CHAPTER I

Introduction
INTRODUCTION

One obviously feels somewhat embarrassed to write something on humus, since the available literature on humus researches is tremendously vast. Also the nature and type of investigations already carried out are extremely varied in character. Humus, the most important soil organic matter, is essentially a complex mixture of high and low molecular weight substances, humic and fulvic acids respectively. Here it would, therefore, not be out of point to have a brief discussion on high polymer chemistry. The development of macromolecular chemistry has given a new impetus to colloid research. In the beginning of the third decade of the present century Staudinger and his school pointed out that the primary particles of synthetic and naturally occurring high polymers are to be regarded not as colloidal aggregates of small molecules but as extremely large molecules of colloidal dimensions consisting of a large number of atoms which are joined together primarily by chemical valency bonds. The micellar theory which once acted as the limelight in the study of the high molecular weight substances, was thus rejected. The properties of polymeric substances are due, rather to the remarkable properties of long chain molecules. This macromolecular concept, which underlies all modern polymer researches, also profoundly and greatly influenced the development of modern views on the nature of a number of biological substances and the organisation of cells and tissues. They are no longer bio-colloids but should logically be termed "biological high polymers or biopolymers".
The bio-polymers carrying electrical charges are extremely important. Proteins, which carry both positive and negative charges (polyampholytes), protamines carrying predominantly positive charges, and a vast group of negative charge carrying macromolecules like nucleic acids, hyaluronic acid, chondroitin sulphuric acid, mucoids and most of the polysaccharides of vegetable and animal origin belong to the group of electrically charged biopolymers. Humic acids of the soil are obviously biopolymers carrying predominantly negative charges. All these biopolymers combine the behaviours of polymers and electrolytes. Though weak, humic acids are essentially polyelectrolytes. The classification of biocolloids as polyelectrolytes instead of associated colloids has given birth to a new era in the history of polymer-research involving the especially intriguing field of biological interaction in the surface of the cell, bacteria and virus.

The term "humus" represents a complex mixture of brown and dark coloured amorphous substances, which have originated during the decomposition of plant and animal debris usually in soils, composts, peat bogs and water basins. Humus is essentially a mixture of several groups of chemical complexes, defined by certain common and characteristic properties. From each group, a large number of different well-defined organic compounds have been separated by various workers, the pioneer among them was Schreiner and Shorey (31,32,33). According to Oden (01) the
soil humus can be separated into four groups of organic complexes. Those are

1. **Humins** - insoluble in alkalies and in alcohol. This fraction mainly consists of undecomposed plant materials. This insoluble residue is variously known as humus coal, humin or ulmin.

2. **Humic acid** - insoluble in alcohol, readily soluble in dilute alkalies and precipitated by acids.

3. **Hymatomelanic acid** - soluble in alkalies and precipitated by acids, the precipitate is soluble in alcohol. This fraction is also known as Umic acid.

4. **Fulvic acid** - soluble in alkalies and acids. This fraction corresponds to apocrenic acids of Berzelius.

Using a selective adsorption technique on activated charcoal, Forsyth (F1) resolved Fulvic acid into four different fractions which are:

5. **Fraction 'A'** - the effluent and washings (0.1N HCl) consisted of water soluble constituents such as sugars and amino acids.

6. **Fraction 'B'** - obtained by elution with 90% acetone was a pigment described as phenolic glycoside.

7. **Fraction 'C'** - eluted with distilled water was a polysaccharide containing glucose, galactose, mannose, arabinose, xylose, and glucuronic acid.

8. **Fraction 'D'** - recovered with 0.5 N NaOH, was a dark coloured material rich in nitrogen and contained pentose sugars and organic phosphorus compounds.

Additional separations of these fractions were made by
dialysis and chemical fractionation procedures by Stevenson (S4).

The above classification of soil humus into its main constituents can be shown as in the following diagramatic scheme:

