-25 gel has been tried for separation of humic acid by Dorado Bernal (29). Schnitzer and Skinner (30) prepared seven fractions of a podzol fulvic acid by using the sequence of Sephadex gel. These fractions differed in their number-average molecular weights, nitrogen content, COOH and phenolic OH content, I.R. and E.S.R. spectra. This method is believed to be a useful method for producing more homogeneous fractions of soil humic substances. Chromatographic separation of different humic acid fractions is also known (31-36).

Molecular weight

Humic acid being a heterogenous mixture of chemically allied substances, molecular weight of a single humic acid cannot be obtained. Attempts have been made to determine the average molecular weight of a fraction of humic acid. Odén (37) reported molecular weight as 960 and 1280 of humic acid fractions with basicity 3 and 4 respectively. Welte (38) obtained molecular weight as 1060 and 1650 for two humic acid fractions by diffusion technique. Fuchs et al. (3[illegible]) obtained values ranging around 1400 by measuring the rise in boiling point of certain acetone soluble oxidation products of humic acids. Schnitzer and Skinner (30) investigated, by application of ultracentrifuge measurements and vapour pressure osmometry, the distribution of molecular weights of fulvic acid extracts from the B_h horizon of a podzol obtained by gel filtration. The molecular weight has been found to be 5[illegible]13 whereas by vapour osmometry the same was calculated as 5[illegible]10. On the other hand Dell'Agnola et al. (40) determined the distribution of molecular weights by extracts of humic acids from a soil by the use of different gels. They found 12 % of the total carbon content in a range of molecular weights between 0 and 4000, 3 % between 4000 and 5000, 23 % between 5000 and 100,000 and 62 % in the range between 100,000 and 200,000. Flaig and Beutelspacher (41) using the ultracentrifuge found the molecular weight in the range of 70,000 to [illegible],000. In examining the humic matter extracted from soils under continuous pasture and a wheat fallow rotation, Posner (4[illegible]) was able to obtain two fractions of average molecular weights estimated as 70,000 and 200,000. He emphasised the importance of electrolyte control in both sample applications and eluting solutions in order to obtain discrete fractions and to minimise irreversible adsorption. Dubin and Wilson ([illegible]3) fractionated humic acids from five Moldavian soil groups on the various grades of Sephadex and obtained two fractions from each humic acid, one having molecular weight less than 5000 and the other ranging from 50,000 to 100,000. Wershaw et al. (44) calculated molecular weight of sodium humate from a sandy soil which ranged between 2.0 x 10^5 to 1.2 x 10^6. Orlov and Govskova (45) determined the molecular weight by light scattering method and found the molecular weight to be 63.3 x 10^3 for humic acid from sod podzolic soil and 66.2 x 10^3 for humic acids derived from chernozem. These large differences in molecular weight of humic acids of different origin determined by different methods and extraction procedure are possible due to unspecific fractionation procedure. It is also known that the molecular weight of high molecular weight substances determined by some cryoscopic methods are very often much lower than the weights determined by osmometry or by ultracentrifuge.

Complexing property and chelation reactions of soil organic matter with metals.

A large number of workers have suggested that many metals are capable of forming stable complexes with soil organic matter (46,47,48). Evidence of chelation stem from the observation that Ba^{2+} and Na^{+} are unable to replace all the Cu^{2+}, Zn^{2+} and other heavy metals those are adsorbed from solution by soil organic matter. According to different possible types of linkages between functional groups of humic acids and the inorganic soil constituents three main types of metal organic derivatives are important (6).

(i) The ionic type, with participation of carboxyl and phenolic hydroxyl groups, leads to the corresponding humates and fulvates by the known reaction of salt formation.

(ii) The semipolar type, formed by coordination linkages with participation of amino, imino, keto and thio-ether groups, leads to complex compounds of the chelate types.

(iii) A type that is formed by polarisation effects and hydrogen bridge linkages with special participation of terminal functional groups, forming compounds of the adsorption type.

But in the humic acid chemistry most of the authors used the term "complex" or "chelate" for all types of linkages mentioned above. In fact, binding of cations to negatively charged groups of an ion exchanger like soil organic matter always involves electrostatic forces to some extent. Such bonds may also have a more or less strongly covalent character. The bond between metal ions of the first transition series and the complexing sites of humic acids is undoubtedly partly covalent in character.

