

# INVESTIGATIONS ON COLLOID CHEMICAL PROPERTIES OF PEAT HUMIC SUBSTANCES

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(Received October 2, 1974)

The molecular weight of peat humic acid samples and metal humates of Keszthely origin were determined by ultracentrifugation as a function of pH and electrolyte concentration. These studies on sedimentation were completed by electron microscopic and gel filtration determinations in the regions of low and high pH, respectively, because in these regions the possibilities of using the ultracentrifuge method are restricted by intrinsic limitations of the method.

The molecular weights and diameter of the particles unequivocally indicate the presence of molecule aggregates in the solution at lower pH values; at higher pH these aggregates disintegrate. Studying the aggregating effect of metal ions on humic acids, it can be stated that the higher degree of aggregation is produced by trivalent ions and Cu(II), while the aggregating effect of divalent ions is more moderate.

The molecular weights obtained by ultracentrifugation and gel filtration show a parallel course. The gel filtration method permits a quick comparative determination of molecular weight and molecular weight distribution.

## *Introduction*

In the last years several workers [1—12] dealt with investigations on physical and chemical properties of humic materials. Besides classical methods of investigation, modern methods of exploring the structure accurately, such as ultracentrifugation, light scattering, X-ray, spectrophotometric, osmometric, gel filtration and electron microscopic methods became more and more dominant.

We used similar methods for investigating humic acids of different origin in the last years [13—15]. The state of aggregation of these samples was studied as a function of pH and time. Some morphological parameters were calculated from results of ultracentrifugation and the effect of different metal ions on the humic acids was investigated.

Ultracentrifugation studies were restricted by the possibilities of using the method; namely, reliable results on aggregation could be obtained only in a comparatively narrow pH range (5—6.5). To clear up the extent of aggregation and dis-

aggregation in the pH range below 5 and 6.5, respectively, electron microscopic and gel filtration methods had to be applied. Electron microscopy permitted to observe the aggregation down to pH 2, and gel filtration to study disaggregation and to determine the molecular weight up to pH 10.

The aggregating effect of different metal ions on humic acids was studied and the molecular weight of the metal humates obtained was determined; in order to assess the fine structure of these humates, some electron micrographs were made, too.

In this paper we wish to give an account of our more recent results.

### Materials and methods

Brown humic acids, extracted from Keszthely peat by pyrophosphate process were studied after adequate extraction, purification and fractionation.

The molecular weights of the samples were determined with an analytical ultracentrifuge Model G-120 of MOM (Hungarian Optical Works), Budapest, using the sedimentation equilibrium method.

The gel filtration was performed on a dextrane polymer, type Sephadex G-75 (PHARMACIA, Sweden), in a column of 2 cm  $\varnothing$  using a fractionating automat type SF-62. Glycine-NaOH buffer of pH 10 was used as eluent, then 5 ml fractions were collected with a rate of 30 ml/hr. The extinctions of the different fractions were measured with a spectrophotometer ZEISS Specol at 450 nm. The following substances were used as molecular weight standards of the gel filtration:

Cytocrome C (CALBIOCHEM AG)  $M = 12500$

Ribonuclease (PHARMACIA AG)  $M = 13600$

Myoglobin (horse) (SERVA AG)  $M = 17800$

Trypsin inhibitor (soy-bean) (CALBIOCHEM AG)  $M = 21500$

Chymotrypsinogen A (SERVA AG)  $M = 25000$ .

The distribution coefficient  $K_d$  and the molecular weights were determined according to [16-19]. The electron micrographs were made with an electron microscope TESLA BS-500.

### Results and discussion

The results of our investigations are presented in Tables I-II and Figs 1-7.

As can be seen from Table I, the molecular weight decreases with increasing pH. Particle diameters between 70 and 130 Å were calculated from sedimentation results, corresponding to the pH values decreasing in this interval. Similar results had been published in more detail in our earlier papers [12-15].

