

Lectures on
Soil Organic Matter

by

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Foreword

To my friends and the friends of the subject.

In this manuscript are some unpublished results and therefore only for friends and not to use for publication.

I would enjoy having any comments on this material.

W. Flaig

Acknowledgments

This manuscript came about as a result of the kind invitation of Prof. Dr. W. H. Pierre, Head, Department of Agronomy, to give lectures about soil biochemistry. I am very thankful for this opportunity.

I would like to express my best thanks also to my colleague, Prof. Dr. Lloyd Frederick, who stood by me helpfully at all times during the writing of these lectures in the English language and I appreciate his suggestions during our many discussions.

Without the help of his co-workers, Messrs. McIntosh Sims, Horton, Brown, and of the secretaries, Mrs. McLaughlin, Misses Sansgaard and Zart, it would not have been possible to mimeograph the lectures. Also to these, many thanks.

June, 1959

W. Flaig

SOIL ORGANIC MATTER

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by

W. Flaig and H. Beutelspacher

Humic substances form one of the most important factors for the fertility of soils. Partly humus consists of a mixture of various compounds of low and high molecular weight. Only a few ingredients could be isolated from this mixture and still these had no uniform composition. Humic substances are defined as substances which can be dissolved out of the soil by means of

- a) dilute alkaline solutions
- b) solutions which form difficult soluble compounds with calcium ions
- c) solutions of neutral salts

Further on humic acids are insoluble in alcohol. They disperse with alkali and can be precipitated by adding mineral acids.

For purification they are dissolved in alkaline solutions and are reprecipitated with acids several times. In between they are centrifuged. For the removal of ions they have to undergo dialyse and an electrodialyse. From different properties of the humic acids we may presume that they own a higher molecular weight at least. Already according to their behavior to the dialyse membrane they must have the properties of colloid substances. Their difficult solubility in different organic solvents points out their higher molecular structure, too.

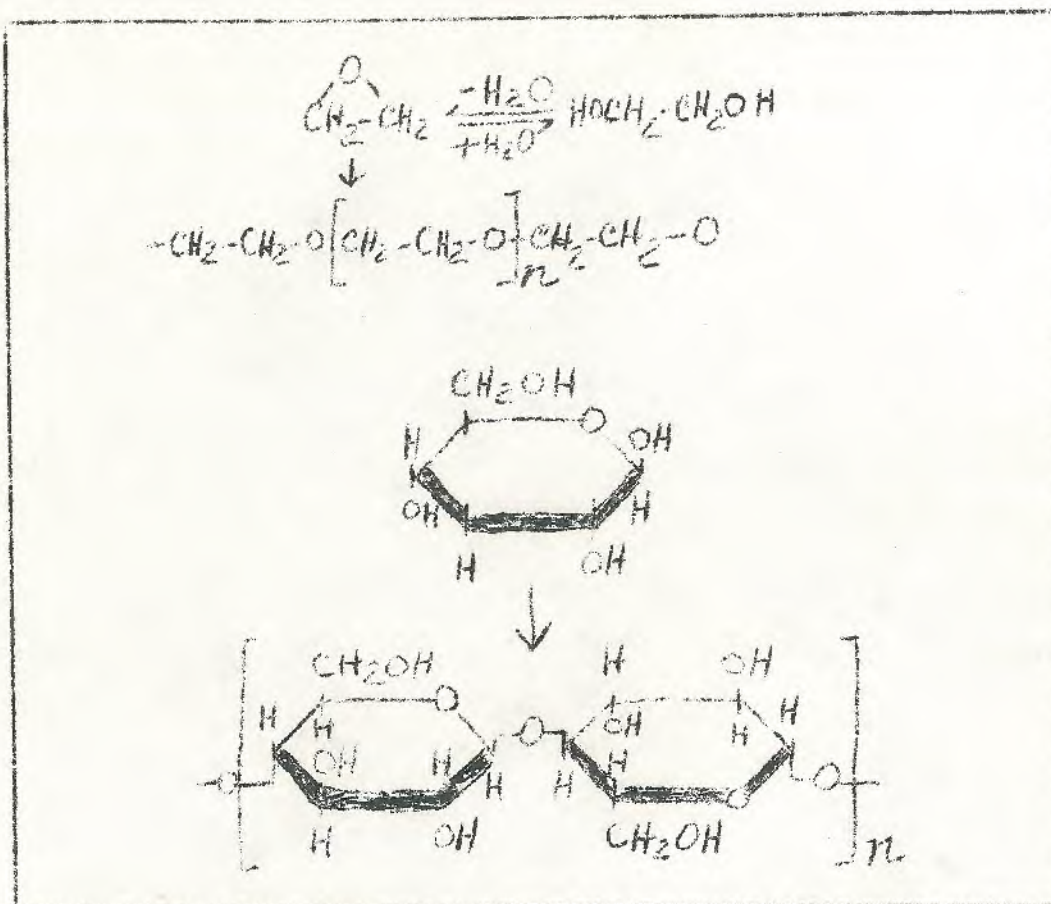
Since for the explanation of the constitution of natural substances with a higher molecular weight the knowledge of the elementary composition and the molecular weight are not sufficient enough so also the shape and size of the particles have to be established by means of physical chemical methods. In addition it is important to know the charge of the particles of colloids. Various chemical reactions point out that humic acids are made of redox-systems. The property certainly has great importance for the processes which take place in the soil and they may be important too for the growth of living beings.

At the same time humic acids can act as ion exchangers. Since humic acids are substances of higher molecular weight, which own different functions, the explanation of the chemical constitution will be very difficult. Certainly there is no other natural product of high molecular weight which owns so different properties.

The varying high content of the part of nitrogen which cannot be split off by means of aqueous alkaline solutions, forms another factor causing trouble in detection of single properties.

When the constitution of other natural products of high molecular weight had been explained, it was possible to gain known cleavage products either by oxidation, in the case of rubber, or by hydrolysis, in the case of cellulose, glycogen, or polypeptides. Concerning humic acids this way seemsto be impossible.

