

# INFRARED SPECTROSCOPIC EXAMINATION OF HUMIC ACIDS I.

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The infrared spectroscopic examination is highly applicable to determine the functional groups, bond-types and structures of humic acid samples of different origins, if the samples are divided into several homogeneous fractions with a suitable method.

The products obtained in the fractionation of humic acid extracted from Oroszlány brown coal display characteristic values. With the increase in the number of the fractions, the molecular weight rises and increasingly intense peaks appear in the infrared spectrum. In parallel with the increase in the molecular weight, besides the development of the aliphatic side-chains, the increase in the aromatic nature is striking. The infrared spectra of the fractions of humic acid extracted from compost soil indicate a considerably different structure compared with that of brown coal humic acid. In these samples the aromatic character dominates.

Infrared spectroscopy is one of the currently most important research and analytical methods, and is widely used in structural examinations. In the past decade an increasing number of papers have been published on the structural examination of humic materials by infrared spectroscopy.

According to certain research workers [1—3], humic materials originating from different places differ in the proportions of certain functional groups, but the similarity of their spectra indicates materials of similar structure. BUTLER and LADD [4], carried out a detailed analysis of the differences in humic acid preparations obtained by different extraction procedures. They found that the method applied during extraction results in essential differences in the infrared spectrum. According to DORMAAR [5], the infrared spectra of different soil humic acids depends to a great extent on the structure of the soil, the thickness of the layer and the molecular weight of the sample. ISHIWATARI [6] examined the structures of fulvic and humic acids extracted from the sediment of lakes and from the soil, also applying spectroscopic methods. He compared the functional group, the molecular weight and the elemental analysis data. He found that in the visible and ultraviolet regions there is no essential difference in the spectra of the samples; however, in the infrared region, significant differences can be found, depending on the extent of humification. KHAN [7] examined heat-treated humic acid fractions. He observed that heat-treatment does not cause a significant change in the infrared spectrum. GOH and STEVENSON [8] compared synthetic and natural humic acids on the basis of their infrared spectra. Certain synthetic materials (e.g. catechol and benzoquinone types) show a slight, but others a strong similarity with soil humic acids. NUTTER and DAVIS [9] examined

preparations obtained by ultrafiltration, as well as metal humate complexes. On the basis of their experimental data, they declared that the course of complex-development can be followed very well by means of the infrared technique.

During the past few years we have carried out series of measurements in which infrared spectroscopy was used to establish the chemical compositions of coal samples of different ages and products made from them and to characterize certain preparations. The method proved applicable in the determination of the characteristic bonds of the samples examined and the nature of the functional groups; it was also possible to deduce the quantity of the latter. With this method it was possible to choose the optimum extracting conditions for certain preparations and, complemented by analytical procedures, to trace the degree of carbonification of coals. These examinations were reported earlier [10—13].

Colloid chemical examinations of humic acids extracted from coals [14—17] proved that, for a clearer picture of the structure, the preparations obtained, must be fractionated further, since they show considerable heterodispersity in their original state. This heterodispersity can be observed in the infrared spectra of the humic materials where only a few, mainly broad overlapping bands can be observed.

Since our previous examinations revealed that, as a result of the interaction of the functional groups, the broad bands in the infrared spectra taken before fractionation are compound ones, our aim was further gel-chromatographic fractionation and examination of the resulting humic acid preparations. The infrared spectra of the fractions were compared with those of the humic acid samples before fractionation.

Our investigations were carried out on a humic acid sample extracted from Eocene brown coal from Oroszlány. The methods of extracting the humic acids [18] and purifying the crude products [19] were described earlier. For comparison, a humic acid extracted from a compost soil was also examined; this was purified by similar operations as for the coal humic acid.

To obtain preliminary information, infrared spectra were taken of the two materials. Figures 1 and 2 show only a few, mainly broad overlapping bands. Such spectra suggest that heterodisperse materials were obtained in the extraction. This is supported by gel-fractionation examinations on the same materials, whose distribution curves indicate considerable heterogeneity. Gel-fractionation examinations were carried out as described previously [20].