<table>
<thead>
<tr>
<th>Humus</th>
<th>Treated with alkali</th>
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<tbody>
<tr>
<td>Insoluble Humin</td>
<td>Soluble alkali metal salts of Humic and Fulvic acids Made acidic to pH 2.0–3.0</td>
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<tr>
<th>Filtrate</th>
<th>Precipitate</th>
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<tbody>
<tr>
<td>Alkali and acid soluble Fulvic acid. Adsorbed on activated charcoal</td>
<td>Crude Humic Acid (crnic acid of Berzelius) Electrolysed, dried and extracted with boiling alcohol</td>
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<table>
<thead>
<tr>
<th>Filtrate</th>
<th>Residue</th>
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<tbody>
<tr>
<td>FRACTION 'A' Eluted with 90% Acetone</td>
<td>Insoluble Humic Acid Adsorbed on activated charcoal</td>
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<table>
<thead>
<tr>
<th>Effluent</th>
<th>Residue</th>
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<tr>
<td>FRACTION 'B' Eluted with water</td>
<td>Alcohol soluble Hymatomelanic acid</td>
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<thead>
<tr>
<th>Filtrate</th>
<th>Precipitate (brown)</th>
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<tr>
<td>Dialysed</td>
<td>FRACTION 'B1' (Soluble in ethanol, 90% Acetone but insoluble in water)</td>
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<tr>
<th>Colloid</th>
<th>Dialysate</th>
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<tr>
<td>FRACTION 'B2' (High molecular weight)</td>
<td>FRACTION 'B3' (low molecular weight)</td>
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<table>
<thead>
<tr>
<th>Effluent</th>
<th>Residue</th>
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<tbody>
<tr>
<td>FRACTION 'C' Eluted with 0.5N NaOH</td>
<td>Neutralized to pH 7 and dialysed</td>
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<table>
<thead>
<tr>
<th>Colloid</th>
<th>Dialysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRACTION 'D1' (High molecular weight)</td>
<td>FRACTION 'D2' (low molecular weight)</td>
</tr>
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</table>
The fractions into which Oden divided soil humus are demonstrably not single substances and it is not yet known definitely how complex they are. Attempts have, therefore, been made in recent times by a number of investigators applying chromatographic and electrophoretic techniques to prove the heterogeneity of humic, hymatomelanic and fulvic acids. These newer techniques of resolution of a complex mixture of closely related substances have given a tremendous impetus to isolative chemical researches for establishing the homogeneity of a chemical entity. Separation of humic acids by paper electrophoresis was begun by Robinson, Martin and Page (R1), followed by Swaby (S5), Bremner and Arnold (B1). The latter workers found only one component by this means whilst it was claimed by many investigators that humic acid is a multi-component system. Pavel et al (P1) observed several fluorescent areas on paper chromatograms. It was observed that polymers produced by the oxidation of polyphenols (B1) showed similar patterns on paper (S6). Continuous paper electrophoresis failed to produce any significantly different fractionation of the humic acids as far as one could judge from the aminoacids contained in the fractions (S5). Coulson, Davies and Khan (C1) have subjected humic acids to both high and low voltage paper electrophoresis over a wide pH range 1.00 - 12.0. They noted separation when the buffer pH was 6.4. Three main fractions were recognized in buffers of pH higher than 6.4 when electrophoresis was carried out at 33 volt/cm. These were:

(i) a small immobile fraction,
(ii) a main mobile component, and
(iii) a streaky fraction with migration values between
those of (i) and (ii). This fraction was usually present in larger amounts than fraction (i). Martin and his associates (M7) have examined by Tiselius electrophoretic technique the organic matter extracted from Brookston clay with neutral sodium pyrophosphate and have shown that it consists of one main component with a second component present in traces.

Paper chromatographic method of resolution of humic acids was tried by Pavel et al (P1), Coulson et al (C1), and Khan (K1). Khan tested some fifty organic solvent systems. Ascending chromatography was found to be much better than the descending one. The paper chromatograms were examined both under ultraviolet and visible light for observing fractionation. The chromatograms were found to fluoresce beautifully in ultraviolet light. Several fluorescent areas or spots were observed by a number of workers. Only a partial success has been achieved in the fractionation of humic components by this technique.

 Adsorption column chromatography was tried by Hook (H1) who attempted to resolve natural humic, hystatomelanic and fulvic acids through columns of alumina, examining for fractionation with both visible and ultraviolet light. Recently Kononova et al (K2), using alumina as well as starch columns, claim an improvement as judged by the same criteria. Forsyth (F1) separated natural humic acid into fractions by selective adsorption on a bed of activated charcoal followed by elution with suitable solvents. Recently Bromfield, Coulson and Davies (B2) attempted fractionation of humic acid on a column of celite '555' using acetone-water and acetone-neutral sodium pyrophosphate as eluants. With a large
number of synthetic ion-exchangers like "zeo-karb" 225, and 226, Amberlite C.G.45, De-Acidite G, the Cellulose ion-exchangers DEAE - Cellulose and ECTEOLA - Cellulose, as well as ion-exchange papers of the carboxymethyl type, phosphorylated type and the aminated type Coulson et al (C1) found no fractionation of humic acids. By developing a column of cellulose by a gradient of isopropanol into ethylacetate (1:1), then water into the isopropanol, Sowden and Deuel (S8) achieved some separation of fulvic acids into several distinct components, some of which were found to fluoresce in U.V. light.