From this reason there is no possibility for a synthesis by means of monomeric substances which have been obtained by the cleavage. So this natural product can be studied only by means of model substances. It is of fundamental importance to have knowledge of the functional groups of the monomeric substance and their principals of linkage, if the structure of natural compounds of high molecular weight shall be explained. We want to take into consideration that e.g. polyoxymethylenes as models for forming cellulose; polyamides as a model for proteins and polymeric butadien (1) forming rubber, brought a lot of new knowledge.

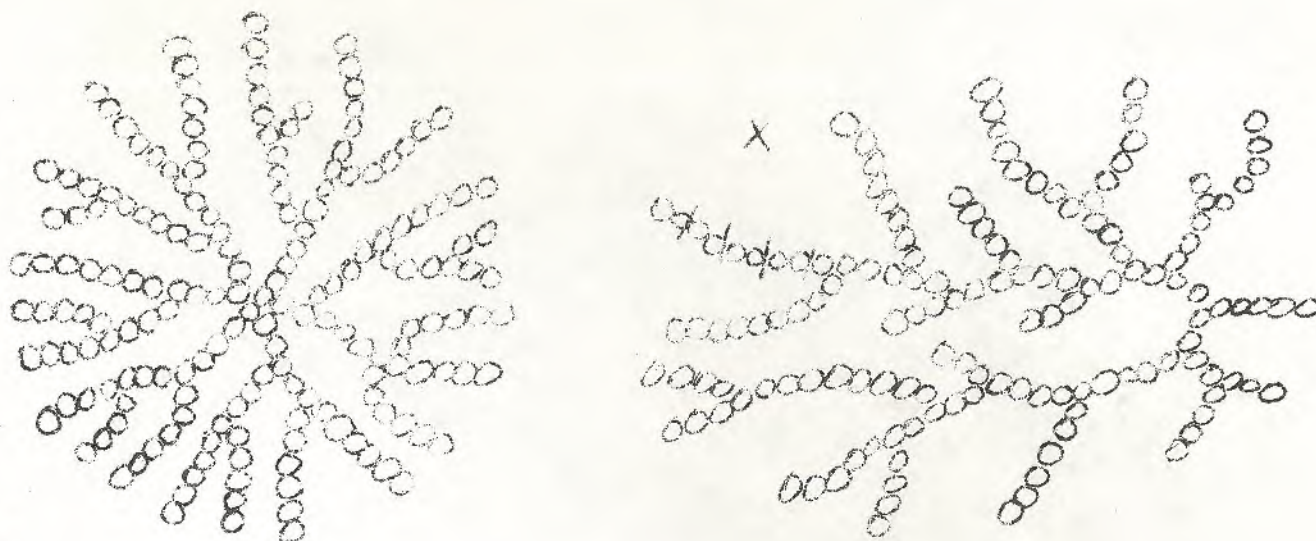


So the synthetic humic acids made by W. Eller (2) have to be regarded as models which have been made of polyoxybenzenes such as hydroquinone, pyrocatechin, and pyrogallic acid. We too have prepared model substances from these raw materials according to Eller's method which had been slightly changed. In the case of model substances, it is important to prove the agreement of the physical properties with those of natural ones.

It may be possible that the same monomeric substance will form polymeric products of different forms depending on the condition of the reaction. We only want to mention the various forms of the molecule which results from the polycondensation of glucose as a typical example.

While the filiform molecule of cellulose results from linear linkage, the molecule of starch has a branching form on the other hand. Glycogen again is a spherocolloid.

The molecules we have mentioned are macromolecules which resulted from preceding condensation of monomeric substances. Their size can reach that of colloids. But it is no micellcolloid as e.g. soaps.



Rechts: Stärke (Verzweigtes Kehenmolekul);

Link: Glykogen (stark verzweigtes Kugel molekul)

Because of the different results we reached the conclusion that humic acids in alkaline solution equal polymeric substances made of monomeric ones. In acid solution processes can take place which very much resemble the formation of micelles caused by aggregation. We will discuss this later on.

The most simple method for determining the shape of molecules is the viscosity. We determined the viscosity of humic acids by means of the viscosimeter by W. Ostwald.

The temperature was kept at $20.00^{\circ} \pm 0.05^{\circ}$ C in a thermostat. As a measurement of the viscosity the time is taken which passes when a definite amount of the solvent (here it was water or the suspension) flows from a mark above the bulb to the mark below it. Relative viscosity $\eta_{rel.}$ is the proportion of the time of flow of the suspension divided by the time of flow of the solvents times density (s).

$$\eta_{rel.} = \frac{t_1 \cdot s_1}{t_0 \cdot s_p} \quad (1)$$

Relative Viskosität nach A. Einstein:

$$\eta_{\text{rel}} = 1 + 2,5 \varphi \quad (1)$$

φ = Volumenanteil des dispersen Anteils.

Spezifische Viskosität nach H. Staudinger:

$$\eta_{\text{spez.}} = \eta_{\text{rel.}} - 1 \quad (2)$$

Aus (1) und (2) nach Staudinger:

$$\frac{\eta_{\text{spez.}} \cdot S}{c} = 0,0025$$

c = Konzentration der Lösung in g/Liter
 S = Dichte des Gelosten

Specific viscosity ($\eta_{\text{spez.}}$) is the increase of viscosity which is caused by the dispersed substance in the dispersing medium.

$$\eta_{\text{spez.}} = \eta_{\text{rel}} - 1 \quad (2)$$

For characterizing colloids H. Staudinger (3) has suggested the viscosity number (Z_{η}). It is the proportion of specific viscosity divided by the concentration (g/l).

$$Z_{\eta} = \frac{\eta_{\text{spez.}}}{c} \quad (3)$$

From the law of hydronamic A. Einstein (4) could derive the following equation for the relative viscosity.

$$\eta_{\text{rel.}} = 1 + 2,5 \Phi \quad (4)$$

Φ means the portion of volume dispersed in the solution.

This equation was derived under the assumption that the dispersed portions are inflexible spheres and that they are bigger than the molecules of the solvent. This equation also points out that the relative viscosity of the dispersion of spheric-shaped particles increases proportional with their concentration. H. Staudinger and E. Husemann (5) remodeled Einstein's equation by putting instead of the concentration by volume, the concentration by weight (g/l), and the density of the dispersed portion and by putting instead of the relative viscosity, the specific viscosity.

$$\eta_{\text{spez.}} = \frac{K \cdot c}{S} \quad \text{or} \quad K = \frac{\eta_{\text{spez.}} \cdot S}{c} \quad (5)$$

Then the constant K is 0.0025.