In the regions of lower and higher pH, ultracentrifugation does not yield reliable results as, at lower pH, aggregates of large dimensions causing rapid sedimentation are present in the solution, and it is difficult to measure the rate of sedimentation under the experimental conditions used. On the other

Table I

Dependence on pH of the molecular weight and particle diameter of Keszthely peat humic acids at  $t = 25^\circ\text{C}$

pH	5.0	5.5	6.0	6.5
$M \cdot 10^{-3}$	3.1	3.0	2.9	2.8
d (Å)	130	100	80	70

hand, at higher pH values the colloidal disperse system begins to become homogeneous, of microscopic character, and the molecular distribution of particles does not permit to measure the sedimentation rate.

In lower pH ranges, we made electron micrographs of the Keszthely peat humic acid sample at pH 2, 4, and 6. These are presented in Figs 1—3.

As can be seen from Fig. 1, the humic acid particles of these samples are globular and of identical dimensions. Their diameter, taking into account the enlargement, is in good accordance

with that calculated from sedimentation values (about 80 Å). It is to be remarked that these humic acid samples appear to be practically monodisperse, both by ultracentrifugation and gel filtration. With decreasing pH further aggregation of the particles occurs. At pH 4 (Fig. 2) the particle diameter is found to be about 200 Å, while at pH 2 (Fig. 3) aggregates of very large dimensions are formed. Similar micrographs were made under the same conditions with coal humic acids; the aggregation shows analogous trends, but the degree of heterodispersity is greater.

Fig. 4 shows the changes in molecular weight of peat humic acid doped with metal ions as a function of electrolyte concentration, while in Table II the molecular weights of different metal humates at the maximum electrolyte concentration, immediately before coagulation, determined both by ultracentrifugation and gel filtration are presented at pH 5 and 10, respectively. A well defined pure fraction of the humic acid samples was used in the investigations.

It can be seen from the data of Fig. 4 that different metal ions exert different

Table II

Molecular weight of metal humates at maximum electrolyte concentration immediately before coagulation,  $t = 25^{\circ}\text{C}$

Sample	Keszthely peat humic acid		
	M · 10 <sup>-3</sup> determined by ultracentrifuge at pH=5	K <sub>d</sub> determined by gel filtration at pH=10	M · 10 <sup>-3</sup>
—	3.1	0.64	2.6
Cu(II)	10.0	0.48	7.5
Co(II)	7.1	0.58	6.0
Mg(II)	2.7	0.65	2.5
Fe(II)	6.4	0.60	5.0
Fe(III)	10.3	0.53	8.0
Al(III)	11.0	0.51	9.0

