INFRARED SPECTROSCOPIC EXAMINATION OF HUMIC ACIDS, III.

By

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Peat humic acid originating from Keszthely was studied by gel-fractionation and by infrared spectroscopy of the fractions. The molecular weights of the individual fractions were determined by osmometry and by light-scattering measurements.

1. The infrared spectra reveal the presence of aromatic components in addition to the aliphatic structure of the sample.

2. The osmometric and light-scattering data show that the molecular weight decreases with the increase in the number of fractions. The low polydispersity values calculated from the weight- and number-averaged molecular weights prove that the gel-fractionation resulted in practically homogeneous samples. The calculated second virial coefficients show the lighter fractions to be better solvated.

3. With the increase in molecular weight of the fractions, the shape of the molecules differs more and more from spherical.

Introduction

Products fractionated by gel-chromatography from humic acid prepared synthetically or extracted from brown coal and soil of various ages and origins were earlier examined by infrared spectroscopy [1, 2]. Significant differences were found between the structures of the individual samples, as indicated both the molecular weight distribution and by the infrared spectra. It was concluded that whereas the coal humic acid samples contained an appreciable aliphatic component in addition to the aromatic structure, the infrared spectra of the synthetic and soil humic acids revealed only aromatic fractions. The molecular weight distributions of the individual samples and the molecular weight intervals of the fractions were determined by gel-chromatography.

We subsequently extended such measurements to an infrared spectroscopic study of humic acids prepared from peat, and to the use of different methods to determine the molecular weights of the individual fractions more exactly. Thus, the number-averaged molecular weights were found by means of osmometric measurements, and the weight-averaged molecular weights by means of light-scattering measurements. Very few references to these methods are made in the humic acid literature. HANSEN and SCHNITZER [3] developed a procedure for the determination

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of low molecular weight fulvic and humic acids with vapour pressure osmometry. However, this method is not suitable for measurements on fractions of higher molecular weight. A further problem is that the measurement is complicated by the dissociation of the ions in the aqueous solution. In this respect, a correction calculation is reported by SCHNITZER and KHAN [4]. Unfortunately, we were unable to obtain well reproducible results with this method, and BERGMANN [5] describes similar negative experiences. Later, with a Knauer membrane osmometer we first succeeded in determining soil humic acid samples of high molecular weight [6], and then, with a special membrane of very fine porosity, the molecular weights of other humic acid fractions of medium and lower molecular weight in the case of samples with low heterodispersity. These samples were prepared by repeated gel-fractionation.

ORLOV and GORSKOVA [7] report data on the study of humic acids by light-scattering measurement. We have performed such measurements on colloid solutions of very dilute, carefully purified peat humic acid sols, obtained by freeze-drying. A comparison of the experimentally determined weight-averaged molecular weights with the values found for the same fractions by osmometry yields accurate information on the extent of heterodispersity. The results of the two methods can also be used to obtain certain thermodynamic parameters, *e.g.* the second virial coefficient, characteristic of the solvation.

Materials and methods

Gel-fractionation, spectrophotometric, osmometric and light-scattering measurements were made on a peat humic acid sample originating from Keszthely. The gel-fractionation and infrared spectroscopic procedures were described previously [1, 2].

Osmometric measurements were carried out with a Knauer membrane osmometer, with the application of Sartorius SM 11536 and SM 11539 regenerated cellulose membrane filters. Solution series were prepared from the individual fractions in the concentration interval 0.2—2 g/100 cm^3, and their osmotic pressures were measured at 298 K. The reduced osmotic pressures (π/c) were plotted as a function of concentration and graphical extrapolation was employed to calculate the number-averaged molecular weights ($\overline{M}_n$). From the slopes of the plots, the second virial coefficient (B) too was determined.

Light-scattering measurements were made with a SHIMADZU instrument at 436 and 546 nm in the concentration interval 0.0005—0.005 g/100 cm^3, with ten different incident angles. The weight-averaged molecular weights were determined by plotting the expression Hc/τ in the Debye relation as a function of the concentration to yield a straight line; extrapolation of this to zero concentration gives an ordinate intercept of $1/\overline{M}_w$. The slope of the straight line again gives the second virial coefficient.