According to the investigations of H. Staudinger and his students the viscosity of sols of stretched particles is dependent on the degree of stretching or on the proportion of the length divided by the thickness. These results could be proved by fundamental experimental work. From this reason the equation reads for linear colloids:

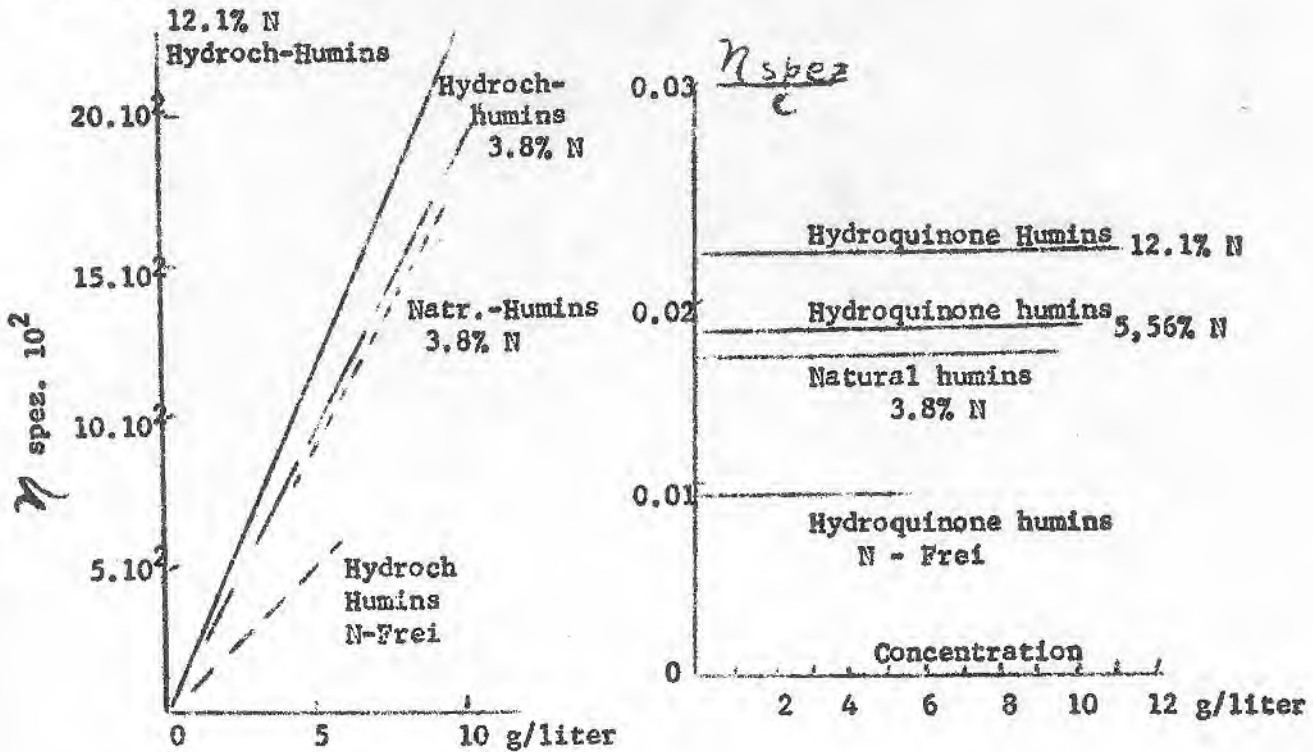
$$z \eta = \frac{\eta_{\text{spec.}}}{c} = K_m \cdot P \quad (6)$$

$c \rightarrow 0$

K is a characteristic constant for every polymeric homologue order and P is the degree of polymerisation. $(c \rightarrow 0)$ means that this relation is only varied for diluted solutions.

As a result of the discussion of spheric and linear colloids we want to point out that the viscosity number of suspensions of spherical shaped particles ranges between 0.0025 and 0.02 and that of threadlike particles between 0.05 and about 5 and even more. From this reason the viscosity of suspensions of sphero colloids is about 20 to 250 times as low as that of linear colloids.

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Influence of Eingebauten nitrogen of humic acids on the viscosity at equal pH.

Abhängigkeit der viscositätszahl $\frac{\eta_{\text{spez}}}{c}$ Bezogen auf die concentration.

Viscosimetric investigations have been made on natural humic acids which had been purified by reprecipitating and dialysing afterwards, and on model substances which had been prepared from hydroquinone in the absence and presence of ammonia in an alkaline medium. Each of these experiments brought out linear curves with a corresponding inclination. The inclination is dependent on the content of nitrogen which is linked to the molecule of humic acids.

(Compare 261)

The dependence of the viscosity number (Z_{η}) or $\frac{\eta_{spec.}}{c}$ referred to the concentration always was a straight line. This result establishes that particles of humic acids have a spherical shape. If they would be filiform particles, the curves must have a trend to rise. The investigations establish further that nitrogen imparts special physical properties to the molecule of humic acids.

We also studied the influence of hydrogen ions on the behavior of humic acids. For these tests humic acids made of hydroquinone have been used. These model substances partly contained nitrogen and partly not.

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Einfluss der Wasserstoffionenkonzentration auf stickstofffrei und stickstoffhaltige Hydrochinonhuminsäuren

HCl-Konz.	0	0,125n	0,5n	1,0n	
rel	1,0270	1,0270	1,0911	1,1390	nach 2,5 Stunden
				1,1390	nach 6 Stunden
				1,1404	nach 9 Stunden
				1,1390	nach 24 Stunden

HCl-Konz.	0 rel	0,125n rel	0,25n rel	0,5n rel	0,75n rel	1,0n rel
1	1,128	1,128	5,326	5,670	5,195	5,156
2	1,128	1,130	5,200	5,574	5,138	5,174
3	1,129	1,128	5,124	5,468	5,116	5,102
4	1,129	1,130	5,031	5,304	5,113	-
5	1,129	1,130	4,986	5,221	-	-
6	1,130	1,130	4,872	5,127	5,083	4,820
7	1,129	1,128	4,746	5,047	5,042	4,762
8	1,129	1,130	4,795	4,990	5,000	4,727
9	1,130	1,130	4,729	4,934	4,986	4,717
10	1,129	1,129	4,684	4,882	4,965	4,689

By maintenance of the volume of the suspension and the concentration of humic acids, hydrochloric acid was added varying in its content from a 0.125 N to a 1 N solution. A separation of the disperse phase from the dispersing solution could be observed after 3 hours. After shaking the sol was again stable for a while. The measurements of nitrogen-free hydroquinone humic acids point out that the viscosity rises on the addition of hydrochloric acid, but it does not change suddenly. Viscosity is still the same after 24 hours, and does not undergo any changes after it has run through the capillary several times.