The soil-factors which influence the formation of humus from organic residues of vegetable and animal origin are the mechanical composition of the soil, its physical conditions, notably, its texture, together with its chemical and biochemical properties, especially bacterial population and presence of available mineral elements. The formation and nature of humus are also influenced by the system of crop rotation, by fertilizer treatment, by utilization of green manures, by abundance of animals in the farm and climatic conditions. Naturally, therefore, variation in the nature and abundance of humus with soil types and within the same soil profiles is expected. And actually there are several types of humus: Tree humus or pure humus; field, orchard and garden soil humus, pasture soil humus; forest humus; garbage humus; water or marine humus. In evergreen forests, the largely organic surface layers are usually not mixed with inorganic soil layers, the former are referred to as "raw humus" or "duff". In deciduous
forests, the organic residues and their decomposition products are well mixed with the inorganic part of the soil, giving rise to a type of humus known as "Mull".

The humus content of soil varies considerably, from extremely small amounts, as 0.1% in some poor sandy soil, to as much as 20% in prairie soils, it may be even higher in peat bogs, say, up to 50-80%. Light-texture soils contain less organic matter, sandy loam 1.03% organic matter, while heavy textured soils contain more. Clay loams contain 1.72% organic matter.

Humus is not distributed evenly through the soil depth. It is largely concentrated in the upper 4-12" or the surface soil layer and decreases rapidly in the subsoil. In brown forest soil and in the red and yellow soils, there is a rapid drop in the organic matter content below the upper 6". In prairie soil, chernozems and chestnut brown soils, there is a gradual decrease of organic matter with depth. Podzolic soils have a zone at the junction of the surface litter layer and mineral soil layer, very rich in humic material. A well marked humus podsol may also have an accumulation of humic matter in the B1 horizon. Here, the total organic matter may be up to 5 or 6% by wt., and 75-80% of this can be extracted as humic acid.

**Solvent Extraction of Humic Matter**

Many investigators have determined the relative proportions of humin, fulvic, hynatomelanic, and humic acids in different soils. However, no consistent pattern of distribution was obtained, either in the profile or in different soils. This might
have stemmed from the different methods of estimation employed. At present, however, the many different extraction methods used make it difficult to correlate the results of different workers.

It is for these reasons, that a little space, in this introductory review should be allowed for a better understanding of the situation.

Humic acids, it is assumed, occur in soils as metallic salts or metallic complexes of polyvalent cations like Ca$^{2+}$, Fe$^{3+}$ and Al$^{3+}$ which are usually present in the soil. It is very likely that humic acids form adsorption complexes with clay minerals by means of molecular and vanderwaals' forces, the mechanism of which is still not quite clear. This lack of knowledge is seriously reflected in the difficulty encountered in isolating organic colloids from the inorganic material of the soil. Any advance in our present state of knowledge regarding the mechanism of the interaction may, therefore, lead to improvements in methods of extracting organic matter from the soil. Soil chemists have usually two main objectives in view in the isolation of organic matter from the soil. These are (i) separation of humic material from unhumified substances, and (ii) complete isolation with minimum chemical degradation of humic colloids from the mineral matter.

The solvent systems which are usually employed in the extraction of soil organic matter can be broadly divided into the following groups: (1) Acidic solvents; (2) Strongly basic solvents; (3) Weakly basic solvents; (4) Chelating reagents as solvents and (5) Basic solvents with reducing character. (1) Acid Solvents: Removal of the mineral matter instead of the organic material by treatment with anhydrous HF has been suggested as an
approach to the problem (33). Hence HF alone and in combination
with HCl has been employed by a number of investigators (39, 22,
B4). Although pretreatment of soils with dilute acids like HCl
is a common procedure (employed to remove exchangeable bases,
carbonates, plant and insect remains), this pretreatment with
dilute acids particularly with HF should be performed with great
cautions, because, considerable quantities of soil organic matter
may thereby be removed. Recently some workers (39, 22) have
reported the removal of organic matter particularly from the B*
horizon of podzolic soils by treatment with HCl and HF acids and
by their suitable combinations. Not only inorganic acids but
organic acids like lactic and anhydrous formic acid (B5, T1)
have also been found to dissolve considerable quantities of the
organic matter from the soil. Systematic investigations with
these newer solvents have not yet been carried out.

