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THERMAL ANALYSIS AND INFRARED SPECTRA OF THE HUMIC ACIDS ISOLATED FROM THE CURATIVE MUDS OF HAAPSALU BAY

Sea sediments (curative muds) have been used in Estonia to cure people for more than a hundred years already [1]. The humic acids (HA) extract from the curative muds of Haapsalu Bay has been used since 1972 for the manufacture of an injection drug under the trade name Humisol. Although it is effective in the treatment of many inflammatory diseases caused by disorders in the immune system [2], very few investigations of this preparation have been carried out from a chemical point of view. Recently, the composition and HA hydrolysates contents of Humisol (amino acids and monosaccharides) were published [3]. The isoelectric properties of the HA of Humisol are also available [4]. However, more evidence is needed to elucidate the chemical background of the curative effect of this preparation. Little is known about the inorganic components present in Humisol and their connections with HA.

In this paper an attempt is made to investigate Humisol using thermal analysis and infrared spectroscopy.

Experimental

Humic acids. The stock solution of Humisol, containing HA, was obtained from the Tallinn Chemical and Pharmaceutical Plant. The stock solution was divided into two fractions (permeate and non-permeate) with 200 nm nylon membranes (Himfil, Estonia). After filtering about 80% (v/v) of the permeate, the same volume of distilled water was added into the filtering unit and the procedure was repeated twice. The permeate fractions were combined, evaporated, and dried at 105°C. The non-permeate fraction was treated in the same way.

Thermal analysis was performed on a Q-1000 Derivatograph (MOM, Hungary). The samples were heated in platinum microcrucibles in the temperature range of 20–800°C at a constant rate of 10 deg/min with an air purge of 200 ml/min; differential thermal analysis (DTA) at a sensitivity of 250 µV; thermogravimetry (TG) and differential thermogravimetry (DTG) in a mass range of 0–50 mg and at a sensitivity of 500 µV. Alternatively the total sample was heated in an electric muffle as follows: four ceramic crucibles, each containing about 110 mg of sample, were heated at 600°C for 2 hours. Then one of the crucibles was taken out, cooled and weighed, the temperature was raised to 700°C during 10 minutes and kept at this level for 2 hours.

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The same was done with the other samples and the procedure was repeated at 800 and 900°C. The temperature was kept with an accuracy of $\pm 24^\circ\text{C}$.

Infrared spectra were taken using a Specord 75 IR Spectrometer (Carl Zeiss, Jena, DDR) from ~ 1 mg of material pelleted with ~ 150 mg of KBr. Ultraviolet spectra were taken from 0.005 and 0.015% water solutions using a Specord UV-Vis Spectrometer (Carl Zeiss, Jena, DDR) and 10 mm quartz cuvettes.

Elemental analysis. The carbon, hydrogen, and nitrogen contents were estimated with a Hewlett Packard 186 CHN Analyser and corrected on moisture by thermal analysis data. Sulphur was determined by the Eschka method according to [5] (Table).

Some characteristics of Humisol dry matter

Sample	Yield, %	Elemental composition, %			H/C
		C	H	N	
Total	100	40.4	4.1	3.2 ^a	1.22
Non-permeate					
>200 nm	74	46.5	4.9	3.8	1.25
Permeate					
<200 nm	26	10.7	0.9	0.7	1.01

^a S content 2.5%

Results and Discussion

Thermal analysis. The weight losses of the samples investigated at temperatures up to 200°C were probably caused by the loss of water. The corresponding endothermic effect with an extreme at 85°C is clearly seen on the DTA curve of the total Humisol sample (Fig. 1, Curve 1') The water loss of that sample is 12%, being 14 and 2% for the non-permeate and permeate, respectively (Fig. 2).