Nitrogen-containing model substances on the other hand show a completely different behavior. The viscosity is raised 40 times, when 0.125 N is changed to 0.25 N. But it does not raise any more if still more hydrochloric acid is added. The increase of the relative viscosity of nitrogen containing compounds is very remarkable, for it raises from 1.13 to 5.39 at a concentration of 0.125 N to 0.25 N. It is remarkable too that the nitrogen-free compound remains almost unchanged. This fact only can be explained in the way that by entering of nitrogen into the molecule of humic acids more chances are given to form hydrogen bonds and so the formation of micell-colloids is favored very much.

If a solution of nitrogen-containing model substances passes through the capillary several times, the relative viscosity continuously decreases when solutions of a high viscosity number are used, but it remains constant if there is used a solution of a low viscosity number. The particles are torn apart by the shearing forces occurring when the suspension runs through the capillary several times. Perhaps this may explain the result.

Increasing values must be found with time if the sol would disintegrate during the measurement. We could notice that a slight coagulation takes place. But we never had any interruptions of the measurements by blocking of the capillary.

These results further prove that nitrogen-containing compounds are faster precipitated than nitrogen-free compounds. This fact was already known from natural ones (6).

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In order to examine the reversibility of the viscosity with the change of the hydrogen ion concentration, without adding alkali, an untreated suspension was diluted with a 0.25 N solution in the proportion of 1 to 1. On mixing suspension I with II a concentration results of 0.125 N hydrochloric acid. Then viscosity falls as to equal I again. If hydrochloric acid is added to suspension III viscosity increases again. The experiments demonstrate that the viscosity is dependent on the concentration of the hydrogen ions, and demonstrate further on that the processes of aggregation and dispersion are reversible. Of course, when all these measurements are carried out, the rise of viscosity of the dispersing medium by hydrochloric acid has to be taken into consideration.

In order to study the behavior in an alkaline medium, we added sodium hydroxide to dialysed humic acids until the solution was 1 N,

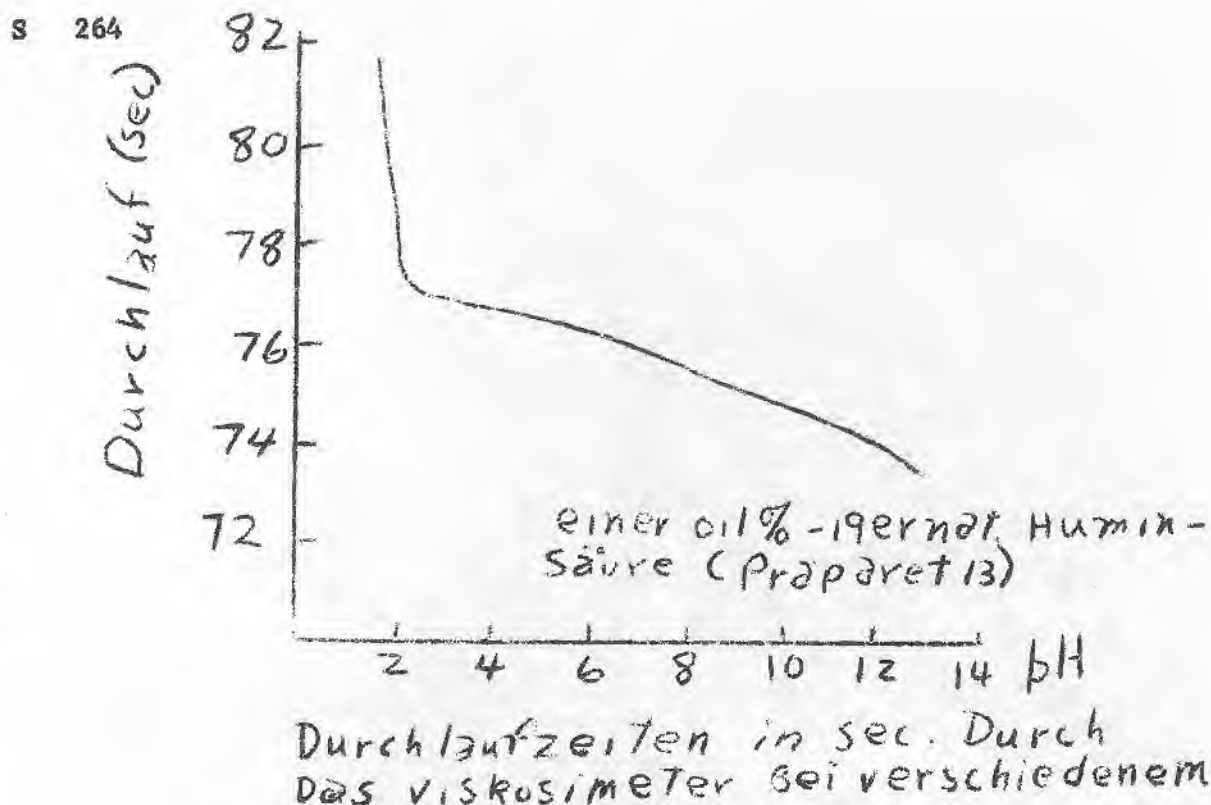
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Aggregation einer Huminsäure bei verschiedenen Salzsäurekonzentration

HCl-Konz.	0	0,25n	0,125n	0,29n
	Losung I	Losung II	Losung III	Losung IV
	rel	I + HCl rel	I + II gem. rel	III+HCl rel
1	1,128	5,326	1,128	5,897
2	1,129	5,200	1,130	5,680
3	1,129	5,124	1,128	5,482
4	1,129	5,031	1,130	5,284
5	1,129	4,986	1,130	5,152
6	1,130	4,872	1,130	5,008
7	1,129	4,746	1,128	4,868
8	1,129	4,795	1,130	4,781
9	1,130	4,729	1,130	4,723
10	1,129	4,684	1,129	4,608

Viskosität im alkalischen Medium

	spez c	in In NaOH	Konz. in g/Ltr.	Viskositätszahlen	
				Wasser	In NaOH
Hydrochinonhuminsäure ohne N			2,95	0,0099	0,0027
Hydrochinonhuminsäure mit 5,56% N			5,70	0,0227	0,0027



These results point out that in an alkaline medium the viscosity of humic acids is independent of the nitrogen content and that these results agree with the constant 0.0025 calculated by Staudinger for sphero-colloids.