The most popular and extensively used solvent is a cold
dilute solution of NaOH. NaOH of different strength has been
employed by a large number of investigators (22) since the days
of Sprengel (310). Although NaOH brings a considerable portion
of the soil organic matter into solution, it has serious drawbacks
in that it is a drastic solvent, and its use may cause deep-seated
changes in the physico-chemical properties of the organic matter
as outlined below. (i) Alkalies dissolve not only the humified
material but also lignin, hemicelluloses and protoplasmic subs-
tances from fresh organic tissues in the soil; (ii) Humic
substances are often contaminated with mineral matter, particularly
silica brought into solution by alkalies; (iii) Auto-oxidation
of the organic matter (26) with consequent release of CO₂ increases
as the alkalinity increases. Finally, it may be concluded that the more alkaline the solution, the longer the period of extraction, and the higher the temperature, the more detrimental will be the effects. Nevertheless, for a fairly complete extraction of soil organic matter, caustic alkali is still the only solution to the problem.

Several workers (T2) had used weakly basic solvents like AmOH, AmgCO₃, Na₂CO₃, NaHCO₃ and their different combinations for the extraction of soil organic colloids. Mattson and Koutler-Anderson (M1) and Sohn and Feach (S11) recently pointed out that ammoniacal solution would be unsuitable for any studies on the chemistry of soil organic matter, because of the side reactions of ammonia not only with lignin but also with other phenolic and quinone compounds, and probably with the carbohydrate components as well. 0.5M Na₂CO₃ has been employed as a solvent for humic colloids by several investigators (T2). However, it is quite clear that weakly alkaline solvents extract much less organic carbon and nitrogen than NaOH of the same strength.

Milder methods of extraction were tried by Simon (S12) and more systematically by Bremner and Lees (B7). The latter workers investigated the extracting abilities of various neutral reagents, particularly the Na-salts of organic and inorganic complex forming acids, and Sodium pyrophosphate, sodium oxalate, sodium fluoride, and sodium citrate were found to be most effective for the purpose. Among these reagents, however, pyrophosphate was by far the most useful, but all of these salts extracted less organic matter than did NaOH, especially from mineral soils.

Hamy and Leroy (H2, H3) surveyed the organic matter extracting
powers of a number of ammonium salts of various carboxylic and hydroxy carboxylic acids in 0.5 N neutral solution. Oxalate, malonate and salicylate were much more effective than succinate, maleate, fumarate, adipate or phthalate. In another study with different metallic malonates the order of extraction followed the series Li > Na > Am > K > Mg.

A number of organic complex-forming reagents such as cupферон, 8-hydroxyquinoline, acetylacetone, and Na₂EDTA were tested recently by Martin and Reeve (M2) who found that these reagents removed large amounts of organic matter from the B-horizon of podzolic soils. Schnitzer, Wright and Nesjardins (S15) observed that Na₂EDTA dissolved most of the organic matter from the B-horizon, but very little was extracted from the A-horizon of a podzolic soil.

Tinsley and Salam (T4) have shown recently that sodium sulphite is as effective as pyrophosphate at comparable concentrations and pH values. A fresh 0.25N Na₂SO₄ solution maintains a pH of 8 - 8.2 during extraction which is high enough to allow dispersion of the organic matter without undue hydrolysis and without oxidation effects on the humus compounds.

On the basis of these informations, it seems very doubtful whether any aqueous reagent can be devised which will dissolve the whole of the organic matter in a single extraction under conditions which are sufficiently mild to prevent serious hydrolysis of the polymeric humus compounds. And in fact, the subject of humus-research has been to some extent handicapped largely due to the lack of a proper extraction procedure.
Molecular Properties of Humus

A large number of attempts have been made in recent years to elucidate the molecular properties of humic acid and their different fractions. All these attempts have taken the help of various physical techniques including electrometric methods, X-ray spectra and electron microscope, ultraviolet and infrared spectrophotometry. Physico-chemical work on humus in recent years has largely centered around the following topics: characterization by viscosity measurements, molecular weight determination, pH titration curves, chemical and physical properties under saturation by various cations, utilization of the ultraviolet, infra-red, X-ray and electron microscope evidences to throw light upon the chemical structure.

Humic Acid as a Polyelectrolyte

It has been pointed out earlier that humic acid is a polyelectrolyte. The most interesting property which distinguishes a polyelectrolyte from a neutral polymer is the way in which the reduced viscosity ($\eta_p/c$) is dependent on concentration. For a neutral polymer, the curve ($\eta_p/c$) vs. $c$ is linear in the low concentration region, having a positive slope with the $c$-axis, while for a polyelectrolyte ($\eta_p/c$) vs. $c$ curve rises rapidly with diminishing concentration. Behaviour typical of polyelectrolytes has been observed by Piret et al (FS) and by Mukherjee & Lahiri (M5) for their peat and coal humic acids respectively. The intrinsic viscosity of peat humic acids was estimated at 11.5 ($c.0. /gm$) by Piret et al whilst that of coal humic acids apparently amounts to only 20% or less of the value.
11.5 (c.o./gm). Sen (314) reported a value of 21 (C.C./gm) for the intrinsic viscosity of humic acid isolated from an Indian soil. From viscosity measurements Piret et al concluded that their humic acids were nonspherical (in shape) and polyelectrolyte in nature. They have also calculated the probable axial and frictional ratios for their humic acids on the basis of some assumed models and assumed hydration. Working with both natural and synthetic humic acids, Flaig and Beutelspacher (23) obtained linear curves showing the reduced viscosity to be independent of concentration leading to the conclusion that the humic particles are spherical in nature.