Clay minerals have definite and well defined values of cation exchange capacities. But humus fractions have no well defined cation exchange capacities which vary from source to source as well as with the method of extraction from soil. It has been found that the complexing of the cations is influenced by its charge~~s~~. Thus Ba^{2+} exchanges more than Na^{+} at any particular hydrogen ion concentration (40). As the extent of exchange follows the lyotropic series it is clear that the exchange mechanism depends on the charge and hydrated volume of the exchanging ions. The rule of Schulze and Hardy is valid for the mechanism of ion exchange. The exchange capacity of humic acid is also strongly influenced by the hydrogen ion concentration of the exchange solution (50). The cation exchange capacity increases with increasing pH values due to the increasing dissociation of the acidic groups (i.e. phenolic and carboxyl acid groups).

The phenolic hydrogen atoms are the sites most acidic in their behaviour in lignin. Again, it has been found that there is a direct relationship between the contents of lignin-like materials of the soil organic fraction and the cation exchange capacity. Thus it is concluded that one of the active sites in soil organic matter must be of phenolic character. Gillam (51) concluded from his results of selective groups blocking that the active groups for ion exchange are carboxylic acid groups. On the other hand, Broadbent and Bradford (52) found by the selective blocking of the exchange sites with dimethyl sulfate and diazomethane that the exchange capacity was inversely proportional to the content of methoxyl groups. They concluded that carboxyl groups as well as phenolic hydroxyl groups participate in the exchange mechanism. Hines and Barber (53) were able to eliminate the whole exchange capacity of the organic matter by treatment with dimethyl sulfate. Thus they concluded that phenolic hydroxyl groups have greater importance for the exchange properties. In the other hand Broadbent et al. (54) found a predominant role of carboxylic acid groups in case of calcium and copper exchange. Schnitzer and Skinner (55) found that blocking of either acidic carboxyls or phenolic hydroxyl caused significant reductions in metal retention. Thus they concluded that both groups reacted simultaneously with the metal ions. Although these authors find no evidence for reactions of the carbonyl groups, according to their opinion these reactions can not be excluded from the interaction mechanism between soil organic matter and metal ions. According to Lewis and Broadbent (56) phenolic groups of model substance with a 0.1 (N) $CuSO_4$ at pH 6.0 fixed no copper; at pH 5.5, 437 meq. of copper were fixed and at pH 4.6, 294 meq. were fixed. It has been concluded that an interaction with phenolic hydroxyl groups is responsible for the resulting exchange.

Upon addition of a metal salt of a strong acid to an aqueous solution of a polybasic humic acid, the pH generally decreases. The magnitude of this decrease depends on the ratio of the affinities of the metal and the hydrogen ion for the humic acid polyanion and on special entropy consideration for polyanion (57). The potentiometric titration curve of a polymeric organic acid has been described by Katchalsky and Spitnik (57) as a generalised Henderson-Hasselbach equation,

$$pH = pK_i + n \log \frac{\alpha}{1-\alpha}$$

where K_i = the intrinsic dissociation constant of the polymeric acid, n = approximately a constant and α = degree of neutralization or dissociation. Van Dijk (58) tested the applicability of this equation to humic acids titrated with sodium hydroxide and Martin and Reeve (59) did the same for organic material extracted from soil with an aqueous solution of acetylacetone. By plotting pH against $\log \frac{\alpha}{1-\alpha}$, no linear curve has been obtained, indicating that the behaviour of these organic substances differs from that of linear polymers such as polyacrylic acid for which the equation has been derived (57). This deviation may be attributed to different reasons, e.g., existence of more than one type of acidic group in humic acid, uneven distribution of these groups over humic acid molecule and interaction of neighbouring acid groups. However, Posner (60) found a reasonable validity of Henderson-Hasselbach equation in the acid region upto pH 6.0 .