Results

The results are listed in Tables I and II, and illustrated in Figs. 1—3.

Examination of the infrared spectra of the fractions (Fig. 1) does not reveal any significant peaks for the unfractionated sample, similarly as found earlier [1, 2]. Only the shoulders around 1700 and 1600 cm^{-1} are well defined; these values corre-
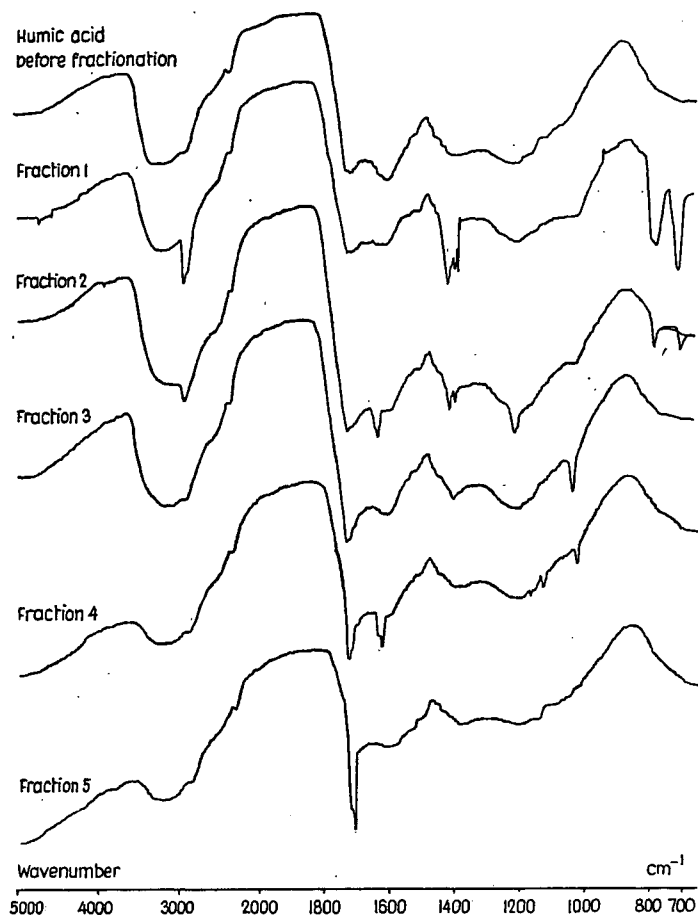
Fig. 1. Infrared spectra of fractions of peat humic acid from Keszthely

spond to the aromatic carboxylic acid C=O group and the carboxylate ion COO⁻ group vibrations, demonstrating the acidic nature of the sample.

In the spectrum of the first fraction, a very intense band is to be seen at 2850 cm^{-1}, corresponding to the aliphatic CH_2 and CH_3 groups. With the increase in the number of fractions, *i.e.* with the decrease in the molecular weight, this band becomes progressively smaller. As for the lignite humic acids, therefore, this sample too contains aliphatic components. In addition, aromatic vibrations also appear from the first fraction on, at 1400, 760 and 680 cm^{-1}. In the spectrum of fraction 2, the stretching vibration of the phenolic OH groups is observed at 1220 cm^{-1}, as are the deformational vibrations of the CN and NH groups. From this fraction on, the band at 1720 cm^{-1} becomes ever larger with the increase in the number of fractions; in fraction 5, virtually only this peak is predominant, as an indication of the presence of molecules with the lowest molecular weight and the most acidic character.

A peak may be seen at $1640\ cm^{-1}$ only in fraction 2, and at $1620\ cm^{-1}$ only in fraction 4; these correspond to the vibrations of chelates and carboxylates. In fraction 3, a sharp peak occurs at $1030\ cm^{-1}$; this can be ascribed to the SiO group of silicates. A small sharp peak is visible in the same wavelength range in fraction 4 too, but with a substantially lower intensity. Likewise in fraction 4, a vibration corresponding to the alcoholic OH groups is observed at $1170\ cm^{-1}$.