For infrared spectroscopy, 1—2 mg sample was homogenised with 200 mg KBr in an agate mortar. Pellets were then formed at 11 atm pressure. Spectra of the pellet preparations were taken with a Spectromom 2000 spectroscope over a 5-minute measuring time in the  $650\text{--}4000\text{ cm}^{-1}$  wavenumber range. The applied concentration and layer thickness gave band transmission values within the 20—70% region.

Preparative fractionation was carried out on the two humic acid samples on a Sephadex G-75 column, 6 cm in diameter and 40 cm in height [20]. 5 fractions of coal humic acid and 4 of compost humic acid were obtained. The quantity of each fraction was 2—3 mg. With molecular weight standards and calibration curves the molecular weights of the fractions were calculated from the gel-fractionation data, and then, on the basis of the percentage compositions of the fractions, the harmonic means of the molecular weights of the two humic acids were obtained. These data are given in the Table I.

*Table I*  
*Molecular weight fractions at different samples*

Sample	Number and molecular weight of fractions ( $M \cdot 10^{-3}$ )					Harmonic mean molecular weight ( $M \cdot 10^{-3}$ )
	1	2	3	4	5	
Oroszlány brown coal humic acid	1.5	5	10	25	40	7.5
Compost soil humic acid	4.0	12	25	60	—	24.0

It is evident from the Table that the brown coal humic acid contains fractions of considerably lower molecular weight than the soil humic acid. As regards the similarity or difference of their structures, an answer can be expected from the infrared spectra.

The spectra taken of the humic acid extracted from Oroszlány brown coal, as well as of the 5 fractions of this sample, are shown in Fig. 1. The characteristic bond and group frequencies in Fig. 1 were evaluated on the basis of the literature and our own experience, as follows.

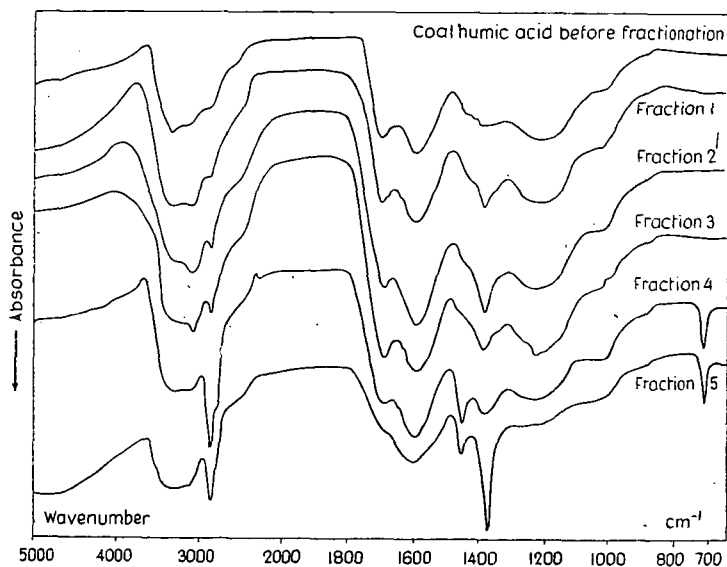


Fig. 1. Infrared spectra of coal humic acid fractions

No sharp peaks can be observed in the infrared spectrum of the sample before fractionation. Only in the  $3000\text{--}3500\text{ cm}^{-1}$  region can there be found a very broad diffuse band, which is of a similar structure in the case of the fractions, too. A small peak can be observed at  $3100\text{ cm}^{-1}$  only in the cases of the 2nd and 3rd fractions. This is due to the presence of amides. A smaller broad band is found around  $1700\text{ cm}^{-1}$ , and a larger one near  $1600\text{ cm}^{-1}$ . The former corresponds to the  $\text{C}=\text{O}$

of the carboxyl groups of the humic acid, the latter to the valency vibrations of the carboxylate ion. A slight shoulder can also be found at  $1400\text{ cm}^{-1}$ , and a very flat, broad band near  $1200\text{ cm}^{-1}$ . The identification of these bands is uncertain with such great heterodispersity.