Potentiometric titration of humic acid in the presence of metal ions has been done by many workers. Beckwith (61,62) found that the order of decrease of pH upon addition of manganese, cobalt and nickel salts to a humic matter suspension is the same as in the series of Irving and Williams (63). Martin and Reeve (59) have suggested that protons titratable in presence of metal salts, may also originate from the hydrated metal ion and not only from the organic substance. They did not find the above order of pH drop. Again, these authors found a horizontal displacement of the titration curve in the presence of copper salt, which could be explained as the formation of a basic copper salt. Khanna and Stevenson (64) found the following order of pH drop for humic acids $Mn(II) < Co(II) < Ni(II) \ll Cu(II) > Zn(II)$, which agrees with the Irving Williams series and concluded that the bonds are mainly electrostatic in nature. These results were in contrast with the work of Martin and Reeve (59), who found that the order of pH reduction for organic matter from a podzol B horizon followed the order

$$Ni(II) < Mn(II) < Co(II) \ll Cu(II) > Zn(II)$$

Wright and Schnitzer (65) and Schnitzer and Skinner (66) found that calcium and magnesium salts did not influence the titration curves of humic and fulvic acids and nickel and copper salts did so only to a small extent. Van Dijk (67) concluded, from his studies with a number of different natural and synthetic model humic acids that metal humates belong to the polynuclear chelates with cations binding predominantly to negatively charged groups. Judging from the magnitude of the pH drop on addition of inorganic salts, no large differences in bond strength for the divalent ions at pH 5.0 were concluded and the bond strength was found to increase only slightly in the order Ba(II) < Ca(II) < Mg(II) < Mn(II) < Co(II) < Ni(II) < Fe(II) < Zn(II) .

But Pb(II), Cu(II) and Fe(III) ions are more firmly bound and follow this order in their extent of interaction. To explain the pH drop on addition of metal ions in humic acid solution, Van Dijk (67) presented convincing evidence to establish two types of reaction mechanisms: one, at lower and another, at higher pH. Thus for Cu(II) - humic acid complex at low pH one valence of the Cu(II) ion is bound to a "free" anionic group, and the other to a formerly protonated, weakly acidic group, e.g., a phenolic hydroxyl group:

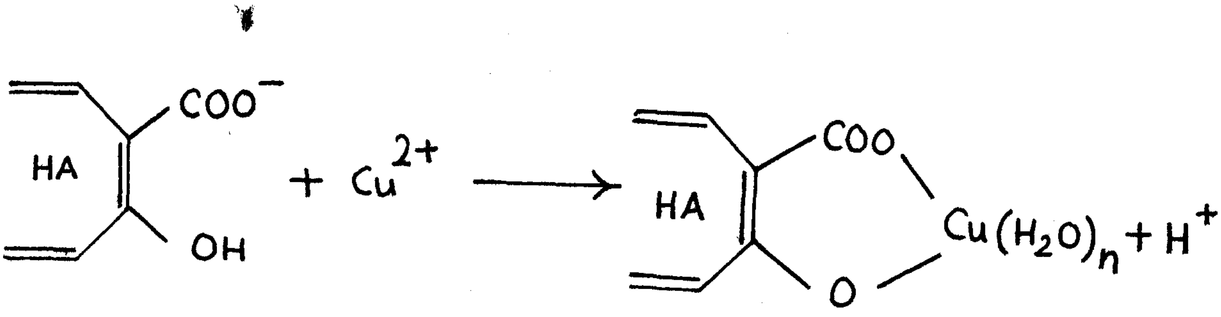

FIG. 1.

whereas at higher pH protons are dissociated from water molecules which were covalently bound to the copper ion, giving a hydroxo complex, e.g.,

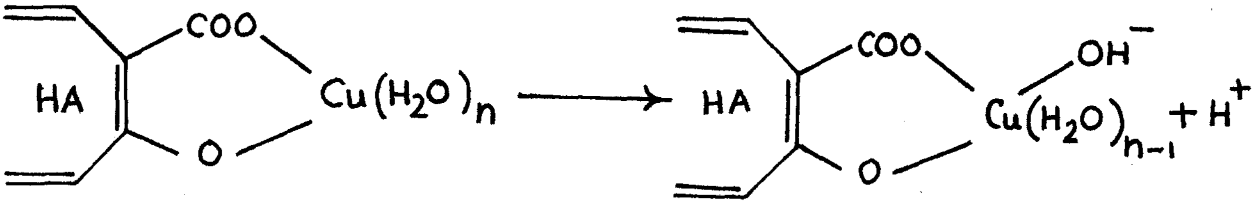

FIG. 2.