Overall, therefore, this sample displays a simpler structure than those of the samples investigated previously [1, 2]; its spectrum contains fewer and smaller vibrations relating to the various functional groups and chemical bonding types.

Table I

Fraction	%	$\bar{M} \cdot 10^{-3}$ determined by gel-fractionation	$\bar{M}_n \cdot 10^{-3}$	$\bar{M}_w \cdot 10^{-3}$	$\bar{M}_w / \bar{M}_n$
1	26.1	>38.0	41.2	46.6	1.13
2	15.8	38.0—28.0	31.9	35.5	1.11
3	11.4	28.0—18.0	22.3	24.0	1.07
4	10.0	18.0— 5.4	10.8	11.9	1.10
5	35.0	<5.4	4.5	5.2	1.15

Molecular weight distribution, molecular weights determined by osmometry ($\bar{M}_n$) and light-scattering measurement ($\bar{M}_w$), and polydispersities ($\bar{M}_w/\bar{M}_n$) of fractions of peat humic acid from Keszthely.

Table I shows that the fractions obtained by gel-fractionation differ considerably in quantity. The amount of fraction 4 (which spans the broadest molecular weight interval) is only 10%, while that of fraction 5 (containing the lightest molecules)
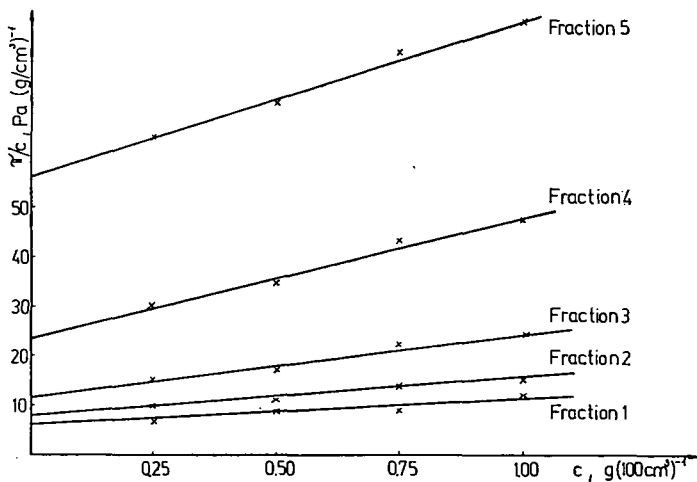

Fig. 2. Concentration-dependence of reduced osmotic pressure (π/c) of peat humic acid from Keszthely

is 35%. Fraction 1 (the heaviest molecules) also accounts for a considerable pro-
portion (26.1%) of the material. However, the Table clearly shows that the individual
fractions have low polydispersities ($\overline{M}_w/\overline{M}_n$), i.e. the gel-fractionation yielded prac-
tically uniform fractions. The molecular weights determined by osmometry and by
light-scattering measurements on the various fractions exhibit a very good parallelity,
with calculated polydispersities between 1.07 and 1.15. The molecular weights
obtained with the two methods likewise correspond well with the molecular weight
intervals given by the gel-fractionation.

The experimental results of osmometry and light-scattering measurements are
depicted in Figs. 2 and 3. Figure 2 demonstrates the concentration-dependence
of the reduced osmotic pressure (π/c). The molecular weights of the fractions were
calculated after extrapolation of these plots to zero concentration, while the second
virial coefficient was determined from the slope; the second virial coefficient is a
very important quantity, for it is characteristic of the magnitude of the interaction
between the solute and the solvent. Its value is the higher, the better the solvation.

It may be seen from Fig. 2 that with the
increase in the number of fractions (i.e.
with the decrease in the molecular weight)
the slope of the straight line progressively
increases, which means that the value of
the second virial coefficient becomes
larger. This permits the obvious conclu-
sion that the fractions with lower molecu-
lar weights are in a better solvated state.