A diagram of the time of flowing through in dependence of the pH shows characteristic curves for humic acids which have a break at a definite pH.

Also the pressure viscosity of suspensions of humic acids points out that they are made of particles of spherical shape. The results of the measurement of the dependence of the temperature of the viscosity prove that with changes of the temperature no special processes take place in the suspensions.

As far as we know several measurements of viscosity of humic acids have been made several times by Sven Oden and co-workers (7), W. Ostwald and A. Steiner (8). Sven Oden gives a value of 8.86 for the innerfriction of 3.78% suspension of humic acids; this would equal a specific viscosity of 7.86 and a viscosity number of 0.208.

According to these investigations particles of humic acids must have a long stretched shape. Now on behalf on detailed experiments, reported on above, we can ascertain that these measurements have been carried out with material still containing acid or with humic acids that had to undergo a process of drying which causes changes as it is known. Wo. Ostwald only examined peat humic acids without studying the given problem.

Viscosimetric measurements of suspensions containing spherical shaped particles only indicate the shape of the particles but not their size. So we tried to get on by means of supermicroscopical investigations. Only because of the fact that humic acids are spherocolloids according to the viscosimetric investigations, this matter could be taken into consideration. Linear colloids, e.g. cellulose molecule, are not visible in the supermicroscope, since the thickness of the molecules is not resolved by the microscope.

S 725 308 (Be)

Figure 9 shows a natural humic acid being very much enlarged. It was extracted from black soil. The print was taken of a suspension at pH 3. The particles of humic acids are forming grape-like aggregates. Synthetic model substances have the same appearance. This is a fact which has not been expected.

Prints also have been taken of the synthetic nitrogen-containing hydroquinone humic acids in suspensions at pH 3. They had been purified in the same way as natural ones. The supermicroscopical print shows under equal conditions equal forms, too.

The nitrogen-free synthetic humic acids have exactly the same behavior as natural and nitrogen-containing humic acids. The increased dispersion when the hydrogen ion concentration is lowered, not only can be observed by measurement of the viscosity but also by supermicroscopical prints. At pH 5.7 there is no rough aggregation and dispersion increases. At higher concentrations of the hydroxyl ions at about pH 8 almost complete dispersion takes place. Larger particles consist of several single particles equal in size.

In order to get a better visualization of the particles and eventually to measure their size we treated them with vapors of platinum. Further on it could be proved that dispersion in strong alkaline medium goes on until there are only single particles.

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Figure 14 is a print of nitrogen-containing hydroquinone humic acids. The suspension was made alkaline up to pH 10 by means of ammonia. Here a complete dispersion can be observed. This had been indicated already by the viscosimetric measurements. However, only the supermicroscopical print can prove that it is a probably monodispersed distribution, i.e., all particles are equal in size.

Figure 15 shows natural humic acids which are dispersed in an alkaline medium in the same way as synthetic ones.

As it is known it is very difficult to isolate natural humic acids from the soil which are completely free of ash. By means of the supermicroscopical print can be indicated that humic acids which have been isolated from black soil still contained inorganic impurities. The inorganic origin could be indicated by means of electron diffraction. It has been very difficult to obtain ash-free humic acids from black soil, even when they were purified with special care. Such impurities disturb optical investigations very much as it is known. So probably all the results of the investigations which have been made on this field up to today have to be checked once more.

Giving a general review I want to show that humic acids being isolated and purified by the usual methods have no differences in the shape of the particles in spite of their different origin.

Humic acids which have been isolated from soils of the black soil type are shown on figure 16. Also at this enlargement being about 1/5 of the preceding figure humic acids can be recognized as structures of spherical shape.

On figure 17, there are humic acids which were extracted by means of sodium hydroxide and examined at pH 3,4.

In contrast to the humic acids shown above these substances have been extracted from compost by means of sodium fluoride. The print was taken at pH 3. Also here the particles of humic acids have the same shape and they are only a little more aggregated at higher concentrations of the hydrogen ion.

As it is known, substances are produced autolysis of microorganisms which have a great affinity (9) with humic acids.

Figure 19 shows humic acids which were obtained from cultures of streptomycetes and which have the properties of natural ones. The supermicroscopical prints differ only slightly.

From cultures of Aspergillus also substances can be isolated which resemble humic acids being isolated from the soil.

Since we were able to make supermicroscopical prints of dispersed humic acids, we also were interested in the size of the particles which have been observed. We could measure a diameter of about 60 to 80 Å.

The result is the same as it had been found by the investigations of E. Jung (11) who found a diameter of about 80 Å by sorbing humic acids on montmorillonite particles of a known size.

He did it in the following way:

10 g of potassium and calcium clay have been shaken with solutions of an increasing concentration of a humate. Three days after-wards the overlying liquid was centrifuged and the concentration of the humate was indicated by the colorimetric measurement or by titration with potassium permanganate solution. With an increasing concentration of the humate, particles have been sorbed up to a limit value almost quantitatively on montmorillonite. After reaching the limit value very small amounts of humate have been sorbed. According to his opinion, the sorbed humic acids consist of a monomolecular layer at the limit value. The number of particles and the size of clay particles was determined by counting in the dark field. By means of the average volume of the particles the thickness of the sorbed layer was determined to be 80 Å according to the method of diffusion by K. Zeile (12)

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Another method of determining the weight and size of spherical shaped particles is the measurement of diffusion. Suspensions of spherical shaped particles obey Fick's Law and the diffusion coefficient increases indirect proportional with the radius of the particle.

$$D = \frac{R \cdot T}{N \cdot 6 \cdot \pi \cdot \eta \cdot r} \quad (7)$$

η = absolute viscosity (it is approximately 1 for water at 20° C). If the radius is known then the size of the particles can be calculated. We have

calculated the diameter of the particles from the great number of diffusion experiments which W. Scheele (13) had made with humic acids and hylatomelanic acids. Scheele had made his investigations by means of a set-up according to Ohm.