Schnitzer and Skinner (63) also arrived at the same conclusion when investigating on fulvic acids. In the reaction involving copper it is assumed that the two ligands acting together in binding metal ions may be neighbouring carboxyl and phenolic hydroxyl groups. That this situation occurs although presumably not exclusively, is confirmed by the investigation of Hime and Barber (69), Schnitzer and Skinner (55) and Davies et al. (71).

From the proposed molecular formula of the organic matter, $C_{20}H_{12}(COOH)_6(OH)_5(CO)_2$, Schnitzer and Desjardin (72) assumed that at pH 3 one carboxylic acid group, at pH 6 five groups, and at pH 9 all six carboxylic acid groups were titrated. They assumed a titration of two phenolic hydroxyl groups of the organic substance at pH 10. The determination of the release of protons during titration of metal complexes shows two protons for Fe^{3+} at pH 3. Therefore, it is concluded that in this pH range $[Fe(OH)_2]^+$ has been combined with one negatively charged carboxyl group by an electrovalent linkage. Beyond pH 9 the complex seems to be destroyed by iron hydroxide formation and by release of three protons. In the case of Al^{3}, one proton has been released at pH 3.6, corresponding to a linkage of $[Al(OH)]^{2+}$ with two carboxyl groups, while the release of two protons at pH 7 is obtained by a reaction between $[Al(OH)_2]^+$ and one carboxyl group. Three protons are released beyond pH 9 to form $Al(OH)_3$ and the organometallic complex is finally destroyed.

<u>Stability constants and stoichiometry of the complex.</u>

The fact that humic substances form stable water soluble complexes with polyvalent metal ions has been well established. An important characteristics of a soluble metal-organic matter complex is its stability constant, the value of which provides a quantitative measure of the affinity of the metal for the ligand.

Numerous attempts have been made to determine the stability constants of metal-soil organic matter complexes. Extensive use has been made by soil scientists (73,69,74-82) of Schubert's (83) ion exchange method but essentially all of the measurements have been made using the simplified approach described by Mortel and Calvin (84). This determination is based on the fact that the quantity of metal bound to a known weight of resin at equilibrium is proportional to the concentration of free ions in solution even in presence of a complexant and the stability constant (K) follows the relationship:

$$\log[(\lambda_o/\lambda)-1] = \log K + x \log Ch,$$

where, the reaction is

$$M + xCh = MCh_x$$

and, λ_o = the distribution constant in absence of the complexing agent.

λ = the distribution constant in the presence of the complexing agent.

K = Stability constant of the complex.

x = the number of moles of complexing agent which combine with 1 mole of metal, M.

Ch = the concentration of the complexing agent in moles per litre.

Randhawa and Broadbent (77) calculated log- stability constant for a Zn - humic acid complex to be 4.42 at pH 3.6, 6.18 at pH 5.6 and 6.8 at pH 7.0. From the different values of x of the chelating molecules in the complex obtained at different hydrogen ion concentrations (x = 1.25 at pH 3.6; x = 1.59 at pH 5.6; and x = 1.70 at pH 7.0), these workers assumed a participation of monovalent and divalent form of ions in the reaction. At low pH values the amount of the monovalent form is predominant, which is lowered with increasing pH values in favour of the divalent form. From the observed results it was also concluded that the concentration of the ligand available for the complex formation is increased with decreasing hydrogen ion concentration of the exchange solution.

Miller and Ohlrogge (76) found that there were at least two complexing agents in soil extract, one or more of which formed a 2:1 type of complex and one or more of which formed 1:1 type of complex, the resulting average being 1.55 : 1. The "apparent" stability constant of the combination of agents for Zn(II) was found to be 7.8. The concentration of the ligands was determined as the maximum complexing ability (M.C.A) of the complexant. Courpron (73) using the same ion exchange equilibrium method found a considerably lower log-stability constant value of 2.87 for Zn-humic acid complex at pH 5.0, whereas a value of

7.0 was calculated for Cu(II) - humic acid complex at the same pH. Schnitzer and Skinner (79) investigated in a similar way the pH dependent stability from B_h horizon of a podzol and the metal ions Cu^{2+}, Fe^{2+} and Zn^{2+} at pH 3.5 and 5.0 and in a further publication (80), the complexing properties with the metal ions Pb^{2+}, Ni^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+} and Mg^{2+}. The stability at pH 3.5 decreases in the following order.