The largest total weight loss in heating the samples in the derivatograph up to 700°C was registered for the organic-rich non-permeate fraction (Fig. 2). The weight loss of the non-permeate fraction was 90% of the initial matter (Curve 2); for the organic-poor permeate fraction this value was 11% (Curve 3), and for the total sample 53% (Curve 1). For comparison, the weight loss at heating the total Humisol in the electric muffle (Fig. 2, Curve 1m) at 700°C was more than 67% of the initial mass (the mean of two experiments). This weight loss of the total sample was higher than that measured by heating the same sample in the derivatograph (53%), but it was very close to the weighted average of the weight losses of the non-permeate and permeate at 700°C (70%). This demonstrates that the permeate reduces the velocity of the thermal decomposition of organic matter during the heating of Humisol. The temporal delay of exothermic effects (these effects appear at higher temperatures) upon heating the total sample may be really caused by the presence of inorganic matter in Humisol. As an example, no exothermic maximum on the DTA curve is observed for the H-substituted

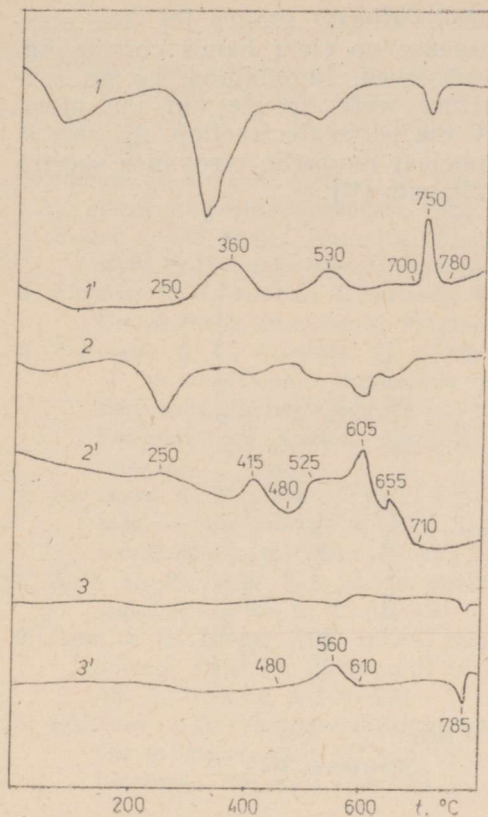


Fig. 1. The DTG (1-3) and DTA (1'-3') curves of thermal analysis. 1, 1' — the total sample; 2, 2' — the non-permeate fraction (>200 nm); 3, 3' — the permeate fraction (<200 nm).

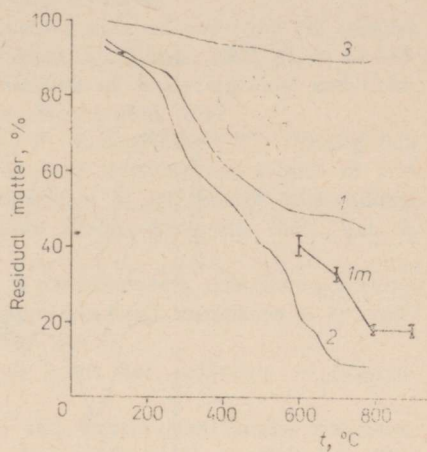


Fig. 2. The TG curves of thermal analysis. 1m — the total sample in the electric muffle; the other designations are the same as in Fig. 1.

humic acids of soil at temperatures above 600°C [6]. On the DTA curve of the total Humisol the last exothermic effect occurs at 750°C (Fig. 1, Curve 1'), while in the case of the non-permeate fraction it lies at 655°C (Curve 2').

The endothermic minimum at 785°C on the DTA curve of the permeate (Fig. 1, Curve 3') corresponds to the fusion temperature of chlorides (782°C for CaCl_2). The simultaneous slight weight loss shows that some compounds were also evaporated or destructed.

It can be concluded that thermal analysis can provide new information about Humisol. Also, it is necessary to analyse the chemical composition of the gaseous compounds evolving upon heating the sample to elucidate the extrema on the DTA curve of total Humisol related to an intensive weight loss of the sample.

Infrared spectra (Fig. 3). The absorbance maxima are at 2920 and 2845 cm^{-1} for all samples. These values correspond to the aliphatic C—H stretching vibrations. The existence of these maxima in the spectrum of the ash sample shows that the organic matter was not destructed completely in the derivatograph. It is not excluded, however, that these maxima may be due not only to the C—H vibrations but also to the $\text{R}_3\text{—Si—OH}$ groups present in clay minerals [7].