In the first fraction of this sample a small sharp shoulder appears at  $2850\text{ cm}^{-1}$ ; this becomes more and more intense in the later fractions. In the 4th fraction a very intense, long peak can be found at the same wavenumber. In the 5th fraction the intensity of the peak decreases slightly. This peak indicates the presence of aliphatic ( $\text{CH}$ ,  $\text{CH}_2$  and  $\text{CH}_3$ ) groups. This tendency is well supported by the data shown in Table I: with the increase in the number of fractions, the molecular weight increases as well. In the higher fractions sharper peaks can be observed, which proves the presence of humic acids of greater molecular weight (more aliphatic chains). There is a seemingly contradictory fact in connection with this tendency, *i.e.* the intensity of the band decreases in the 5th fraction. At the same time, a very sharp peak appears in this fraction at  $1360\text{ cm}^{-1}$ ; this is due to the presence of aromatic carboxylic acids. (This peak appears in very weak form in the 4th fraction). Furthermore, there is a sharp band at  $720\text{ cm}^{-1}$ ; according to the literature, this corresponds to a C—C skeletal vibration and to a  $(\text{CH}_2)_n$  grouping, where  $n=4$  or more. In the case of fractions 1—4 the vibration of the carboxylic C=O groups can be observed in the range  $1700\text{—}1720\text{ cm}^{-1}$ , with increasing intensity between fractions 1 and 3. In the 4th fraction this peak is smaller, and it has disappeared completely in the 5th fraction. In the 4th and 5th fractions a sharp peak appears at  $1460\text{ cm}^{-1}$ . This corresponds to the deformation vibrations of the  $\text{CH}_2$  and  $\text{CH}_3$  groups on aromatic rings. This is in accordance (especially in the case of the 5th fraction) with the development of the aromatic character found in the region of  $1360\text{ cm}^{-1}$ . According to the spectrum, besides the aromatic characteristics, sidechains can also be found on the ring in the case of these two fractions of highest molecular weight. Each fraction has a characteristic band of the same width and intensity around  $1600\text{ cm}^{-1}$ . This corresponds to the C=C bond and the carboxylate ion valency vibration. The presence of these bands might mean that the system contains not only covalent bonds, but also ionic  $\text{COO}^-$  groups. This fact is well supported by some of our metal ion-induced aggregation examinations carried out previously [21], in which it was found that a proportion of the metal humates are ionically bound.

In the 3rd fraction a small peak can be observed at  $1240\text{ cm}^{-1}$ ; this indicates the presence of phenolic OH groups. There is a shoulder at  $1020$  and  $1030\text{ cm}^{-1}$  in the case of the 1st, 3rd and 4th fractions, which might mean silicate-contamination or polysaccharides in the sample.

Similar to the coal humic acid sample, the humic acid sample extracted from compost soil shows a spectrum containing scarcely evaluable, flat, broad bands before fractionation. After fractionation, however, evaluable, characteristic bands appear in the spectra of the fractions. These can be seen in Fig. 2. On comparison of these spectra with those demonstrated in Fig. 1, the difference is obvious. The fractions of soil humic acid show completely different characteristic values. Only the region above  $3000\text{ cm}^{-1}$  is similar to the spectrum of the fraction of brown coal humic acid. The  $3500\text{—}3600\text{ cm}^{-1}$  region for the soil humic acid differs slightly from this, *i.e.* here the broad band is divided into two parts. It may be assumed