$$Cu^{2+} > Fe^{2+} > Ni^{2+} > Pb^{2+} > Co^{2+} > Ca^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$$

At pH 5.0 the order is

$$Cu^{2+} > Pb^{2+} > Ni^{2+} > Mn^{2+} > Co^{2+} > Ca^{2+} > Zn^{2+} > Mg^{2+}$$

In these cases the authors reported x values from 0.5 to 2.0 for complexes of fulvic acids with nine divalent metal ions. In the later publications they reevaluated the earlier work on metal-fulvic acid complexes (79,68) and concluded that the deviations were due primarily to analytical errors but also that the concentration of the metal complexes had not always been negligible compared to the ligand. By using an improved analytical method and low concentrations of metal ions Schnitzer and Hansen (78) reported that "x" values were unity for nine metal fulvic acid complexes at pH 3 and 5 and at ionic strength of 0.1. At lower ionic strengths, however, x was < 1.0, presumably due to the formation of mixed ~~for~~ polynuclear complexes.

Among other methods used so far for determining the stability constant and composition of the metal-humic matter complexes, spectrophotometric and the potentiometric methods are important. Schnitzer and Skinner (66) used Job's method of continuous variation in an attempt to determine the composition of complexes formed between a soil fulvic acid and a number of metal ions. Schnitzer and Hansen (78) employed Job's method in an attempt to determine both composition and stability constants of fulvic acid-metal complexes. These authors found it to be a 1:1 type of complex for Cu(II), Ni(II), Pb(II), Co(II), Mn(II), Zn(II), Ca(II) and Mg(II) complexes at pH 5.0 and the ionic strengthe of the solutions were maintained at 0.1. These authors also found that at pH 1.70 and $\mu = 0.1$, Fe(II) and fulvic acid formed a molar 1:1 complex; similarly at ionic strength 0.1 and pH 2.35, Al(III) formed a 1:1 complex with fulvic acid. Gamble et al. (85) studied the Cu^{2+} - fulvic acid chelation equilibrium in 0.1 M KCl at $25^{\circ}C$ and concluded that Cu^{2+} reacted with fulvic acid to from a site bound salicylic acid type complex. The result has been supported by a Job's continuous variation plot.

Conditional stability constante (80) of some metal fulvate complexes have been determined by Cheam et al. (86) at $25^{\circ}C$ using specific ion electrode. It has been assumed that metals form site bound complexes and found the order of affinity of some metals toward fulvic acid as Hg > Cu > Cd. Cheam (87) also studied the chelation reaction between Cu(II) and fulvic acid using this relatively new direct approach, i.e., titration with a cupric ion selective electrode and found the equilibrium quotient of 2.2± 0.1 at 25°C and pH 3.0.

Recently Stevenson et al. (88), Stevenson (89,90) used potentiometric method and Takamatsu et al. (91) used a new technique based on the combined use of potentiometric titration and ion-slective electrodes for the determination of stepwise formation constants of different metal-humic acid complexes at different pH and ionic strength employing the Bjerrum's (70) approach as modified by Gregor et al. (99). For a bivalent metal ion, a two step process of the reaction has been assumed, thus

$$HA + M^{2+} \underset{}{\overset{b_1}{\rightleftharpoons}} MA^{+} + H^{+}$$

$$HA + MA^{+} \underset{}{\overset{b_2}{\rightleftharpoons}} MA_2 + H^{+}$$

where the successive constants b_1 and b_2 are given by

$$b_1 = \frac{(MA^{+})\ (H^{+})}{(HA)(M^{2+})}$$

$$b_2 = \frac{(MA_2)(H^{+})}{(HA)(MA^{+})}$$

and the overall constant B_2 is given by

$$B_2 = b_1 b_2 = \frac{(MA_2)(H^{+})^2}{(HA)^2\ (M^{2+})}$$

Values obtained for the formation function $\bar{n}$, and $P(HA/H)$ by analysis of the titration curve of humic acids in presence of metal salt are used to draw the formation curve. It was concluded that 2:1 complexes were formed simultaneously with 1:1 complexes. Thus for a copper-fulvic acid complex log K values obtained ranged from 3.1 to 3.4 at pH 3.0 and ionic strength 0.1 (88). On the other hand for a Pb-humic acid complex the log K_2 values were found to be as high as 8.2 to 8.8 at ionic strength less than 0.01 where K_2 is the $(MA_2)/(A^-)^2(M^{2+})$.