MOLECULAR WEIGHT

Knowledge of the molecular weights of humic acids and their different fractions is of the utmost importance for the elucidation of their structures and consequently attempts, both direct and indirect, have been made to determine it. The term "humus" does not represent a definite, uniform compound, but is a collective term for a group of complex polymeric substances of nearly similar chemical properties but of very different molecular weights. It is not surprising, therefore, that the molecular weights of humic acids reported in the literature vary widely. From the measurements on viscosity and conductivity of sodium humate solutions with dilution, Oden (61) suggested a molecular weight of 960 or 1280 of humic acid fraction. These pointed to a basicity of 3 or 4. Hymatomelanio acid was assigned a somewhat lower value corresponding to an equivalent wt. of 200. These estimates of molecular weight are very
uncertain as Oden himself recognized, since the arguments used in their support apply strictly to pure compounds and not to complex mixtures. The molecular weights cannot be extremely high, since the sodium salts of humic and hymatomelanic acids and the alcoholic solution of hymatomelanic acid itself, are all capable of passing through dense ultrafilters. Assuming that the ammonium salts of humic and hymatomelanic acids are unionized, Sameo and Pirkmaier (S15) were able to reconcile their results with those of Oden. The assumptions are, however, extremely uncertain. Recently from osmometric measurements on the organic matter extracted by dilute inorganic acids (0.5% HF) Wright et al (W1) obtained number-average mol.wt. of 47,000. Fuchs et al (F3) measured the rise in boiling point of certain acetone-soluble oxidation products of humic acid. Values of the molecular weights ranging around 1,400 were obtained. There was no direct evidence of the degree of possible molecular breakdown caused by the mild oxidation employed. Measuring the diffusion coefficient of sodium humate in 0.085 N NaOH in the Northrop diffusion cell Zeile (Z1) obtained molecular weight values of 1,060 and 1650 for two humic acid samples, the first prepared by extraction of soil with hexamethylene-tetramine, the second from the same soil treated with urea to peptide the humic matter. Mukherjee et al (M4) estimated the molecular weight of coal humic acids at 530. Stevenson et al (S16) reported that the humic acids extracted with neutral sodium pyrophosphate from Miami clay have a sedimentation average molecular weight of 53,000 whilst a value of 25,000 for the molecular weight of peat humic acids was reported by Firet et al (F3) from
ultracentrifuge data. Flalg and Beutelspacher determined "particle - weights" for soil humic acids via the ultracentrifuge (P2). Their values of $3 \times 10^4$ lie just between those given by Firet and Stevenson.

ELECTROCHEMICAL AND ION EXCHANGE PROPERTIES

Humic acids are essentially weak polyelectrolytic acids containing several functional groups such as carboxyl, hydroxyl (both phenolic and alcoholic), free amino and imino groups some of which can be estimated by titration. But very little is known about the exact number and stereochemical positions of these groups in polymeric humic compounds. Even we do not know the relative distribution pattern of these groups on the non-protein and protein nuclei which constitute the humic acid molecule. To get an answer to these questions, several attempts have been made by a number of workers from time to time. But their various findings have resulted in a considerable divergence of opinion. The exploratory attempts so far made can be classified into three groups.

(1) Inorganic methods : e.g., Ubaldini (Ul), Kononova (K3). The total acidity is determined by potentiometric or conductometric titration in aqueous solutions or suspensions with baryts. Some investigators have taken the amount of carboxyl groups as equivalent to the amount of CO$_2$ liberated by humic acid solutions from CaCO$_3$ or the amount of acetic acid set free in a calcium acetate solution. Some workers simply suppose that the acid groups which have not reacted with a base at pH 7 are phenolic hydroxyl groups and that
those neutralized are carboxyl groups. Others have taken this limit at pH 8.2. The assumptions involved are extremely crude and neglect the fact that the content of phenolic OH groups would be a function of the concentration of the humic acid solution; more acid groups being dissociated at a certain pH as the solution is diluted. Also the influence of neighbouring groups on phenolic acidity has not been taken into consideration. Van Dijk (VI) has recently shown that conductometric and especially high frequency titration of humic acids in dimethyl formamide with Na-isopropylate enable one to distinguish clearly and determine exactly the carboxyl and phenolic acidities. The maximum in these curves corresponds to carboxylic acidity. The minimum in the H.F. titration curves appeared to correspond more or less with the amount of very weak acid groups (phenolic hydroxyl groups) judging from the total exchange capacity determined by other methods.