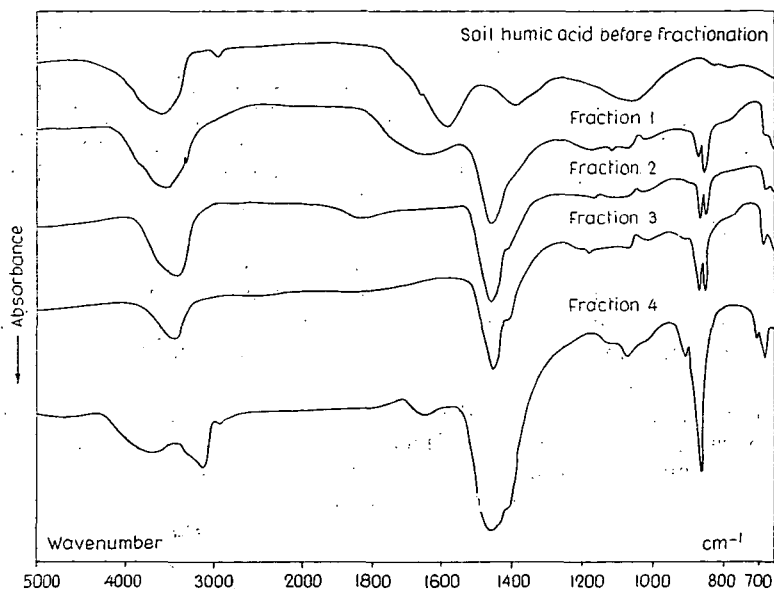


Fig. 2. Infrared spectra of soil humic acid fractions

that in this fraction the humic acid in the solid sample has a cyclic dimeric structure, and its OH valency vibration band is different from that of the other intermolecularly-associated compounds.

The 1720 and the 1620  $\text{cm}^{-1}$  bands in the spectrum of the coal humic acids, which prove the presence of carboxyl groups and carboxylate ions, are absent here from each fraction. However, the deformation vibrations of the  $\text{CH}_3$  and  $\text{CH}_2$  groups on the aromatic ring appear as very intense bands in the spectra of each fraction, at 1450  $\text{cm}^{-1}$ . In the 2nd, 3rd and 4th fractions, the valency vibration of aromatic carboxylic acids can be observed with a small shoulder at 1380–1400  $\text{cm}^{-1}$ . The mainly aromatic nature of the samples is proved by the characteristic value of the deformation vibration at 860  $\text{cm}^{-1}$ , which appears with increasing intensity, parallel with the increase in the number of the fraction; this is typical of 1,3,5-tri-substituted aromatic compounds. A band characteristic of substituted aromatic compounds can be found with the same tendency at 680  $\text{cm}^{-1}$ . In the 3rd fraction there is only a small shoulder at 1060  $\text{cm}^{-1}$ , but in the 4th it is a small peak; it is characteristic of polysaccharides indicative of plant fossil remains. It is absent from the earlier fractions, probably because of decomposition. In the 3rd fraction, and to a smaller extent in the 2nd fraction, there is a deformation vibration at 1220  $\text{cm}^{-1}$ , characteristic of the C—N and N—H groups.

The 2850  $\text{cm}^{-1}$  region, indicating aliphatic character, shows absorption in the form of a small shoulder in the 4th fraction, which has the highest molecular weight.

The examinations permit the statement that the infrared spectra of the humic acid samples before fractionation cannot be evaluated unambiguously, owing to their heterodispersity. The broad bands found in these spectra are highly complex

as a result of the interaction of the functional groups of the fractions; the characteristic bond and group frequencies overlap.

The products obtained in the fractionation of humic acid extracted from Oroszlány brown coal display characteristic values. With the increase in the number of the fractions, the molecular weight rises and increasingly intense peaks appear in the infrared spectrum. In parallel with the increase in the molecular weight, besides the development of the aliphatic side-chains, the increase in the aromatic nature is striking.

The infrared spectra of the fractions of humic acid extracted from compost soil indicate a considerably different structure compared with that of brown coal humic acid. In these samples the aromatic character dominates. The intensity increase of the band runs parallel with the increasing molecular weight in the case of these samples, too.

To summarize, it can be stated that the infrared spectroscopic examination is highly applicable to determine the functional groups, bond-types and structures of humic acid samples of different origins, if the samples are divided into several homogeneous fractions with a suitable method.

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## ИНФРАКРАСНАЯ СПЕКТРОСКОПИЯ ГУМИНОВЫХ КИСЛОТ, I

*Ш. Шупов и Э. Шупов*

Авторы провели ИК-спектроскопическое изучение различных фракций гуминовых кислот, разделенных гель-хроматографией. ИК-спектры фракций сравнивали со спектром исходных образцов. Найдено, что только фракционированные образцы имеют спектры, обладающие характерными полосами пригодными для соответствующего отнесения. Показано, что в гуминовой кислоте из других углей, вместе с увеличением молекулярного веса, наряду с появлением алифатических боковых цепей появляется также ароматический характер. В фракциях гуминовых кислот из компостных почв показана преобладающая роль ароматических участков. Используемый метод, по полученным данным, показывает его применимость для определения структуры и состава различных гуминовых кислот.