Figure 3 illustrates the concentration-
dependence of the Hc/τ values determined
by light-scattering measurement. Here
too the molecular weights of the indivi-
dual fractions were calculated by extrap-
olation to zero concentration, while the
second virial coefficient was obtained
from the slope of the straight line. The
results are given in Table II. The values
of the second virial coefficient calculated
from the results of light-scattering meas-
urements display a tendency similar to
that observed in the case of osmometry:
this parameter becomes larger with the
increase in the number of fractions (the
slope becomes progressively steeper as the
fractions become lighter in Fig. 3).

The intensities of the scattered light,
measured from various directions for the
individual fractions, were employed to

Fig. 3. Concentration-dependence
of light-scattering measurement data on
peat humic acid from Keszthely

calculate the asymmetry factor, the values of which are also reported in Table II.
From these values it may be concluded that as the molecules become heavier, their
shape differs more and more from spherical.

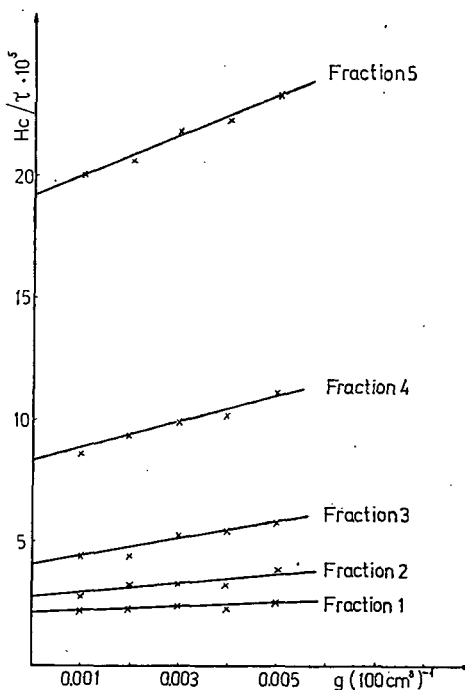We should subsequently like to supplement these investigations with molecular

Table II

Fraction	$\overline{M}_n \cdot 10^{-3}$	B (mol cm³/g²·10⁴) determined by osmometry	$\overline{M}_w \cdot 10^{-3}$	B (mol cm³/g²·10⁴) determined by light scattering	$Z = \dfrac{i_{45}}{i_{135}}$
1	41.2	6.2	46.6	2.5	4.27
2	31.9	8.1	35.5	5.2	3.85
3	22.3	15.4	24.0	9.5	3.23
4	10.8	25.2	11.9	13.3	2.18
5	4.5	34.3	5.2	22.7	1.56

Second virial coefficients (B) determined by osmometry and by light-scattering measurement, and asymmetry factors (Z) of fractions of peat humic acid Keszthely.

weight measurements involving ultracentrifugation, and with alternative fractionation techniques. Such measurements could be used to calculate other thermodynamic parameters too, which would provide additional data towards a more exact understanding of the structures and the colloid chemical properties of the humic acids.

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ИССЛЕДОВАНИЕ ГУМИНОВЫХ КИСЛОТ МЕТОДОМ ИНФРАКРАСНОЙ СПЕКТРОСКОПИИ, III.

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Проведено гель-фракционное разделение образца торфяной гуминовой кислоты и сняты ИК спектры отдельных фракций. Определены средние молекулярные массы отдельных фракций методом осмометрии и светорассеивания.

I. На основании ИК спектров показано, что исследуемый образец содержит не только алифатические, но и ароматические структурные элементы.

II. Согласно данным осмометрии и светорассеивания с увеличением порядкового номера фракции молекулярная масса их уменшается.

Степень полидисперсности, рассчитанная на основании средних отношений весовых и численных молекулярных масс весьма мала, что свидетельствует о практической монодисперсности фракций, выделенных методом гель-фракционирования. Рассчитанные значения вторых вириальных коэффициентов указывают на большую меру сольватации у фракций с меньшими молекулярными массами.

III. С возрастанием молекулярной массы фракций отклонение формы молекул от сферической увеличивается.