The apparent pk values of humic acid preparations in the absence of neutral electrolyte as reported by Dawson et al (D1), Marshall and Pattnik (M5), and Gillam (G1) are in the range 5.4 - 6.1. For Merck humic acid a pk of 6.8 to 7.0, and a titration exponent of about 4.8 has been reported recently by Fommer and Breger (P4). On the other hand, Martin and Reeve (M6) reported a pk value of 5.0 for their humus preparations of low Al content titrated in 0.1 N KCl medium. This difference in pk has been attributed to real differences between the constitution and properties of illuvial podsol humus (used by Martin and Reeve) and humic acids derived from more alkaline soils.

(2) Organo-chemical methods: These are indirect in nature.
Phenolic hydroxyl groups with pKa values less than 6 are determined by blocking the sites with (CH$_3$)$_2$SO$_4$, and determination of the increase in methoxyl content, or by acetylation, and determination of the increase in acetyl content. Gilliam (61) titrated both methylated and acetylated derivatives and compared them with the original humic acid fractions isolated from a prairie, a muck, and a forest soil. In all three cases the acetylated product was found more highly buffered than the untreated humic acids above pH 5. On the other hand, the methylated products showed very little buffering effect above pH 5, the curves being very steep from this point onward. An estimation of the carboxyl group content is made directly by determining the increase of methoxyl groups upon esterification with CH$_3$OH-HCl. The drawbacks of these methods include tediousness, incompleteness of methylation or acetylation, and side reactions with other groups when CH$_3$OH is the methylating agent.

(3) Cation exchange methods: Recently Broadbent and Lewis (28) have discussed in great detail the nature of the functional groups and their most probable pK values by comparing cation-retention experiments with pure aromatic compounds resembling organic-matter in their low solubility and with carboxyl and phenolic groups attached.

The fulvic fraction of soil organic matter has received much less attention than the humic fraction and very little is known about its chemistry. Ponomareva (26) reported an equivalent weight of 160 for this fraction. On the other hand Tiurin, (26) reported that fulvic acids are nitrogenous complexes with an equivalent weight of about 300.
One of the most important properties exhibited by soil humus is its exchange capacity which is quite high when compared with those of silicate clays, its mineral counterpart in the soil. Numerous investigations have been conducted on the exchange reactions and physical properties of humic acids saturated with different cations. Therefore, a very brief discussion on exchange reactions of humus is presented below. Working with K-saturated peat humus Zadmard observed the following orders of exchange for

(1) monovalent cations \( \text{H} > \text{Cs} > \text{Rb} > \text{K} > \text{Na} > \text{Li} \)
(2) divalent cations \( \text{H} > \text{Sr} > \text{Ba} > \text{Ca} > \text{Mg} \).

Working with a mineral soil humus Basu (120) found that the adsorption of trace element cations for H⁺ of crude humic acid follows the order

\[ \text{Zn} \gg \text{Mn} \gg \text{Ni} \gg \text{Cu} \]

and the order of release of these cations from the respective humates for H⁺ and Mg⁺⁺ is : \( \text{Mn} \gg \text{Ni} \gg \text{Cu} \).

Selectivity coefficients calculated from the above ion-exchange data have, however, been found to vary in an irregular fashion (119). With Ca-saturated peat humus the following series : \( \text{H} \gg \text{Ba} \gg \text{Sr} \gg \text{Mg} \) was observed for the divalent ions. Monovalent cations entered K-humus somewhat more strongly than the divalent cations of comparable size, especially at the higher concentrations. The reverse was the case with Ca-humus. Hydrogen entered the calcium system more energetically than it did with the potassium humus. Working with clay minerals, crude humic acid, and a soft coal Schachtschabel (117) clearly envisaged that in natural soils the exchange cations should be present in different proportions on the different constituents. The cation distribution would be by discrete areas or zones.
OPTICAL PROPERTIES

In literature numerous x-ray and electronmicroscope studies on the structure of humic acids have been reported, but these studies have yielded no fruitful information, rather they have led to considerable divergence of opinion. Thus Sedletsky and his co-workers (S18-21) have concluded from x-ray, electronmicroscope and thermal investigations that humic acids from soils and peats are identical in constitution and have a crystalline structure that is basically aromatic (graphite lattice). Gemmerling and Zyrin (G2) have deduced from their x-ray studies that humic acids from different soils are not identical but there is a crystalline component which does not vary much from soil to soil. On the other hand, Jung (J1), Gorbunov (G3), Flaig and Beutelspacher (F4), Botteri (B9), and Schuffelen and Bolt (S22) have all been unable to detect signs of crystal structure in humic acids by x-ray or electron microscope examination. According to Gorbunov (G3) Debye rings are apparent only in samples of humic acids containing more than 2% of mineral salts, and the diffuse rings sometimes obtained with humic acid preparations are caused by intramolecular diffraction. Only two characteristic diffuse rings have been recognised and these are only sufficient to suggest a similarity to planar polycyclic benzenoid compounds in general. 