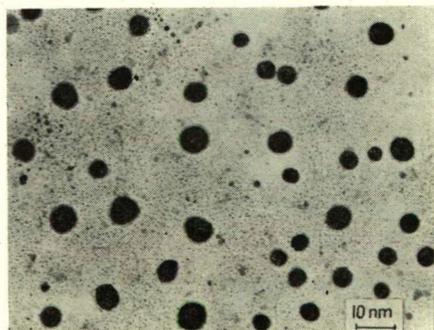


Fig. 1. Electron micrograph of Keszthely peat humic acid at pH 6

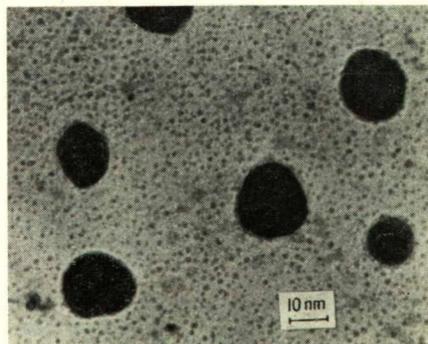


Fig. 2. Electron micrograph of Keszthely peat humic acid at pH 4

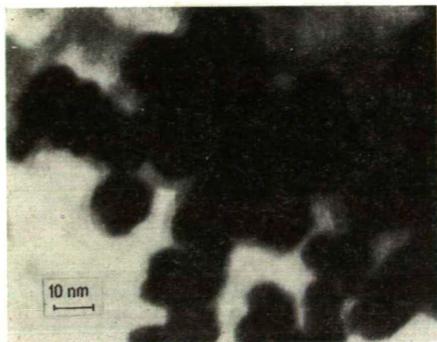


Fig. 3. Electron micrograph of Keszthely peat humic acid at pH 2

effects on the humic acids. Among the metal ions studied, the highest degree of aggregation is caused by Al(III) and Fe(III) ions. The sequence of the aggregating effect of divalent cations is  $\text{Cu(II)} > \text{Co(II)} > \text{Fe(II)}$ , corresponding to the stability of the metal complexes of the humic acid. Mg(II) ions, on the other hand, had no aggregating effect, even with electrolyte concentrations higher by an order of magnitude.

Taking into account our earlier investigations, too, we could not find any significant differences in the aggregation due to cations of humic acids extracted from material of different degrees of carbonification.

It can be seen from Table II that the molecular weights determined with the gel filtration method, using well defined molecular weight standards, and extrapolating the  $K_d$  values in the way shown in Fig. 5, are all lower than those obtained by ultracentrifugation. This is consistent with the result to be expected, as the gel filtration was performed in a higher pH range (pH 10) where the particles are more disaggregated. In this pH range ultracentrifugation does not lead to reliable results. On the other hand, at lower pH values gel filtration cannot be used due to the pH of eluent (which, according to our observations, has an optimum of pH 9–10 in the case of humic materials). The molecular weights obtained with both methods show a good parallelism, and, though the gel filtration cannot be considered as

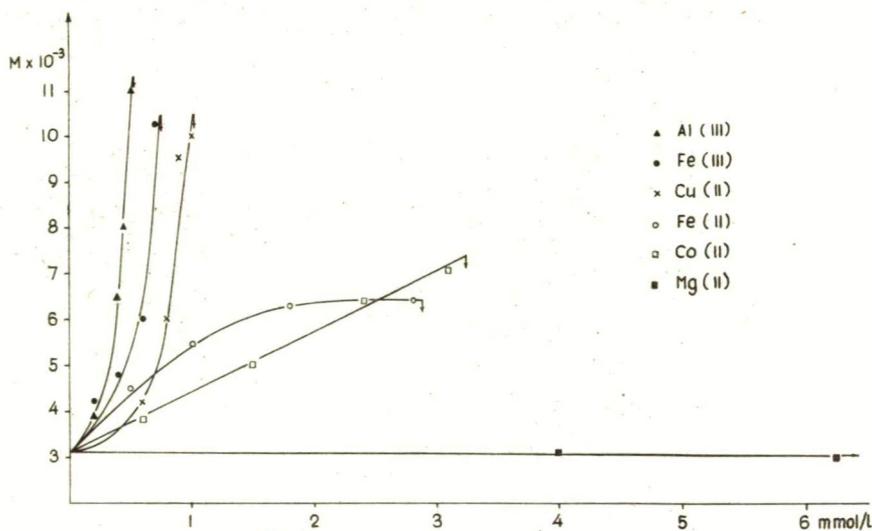


Fig. 4. Dependence on electrolyte concentration of the molecular weight of Keszthely peat humic acid samples doped with metal ions (Basis mol. wt. =  $3.1 \cdot 10^{-3}$ , pH = 5)