Determination of stability constants was based on the assumption that the protons which are liberated when metal ions react with humic acids at low pH arose from undissociated acidic functional groups. With an increase in pH additional protons have been liberated from hydration water of the metal ions (67). The validity of this assumption was tested in experiments in which sequential addition of metal ion were made to solutions of the humic acid at constant pH (pH returned to initial starting point after each addition). Each addition of metal ion depressed the pH to a lesser extent than the previous addition and less and less KOH was required to neutralise the liberated protons. For Pb^{2+} - humic acid complexes it was found that more protons were liberated at pH 5 and 6 than would be accounted for by acidic functional groups. A similar result was obtained for Cu^{2+} whereas for Cd^{2+} the amount of base required did not exceed the amount of base consumed for neutralisation of acidic functional groups even at pH 6.0. This has been explained on the basis that extra proton resulted from the formation of lead and copper oxide hydrate whereas cadmium oxide hydrate has not been formed at this pH. In later publications, Stevenson (89,90) used the data obtained from the sequential addition of metal ions at constant pH to calculate the stepwise formation constant of Cu^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} - humic acid complexes and found these to follow the order:

$$Cu(II) > Pb(II) > Cd(II) > Zn(II).$$

This modified technique was used for particularly troublesome features of the potentiometric titration curves e.g., the horizontal displacement of the curves when titrated in the presence of metal ions. It has been suggested that mixed complexes are formed, one being phthalic acid type and the other being salicylic acid type complex, as also suggested by other workers. The values of log K obtained from these studies were of several orders of magnitude than those obtained by other methods. Again, in contrast to other studies, these authors found a very insignificant dependence of log K_2 on the pH of the solution.

Although a number of methods have been used in determining the stability constants of metal-humic matter complexes, none of them seems to be unequivocal. Application of the simplified approach (84) of Schubert's ion exchange equilibrium method to the determination of the stability constants of metal - humic acid complexes has been criticised by many workers (92,93,94, 78). Clark and Turner (92) stated that the extracted soil organic matter will form metallic complexes when mixed with metal ion solutions where the central group would be the organic molecule coordinated with more than one metal ion. But the Schubert's ion exchange equilibrium technique is only applicable to systems where only MCh_b type of complexes have been formed (where M = Metal, Ch = ligand and b is an integer $\geqslant 1$). Zunino et al. (93) supported the views of Clark and Turner (92) and explained his view in terms of the earlier reported experimental data. They concluded that the metal ions will form complexes with the polyfunctional macromolecules like humic acid of the type M_aCh, where $a \geqslant 1$. The new interpretation of the ion-exchange equilibrium method made by Ardakani and Stevenson (94) was developed from the general equation of Schubert (83). Also Zunino et al. (93) presented a new approach to the ion exchange method considering proper experimental conditions. Stability of the complex has been explained in terms of maximum complexing ability (M.C.A.). They also demonstrated that some erroneous assumptions were made when the ion exchange equilibrium method for the determination of stability constants of metallic complexes was originally developed (83). According to them, these assumptions compensate with each other but only when the complex involved is of the MCh_b type, which is not formed generally with polyfunctional macromolecular substrates.

In a recent publication McCarthy et al. (95) reviewed the literature dealing with the application of Job's method of continuous variations to the study of soil organic matter-metal ions interactions and believed that this method is not directly applicable to the study of soil organic-matter-metal ion interactions. Explanation was also proposed to account for the deceptive results which were obtained by the application of this method by other workers. Those authors concluded that for highly complicated structure and heterogenity of humic acid molecules, studies of the metal complexes in terms of Stoichiometry and thermodynamic stability constants are hardly possible. Possible interferences, due to scattering of light, during the study of soil organic matter-metal ion reactions by U.V.,visible absorption spectroscopy were considered to be a serious limitation of the spectroscopic study.