U.v. spectra of humic acid samples were too diffuse to reveal any significant structural detail. The u.v. spectra taken by Scheffer and Welte (S23) and by Baudnitz (B2) contained a shoulder in the 250 - 270 m\(\mu\) region. Scheffer and his associates (S25) concluded from ultraviolet spectroscopic
examination that the dark brown substances present in autolyses of Aspergillus niger and of an actinomycete strain from a Calcareous soil had structures similar to that of the grey humic acid of soil. For purpose of comparison and characterisation German and Russian workers have taken the help of optical properties. For example, Frömel (F5) compared the absorption of light by humic acids from various sources and deduced from this and from a comparison of their base exchange capacities that all the preparations examined had similar chemical structures. Kononova, Pankova and Belchikova (K4) measured the absorption spectra of humic acids from manured and unmanured podzol and chernozem soils and concluded that there was no significant difference between the humic acids of unmanured and manured soil, but that of podzol humic acid differed markedly from chernozem humic acid. Aleshin and Zhupakhina (A1) studied the absorption of light by NaOH and Na-oxalate extracts of various soils and deduced that the humic acids in these extracts had identical optical properties.

Attempts have been made in recent years to obtain useful information from a study of the infrared spectra of humic substances. Durie and Murray (D2) have indicated the probable structures giving rise to absorption bands in the spectra of the organic fractions of a peat and of a soil, after separation by hydrofluoric acid treatment; Kumada and Aizawa (K5) have recorded the spectra of humic acids from various soil types including peats, together with artificial humic acids, and Elofson (E2) has compared the spectra of humic acids from peat, lignite, and oxidized hydroquinone with those of various
chars and coals. This work has shown that there is a broad similarity between the infrared spectra of humified material from diverse sources. The spectra indicate that a largely unsaturated structure having both phenolic and carboxyl hydroxyl groups is formed. Farmer and Morrison (F6) have studied phragmites peat and its humic acid by infrared spectroscopy. They have obtained information about the presence of carboxyl and phenolic hydroxyl groups and of aromatic rings derived from lignin. Changes in the infra-red absorption of the humic acid, following methylation and reduction have given information on the acidic unsaturated structures present. Infrared spectra of lignoproteins isolated from soils and composts by Sevag technique have been recorded by Jenkinson and Tinsley (J2). The effect of acid hydrolysis on the ligno-proteins was also followed through infrared spectra. These authors have detected the amide I and II bands and the less pronounced aromatic bands in the materials studied.

THEORIES OF HUMUS FORMATION

Our understanding about the chemical structure of the constituents of soil humus and their nature of formation is still very obscure. There are now two distinct theories regarding the origin of the humic substances of soil. One is that they are formed by the alteration of plant lignins entering the soil; the other being that they are products synthesized by, or formed by autolysis of, soil micro-organisms. The former is represented by the ligno-protein theory of Waksman (W2) and the lignin-ammonia theory of Mattson and Koutler-Anderson
During 1920 to 1930 the German coal chemists Fischer, Schrader, Puchs et al (F8) observed that simple sugars, starches, hemicelluloses and cellulose of plant residues can not participate in humus formation since they are easily decomposed by micro-organisms with completely mineralized end products and low-molecular weight organic acids. Independently in 1930 Waksman came to the same conclusion from studies of decomposing plant materials, particularly in composts. Lignin, the more resistant of the plant components, was thought to be the main precursor of humic matter. Lignin is converted into humic matter by oxidation and simultaneous condensation with protein, a product of microbial metabolism.

The Swedish scientists, Mattson and Koutler-Andersson, held a more or less similar view that humic complexes of the soil are derived from lignin by auto-oxidation and ammonia-fixation, ammonia being liberated during the decomposition of plant residues. Nitrogen enters irreversibly into the aromatic rings of lignin and forms complex cyclic compounds characteristic of humic substances.