It is striking in these investigations that when q is higher than 1,25 a decrease of the weight of the particles takes place to the half of the original one. Humic acids from Kasseler Brown (according to Scheele it is a mixture of humic acid and hylatomelanic acid) the weight of the particles decreases from 10400 to 5800, humic acids from Kasseler Brown from 12600 to 6000, humic acids from Dachauer Moor from 26000 to 11000. The calculated diameters of the particles range between 30 and 70 Å. That is almost 50 Å if one takes an average diameter.

Scheele's investigations are remarkable from certain reasons. The weight of the particles increases when an excess amount of sodium hydroxide is added and this process is reversible. We have found a similar phenomenon measuring the viscosity. These results lead to the conclusion that humic acids are probably molecular colloids in an alkaline medium, while they act as micelles in an acid medium. The reversibility of these processes is another proof of the hypothesis, that micelle colloids result by hydrogen linkages in an acid medium.

Dialysis Coefficient

We could determine the order of magnitude of weights of the particles in an acid medium by a dialysis method of which H. Brinzinger (15) reported, which had been modified by G. Jander and H. Spandau (16). H. Jander and co-workers described this method as useful for humic acids by orientating experiments.

If particles are allowed to diffuse through a permeable membrane into a pure solvent because of a concentration gradient, then the dialysis coefficient can be determined.

The dialysis coefficient λ is a measurement for the migration velocity and is calculated by the following equation

$$\lambda = \frac{\log C_0 - \log C_t}{t \cdot \log e} \quad (8)$$

t = time of dialyse;

c₀ = concentration in the beginning

c_t = concentration at the end of the dialysis

The dialysis coefficient is dependent on values of the apparatus, such as the membrane, the volume of the liquid and the temperature of the solution. On the other hand λ is only a function of the size of the molecules. H. Brinzinger gives the following equation for the dependence of the dialysis coefficient on the weight of the particles (M).

$$\lambda \cdot \sqrt{M} = K$$

The constant K is mostly dependent on the dialysis membrana which is used.

The results of the investigations are listed in table 6.

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Berechnung der Teilchengewichte aus dem Dialysenkoeffizient

Huminsäure extrahiert aus	Extraktionsmittel	pH	Huminsäurekonzentration in %	Asche in %	K	λ	Teilchengewicht
Schwarztorf	1%Na ₂ CO ₃	10,35	2,96	1,4	5,968	0,07187	6896
					5,968	0,07183	6904
					5,968	0,07161	6947
Schwarzerde	1%NaOH	11,65	2,07	1,2	4,441	0,06395	4822
					4,441	0,06488	4685
					4,441	0,06464	4719
Brauntorf	1%NaOH	12,6	1,92	1,1	5,968	0,07461	6398
						0,07490	6349
						0,05548	6318

The carefully dialysed humic acids were peptised with the original dispersing agent such as 1% sodium hydroxide or 1% soda solution. In spite of the electrolysis the preparations had a content of ash of 1.1% to 1.4%. Parallel investigations yielded a comparatively good agreement. The weight of the particles ranges between 5000 and 7000 in an alkaline medium.

We hope to gain further experimental support by further dialysis and diffusion experiments in order to explain certain contradictions about the size of the particles and their weight in the literature. But nevertheless one can point out that the agreement of the diameter of the particles is comparatively good, taking into consideration the different methods used. At any rate the results for the size of the particles prove that alkali humates are colloidal disperse systems as it was suggested by Wo. Ostwald. They are not maximal disperse systems as it is supposed by Sven Oden.

The reports on the weight of the particles so far are in contrast to the molecular weight of humic acids, which has been determined by various authors by different methods.

The main difficulty is, how to find a suitable solvent for humic acids. W. Fuchs (2) succeeded in producing the so-called "nitric humic acids" by nitration. They are soluble in acetone. He determined the molecular weight of the substances at 1260 by means of the boiling-point elevation and that of the corresponding methyl derivatives at 1300. But it is not impossible that an oxidative cleavage had taken place by the treatment with nitric acid.

Determination of the dialysis coefficient:

The needed set-up can be seen from figure 21. In general it is constructed according to the data given by H. Brinzinger (17) as well as of H. Spandau and W. Gross (18). The container for dialyse consisted of a glass tube in a shape of a cylinder. This was put into a glass container filled with 20 liters of distilled water as the solvent. The temperature could be regulated at $21.5^{\circ} \text{C} \pm 0.1^{\circ} \text{C}$ by means of a Hoppler thermostat. The permeable membrane had been fastened

between two rubber rings on the grindings of the glass tube by means of two rings made of V2A steel and 3 bolts. In order to balance the hydrostatic pressure on both sides of the membrane the container for dialysis was dipped into the other container so deep as to reach the same level of both liquids. In order to avoid the electrostatic linkage of the molecules NaF was added to the dispersion according to H. Spandau.

The Cella-membranes (width of the pores 500 Å) were adjusted by means of NaCl. The value of the constant K thus obtained had been used for calculating the weight of the particles of humic acids. In the beginning and at the end of the experiments after about 6 hours the humic acids were titrated conductometrically according to Scheele (19) and co-workers.

M. Samec (21) as well as C. L. Arnold (22) calculated a molecular weight of about 1350 by means of osmometric data of the alkali salts of humic acids. This method still needs further improvement, for a part of alkali humate diffused through the membrane.

Also measurements which are based on the lowering of the melting point of urea, have given a molecular weight of about the same value.