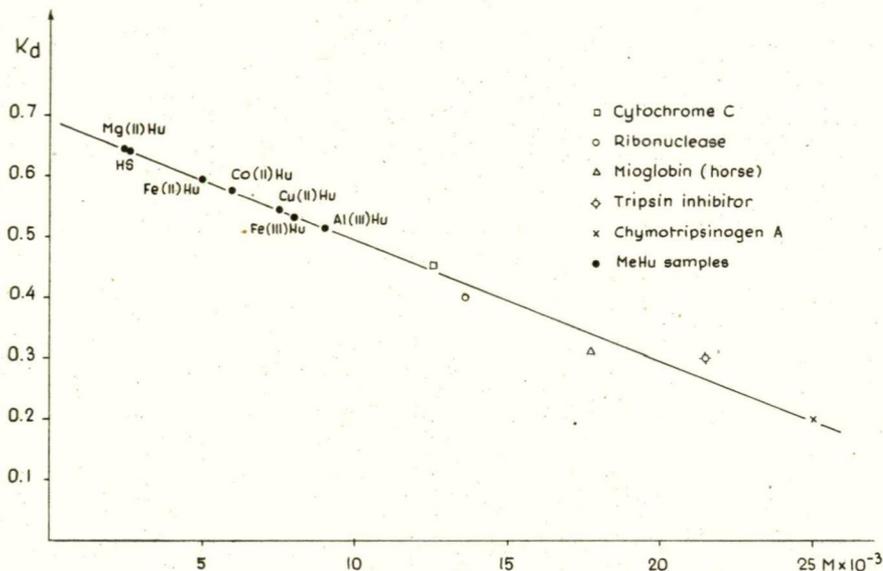
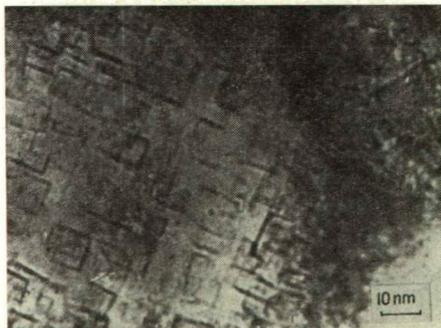
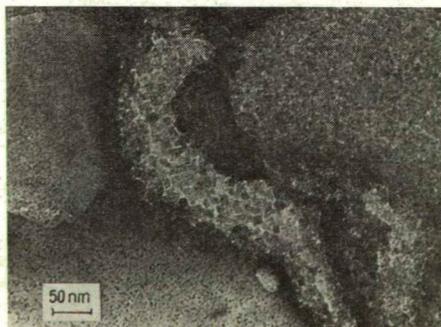


Fig. 5. Gel filtration calibrating curves of molecular weight standards in a Sephadex G-75 column (pH = 10)

an absolute method for determining the molecular weight, it permits a rapid comparative determination of the latter.

For further orientation, we made some electron micrographs of metal humates to assess the structural changes in the humic acid due to the presence of the metal. In the electron micrographs made from Cu-humate particles (Figs 6 and 7) crystalline structure could be observed. These micrographs were made from Cu-humate precipitated by Cu(II) ions from Keszthely peat humic acid, than carefully dialysed, imbedded in Araldite, without any contrast material.



Figs. 6—7. Electron micrographs of Cu-humate precipitated by Cu(II) ions from Keszthely peat humic acid

In Figs. 6 and 7 microcrystalline structures of irregular form can be seen beside amorphous particles.

We intend to extend our investigations to humic acids extracted from peat and brown coal samples of different origin and provenience, and to gel filtration, osmometric and electron microscopic investigation of metal humates with special respect to the aggregation process, in order to clear up the fine structure.

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#### ИССЛЕДОВАНИЕ КОЛЛОИДНО-ХИМИЧЕСКИХ СВОЙСТВ ГУМИНОВЫХ КИСЛОТ ТОРФА

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Проведено определение молекулярных весов образцов гуминовых кислот и их солей из торфа района Кестхель, методом ультрацентрифугирования в зависимости от рН и концентрации электролитов. Седиментационный метод дополнен электронно микроскопическим изучением в области низких рН и гельфильтрационным в области высоких значений рН. Необходимость привлечения этих методов обуславливалась ограниченностью применимости ультрацентрифугического метода.

Молекулярные веса и размеры молекул однозначно указывают на наличие агрегатов при низких рН, которые с повышением рН среды распадаются. Наибольшее агрегирующее действие производят трехвалентные катионы и  $\text{Cu(II)}$ . Другие двухвалентные катионы имеют меньшее агрегирующее действие.

Молекулярные веса, определенные ультрацентрифугированием и гельфильтрацией, хорошо совпадают. Метод гельфильтрации позволяет провести быструю сравнительную оценку молекулярного веса и молекулярно-весового распределения.