Support in favour of the lignin origin of soil humus has been obtained from recent works of Morrison (M9), Steelink et al (S21). These workers have isolated aromatic compounds of the polyphenol type which are known to be the structural constituents of lignin molecule. The isolation in traces of a genuine ligno-protein complex from the soil by Tinsley and Maung (T6) however, shows that such complexes do not form a
major part of the soil organic matter. The observation by Bremner and Shaw (H10) that the rate of mineralization of nitrogen in a soil humic acid is intermediate between that of nitrogen in the form of lignin-ammonia and that in the form of lignin protein can be explained by incorporating both the theories of humus formation just mentioned.

The ligno-protein and lignin-ammonia theories of humus formation have been strongly criticized by Kononova, Swaby, Flaig and others on the ground that these concepts have limited not only the possibility of various plant constituents in humus formation, but also the function of micro-organisms in this important natural process. The oxidation of lignin with simultaneous condensation with microbial protein or ammonia was regarded as a complex physico-chemical process occurring without the help of micro-organisms.

Kononova, Swaby, Flaig and others have considered that the initial stage in humification is a microbial breakdown of organic residues of vegetable and animal origin into simpler constituents. Polymerization of these simpler units to particles of specific high molecular weight organic substances, which appear to be humic substances, is the final stage of the process.

Kononova postulates a polymerization of polyphenolic units liberated during the decomposition of lignins, tannins, plant respiratory enzymes having an aromatic structure and certain products of microbial metabolism. Swaby and Flaig have postulated an oxidation of polyphenols to quinones, and a coupling of these with amino acids or peptides derived from
microbial protoplasm. The oxidation of polyphenols to quinones has been considered by Kononova a biochemical process occurring with the help of enzymes of the phenol-oxidase type. Enders (E3, E4) has advanced the theory that humic acids are formed by condensation of amino-acids and methylglyoxal produced by autolysis of micro-organisms and has much in common with the formation of melanoidins during the manufacture of beer. These newer concepts of humus genesis clearly regard the nitrogen as forming a structural part of the complex humus molecule. But some workers like Tyurin (T7) believed "that humic acid per se as a specific compound is nitrogen free substance". Evidence in favour of this view has recently been presented by Burges (B5) who has shown that humic acid isolated from the B2 horizon of a podzolic soil by lactic acid dispersion contains only 0.7% nitrogen which is clearly indicative that the essential structure of humic acid does not contain nitrogen. The view that relatively simple substances like glucose can participate in humus formation has been supported by the recent work of Mayaudon and Simonart (M10). The isolation of humic acid like substances from micro-organisms grown on glucose and mineral salts (M10) has led some workers to regard humic acid as a product of microbial synthesis. It has been pointed out that the process, which has been postulated as producing humic acids, can be separated into three groups, all involving polyphenols or polyphenol producing materials to a greater or lesser extent. The relative contributions of these groups to humic acid formation could well depend upon the actual conditions prevailing in the site.
IMPORTANCE OF HUMUS IN SOILS

For the sake of completion of an introductory review on humus, mention should be made of its importance in the soil. The functions of humus in the soil are manifold. These may be considered as:

1. **Physical** - It has important physical effects upon the soil, e.g., it modifies the soil colour, improves the soil structure, provides better aeration; has a binding effect upon the soil particles; increases the water holding capacity of the soil; helps the soil to absorb more heat and lastly it increases the buffering properties of the soil, preventing rapid increase in acidity or alkalinity.

2. **Physiological effect** of humic substances on plants is shown by increased penetrability of the cell membranes, by participation in metabolism and also by stimulation of the plant enzyme systems. This stimulation is connected with the presence of quinone groups in the humic substances. Chaminade and Blanchet, in experiments with the epidermis of viola cornuta flowers, have found that the presence of humic substances in a solution of mineral salts hastened the occurrence of deplasmolysis in the plant cells and favoured the penetration of mineral elements through the cell membrane. This statement was confirmed by sand and soil cultures in glass house experiments (ryegrass and rye), whereas application of small amounts of humic acid increased the uptake of mineral nutrients, particularly N and P, and in a number of cases increased 'Crop Yields'.

3. **Nutritional effect**. It serves as a store house of
plant nutrients, the slow but gradual decomposition of the organic matter by micro-organisms results in the liberation of a continuous stream of CO₂, of available N as NH₃, which is soon changed to nitrate & of phosphorous, and other elements essential for plant growth.

(4) Chemical effect. It has certain chemical effects upon the soil constituents, such as rendering P and other elements required in plant nutrition more soluble. This capacity to mobilise nutrients is rated as one of the outstanding characteristics of acid humic colloids functioning in the soil. It neutralizes substances which tend to be toxic to plants. Substances present in the fulvic portion may effect the mobilization of sesquioxides during podzolization.

(5) Biological effect. It has an important effect upon the biological state of the soil, making it a more favourable medium for the development of the root systems of plants, and for the growth of micro-organisms essential for soil processes.