These low molecular weights only agree a little with the other properties of humic acids. According to our own experiences, the method of drying the substances seems to be of importance. For carbondioxide is split off in the presence of air which causes changes. Humic acids which have been dried in a drying oven at 80 to 100° C can not be dispersed in diluted alkaline solutions afterwards.

It must be mentioned that the determination of the molecular weight by means of the cryoscopic method is only possible up to a molecular weight of 5,000 maximum according to H. Staudinger and his students (1) who made exhaustive examinations with this method. He already had observed that besides the linear compounds of high molecular weight also more or less spherical shaped molecules of high molecular weight such as starch and starch derivatives give values which are much too low if this method is applied. Recently W. Kern (24) again reported on similar differences of the molecular weight determined by means of osmometry and the ultracentrifuge compared with the molecular weight determined by means of the cryoscopic method of methyl substituted polyenes.

He determined the molecular weight of a fraction with the ultracentrifuge and the osmometer, and he got a molecular weight of 32,000 while the cryoscopic method only gave a value of 820. There seems to be a difference in the cryoscopic behavior of substances of low and high molecular weight which have not been explained up till now. Values which have been obtained by this method must be proved by other ones if they shall be correct.

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Special studies of the viscosity number point out that the viscosity number of electrolysed humic acids differs from the value of 0.0025 that was calculated by H. Staudinger. But, however, the values never reached that of linear colloids. If one calculates the requirement of volume by means of the proportion of the viscosity number found divided by the theoretical number of 0.0025, one may get an idea of the inner structure of a particle of humic acids.

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$$\text{Volumbeanspruchung} = \frac{\text{Gefundene Viskositätszahl}}{\text{Theoretische Viskositätszahl}}$$

	Z	Volumbeanspruchung
pH ca 3		
Hydrochinonhuminsäuren stickstofffrei	0,0097	3,9
Hydrochinonhuminsäuren, 5,56% N	0,0185	7,4
Hydrochinonhuminsäuren, 12,1% N	0,0228	9,0
Natürliche Huminsäuren, 3,81% N	0,0172	6,9
pH ca 12		
Hydrochinonhuminsäuren, stickstofffrei	0,0027	1,1
Hydrochinonhuminsäuren, 5,7% N	0,0027	1,1

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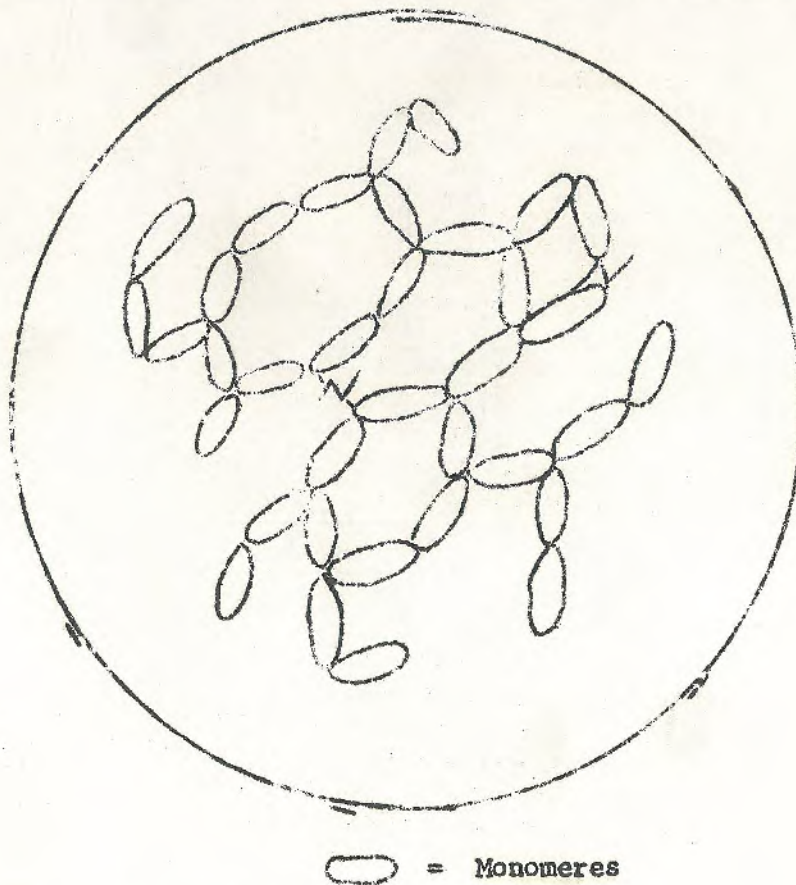


ABB. 23 Schematic Humic Acid Model

Providing that humic acids are lyophilic colloids, solvation has no influence of the viscosity in dilute solutions as H. Staudinger (25) could demonstrate with glycogen and its derivatives. From this reason particles of humic acids can not be solid spheres but must have a great lot of pores and intermediate spaces in the inside. The building stones which are lying in the inside are also hydrated and can bind water molecules. Probably still more water molecules are bound mechanically by these inflexible particles. They are immobilized according to Wo. Ostwald (26). With increasing nitrogen content also the viscosity number increases, that is, the requirement of the volume gets higher or put it in another way, with constant size of spherical shaped particles the pores in the spheres become smaller and so also more water can be retained, i.e., immobilized.

Also this hypothesis can be proved by means of supermicroscopic prints. Differences can be observed, if barium salts of a nitrogen-free and a nitrogen-containing humic acids are prepared under exactly equal conditions and with special care.

While the barium salt of nitrogen-free humic acids, made of hydroquinone is a comparatively loose structure, the salt of the nitrogen-containing humic acids has a closer structure.

As it is known, the salts of ions of a higher atomic weight become denser electronic optically,

So the spherical shape of natural humic acids which have been carefully precipitated with barium salt, and the partly chain-like aggregation can be observed very well,

The result, that particles of humic acids own a low weight compared with the diameter can be demonstrated by supermicroscopical prints.

At pH 6, humic acids have a very loose structure and can be almost completely penetrated with rays.

By contrast gas soot particles, having almost the same size, are less penetrable for electronic radiations; the soot particles have a denser packing.

On behalf of electronic diffraction humic acids have a crystalline structure. The prints by no means differ from an untreated collodium film as figure 29 and 30 prove.

Bungenberg De Jong (27) and co-workers have given a detailed report of flocculation. The effect also could be observed with humic acids.

As it is known of this effect, several colloid particles coalesce under a common water film. The formation of floccules specially can be observed when humic acids are careful purified in an acid medium.

An account on the factor of stability of colloids shall help to explain what conditions are to be found with humic acids. Colloid particles can either be stabilized by a hydrate cover or by the charge.

In a strong alkaline medium there can not be a hydrate cover, because of the low viscosity number which corresponds with the theoretical number of 0.0025. So it cannot be a factor of stability.

The acid groups present such as the phenol or carboxyl group infer that humic acids do not precipitate in an acid medium, and they can be compared with polyvalent acids at this pH. This explanation can only be given when an agreement with the above mentioned results can be assumed, namely humic acids are not solid spheres but appear as almost spherical shaped sponges.

As the factor of stability of humic acids the charge of the particles is to be discussed. The electro-kinetic potential further on gives important criteria on the reciprocal action between inorganic and organic colloids in the soil.

We carried out electrophoresis according to W. Nernst (28). Humic acids were covered with a layer of ultrafiltrate or with distilled water in a U-shaped tube. Then the migration velocity was observed at constant voltage and constant amperage per unit of time.

According to the relation given by P. Debye and E. Huckel (29) the migration velocity of the ζ potential was calculated for spherical shaped particles.

$$\zeta = \frac{6\pi \cdot \eta}{D \cdot E} \cdot w \cdot 300^2 \cdot V \quad (10)$$

E = field intensity

D = constant of electricity

η = viscosity

w = migration velocity in the electric field

300^2 = factor for calculating C.G.S.-units.

Since only a clear separating layer was formed in the cathode part of the tube, we could only here measure the migration velocity.

In the anode part of the tube it was difficult to make any measurements because of the electrocoagulation. Here both natural as well as synthetic humic acids showed a second separating layer migrating much faster.

The ζ potential of humic acids from black soil was almost constant and was determined between -21.6 and -22.7 milli volts. They had been extracted from the soil with 2% sodium fluoride, 1% sodium carbonate and 1% soda solution. The migration velocity of these three humic acids was also equal. Nitrogen-containing humic acids, made of hydroquinone had a higher ζ potential and a higher migration velocity.

It was observed that humic acids did not migrate in the electric field being measured in an alkaline medium. So it can be determined that alkali humates are not stabilized by the charge.

The electrophoretic migration velocity can almost be used as a criterion for how far cations are removed from suspensions of humic acids.

In the table above the migration velocities are listed of a well purified, i.e., electrodialed; a purified, only dialysed humic acid; and one humic acid only titrated with sodium hydroxide, in dependence of the time. While the well purified, electrodialed humic acid after 15 minutes had a migration velocity of $\frac{14}{\text{sec}} \cdot \frac{\text{V}}{\text{cm}}$, on the other hand from the dialysed solutions first sodium ions had

to be removed by electrodialese, before humic acids migrated under the influence of the voltage after 85 minutes. The process of removing sodium ions from humic acids titrated with sodium hydroxide, lasted for about 150 minutes. Only after this time the usual migration velocity for humic acids was reached.

At the moment we are still examining the dependence of the migration velocity of addition of different ions. We noticed that calcium humate shows a fraction of slight positive charge (11.9 to 3.1 milli volts) if an excess of calcium chloride is present. Against the usual opinion we made the surprising observation that humates have a negative charge, and that they cannot be sorbed by clays of a negative charge. Perhaps there may be a possibility of explaining the clay humus complex by studying this effect.

According to investigations of F. Zetsche and H. Reinhart (30) humic acids can be reduced over several color gradients and can be oxidized to brown substances again. Further on quinoid keto-groups can be determined beside phenolic hydroxyl groups by means of phenylhydrazinecarbamate (31). For characterizing humic acids physical chemically it is also necessary to determine the redox potential.

For the determination of the redox potential of humic acids a clean platinum electrode was dipped into a solution of humic acids and was compared with a potential of a calomel electrode referring to the zero value of the normal hydrogen electrode.

The r_H determinations have been made with a set-up as it can be seen from figure 33.

As it is known all systems which strive to get rid of their electrons have a positive or oxidation potential while those easily taking up electrons have a negative or reducing potential. Clark (32) has introduced the r_H value as a measurement for the reducing or oxidation power.

Usually this value is independent on the pH. The neutral point of a redox-system is a r_H value of 27.2 at 20° C. Higher values up to 41 are oxidizing, those below are reducing.

$$r_H = -\log P_H = \frac{E + a \cdot \text{PH}}{b} \quad (11)$$

In table 10 measurements of natural, biological (gained from cultures from microorganisms) and synthetic humic acids are listed.

Generally speaking all sorts of humic acids have reducing properties when nitrogen is passed through their acid solution. In the presence of air on the other hand the r_H value of natural humic acids reaches the neutral point. Also humates show the same behavior at an alkaline reaction. Here it is apparent too that the oxygen content of the solution at an alkaline reaction has no longer the same influence on the r_H as in an acid reaction. If nitrogen is passed through an alkaline medium only natural humic acids, as well as 13 and 9 months old humic acids, which have been obtained from cultures of streptomycetes, lie near the neutral point; on the other hand, hylatomelanin acid, humic acids of hydroquinone

as well as those of young streptomycetes and *Aspergillus* humic acids still show reducing properties. This points out that probably a certain aging has to take place with synthetic as well as with biological humic acids in order to show the same properties as natural ones.

We were interested in what connections exist between the redox potential and the pH of suspensions of humic acids. Table 11 gives the pH dependence of the r_H of humic acids isolated from black soils. The nearer the pH value approaches pH 7, the nearer the r_H gets to the redox neutral point.

At first we were interested in the question how humic acids can take place in the shift of the redox potential. According to different authors, e.g. B. Birks (34), N. J. Volk (35), A. Quispel (36), and others the redox potential of soils shall be an important factor of the productivity. These authors report that the wanted redox neutral point of soils is lowered by excess water, lack of air and by acidification. After elimination of these lacks the old redox neutral point is reached again.

The reported physical chemical investigations are not complete, for they only shall demonstrate that it is not hopeless in the field of humic acids to gain new knowledge by means of the different methods.

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