All the spectra have an intense maximum near 3400 cm^{-1} due to the NH and H-bonded OH stretching bands. The latter must belong to the COOH group because there is a clear maximum at 1225 cm^{-1} on the spectrum of the total sample. The maxima at 1630 cm^{-1} observed in the infrared spectra of humic substances of Baltic Sea sediments [8] can be attributed to the amide NH stretching plus some contribution from

the asymmetrical stretching of ionized carboxyl groups [9]. The presence of phenolic OH is not likely because no clear bands corresponding to the aromatic compounds are observed. In addition, no remarkable signs indicating aromatic structure were detected in the ultraviolet spectra of the total sample and the permeate fraction. All this is in good agreement with ^{13}C and ^1H nuclear magnetic resonance spectra of the HA isolated from Baltic Sea sediments [10].

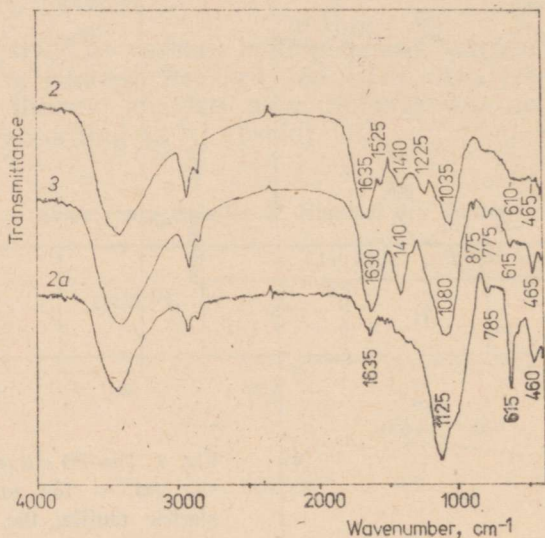


Fig. 3. The infrared spectra of Humisol fractions. 2a — the ash of the non-permeate fraction; the other designations are the same as in Fig. 1.

The intensive absorbance at 615 and 1125 cm^{-1} in the ash spectra (Fig. 3, Spectrum 2a) can be assigned to the sulphate group, which is the most stable form of sulphur in the oxidizing media. On the spectrum of the non-permeate fraction (Fig. 3, Spectrum 2) there is a very weak peak, which is slightly more intense in the permeate sample. This means that the sulphate in ash was formed on the oxidation of organic sulphur in Humisol. As the amount of amino acids containing sulphur is small in Humisol [3], the sulphur must be present in other organic fragments.

No absorbances of sulphones were detected in the spectrum of Humisol. The other organic sulphur compounds are difficult to detect because the absorption bands for these groups below 1000 cm^{-1} are weak and the strong bands over 1000 cm^{-1} are overlapped with other bands.

The maxima at 460–465 cm^{-1} are most likely due to silica-containing groups ($\text{Si}(\text{OCH}_3)_3$ or cyclic SiC band — 440–480 cm^{-1} or silicate ion — 460–470 cm^{-1}). The characteristic bands at 815–825 cm^{-1} and 775–785 cm^{-1} can be attributed to silico-organic compounds. The same conclusion was made on the basis of infrared spectra of the HA of Baltic Sea sediments in [8].

It can be concluded that in some aspects the HA in the Humisol are similar to HA isolated from the bottom sediments of the Baltic Sea.

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HAAPSALU LAHE RAVIMUDAST ERALDATUD HUMIIINHAPETE TERMILINE ANALÜÜS JA INFRAPUNASED SPEKTRID

Analüüsiti Haapsalu lahe ravimudast valmistatud ravimi — humisooli — kuivaine ja tema fraktsioonide (200 nm poorigega membraani läbivat permeaati ja mittepermeaati) derivatogramme ning infrapunaseid spektreid. Selgus, et humisoolis leiduv mineraalne mõjutab oluliselt preparaadis olevate humiinhapete käitumist kuumutamisel. Infrapunaste spektrite alusel on näidatud, et humisoolis leiduvad humiinhapped on sarnased teiste Läänemere setetest eraldatud humiinhapetega.

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ТЕРМИЧЕСКИЙ АНАЛИЗ И ИК-СПЕКТРЫ ГУМИНОВЫХ КИСЛОТ, ВЫДЕЛЕННЫХ ИЗ ЛЕЧЕБНОЙ ГРЯЗИ ХААПСАЛУСКОГО ЗАЛИВА

Проанализированы дериватограммы и ИК-спектры сухого вещества лекарственного препарата гумизоль и его фракций. Найдено, что минеральное вещество, содержащееся в гумизоле, существенно влияет на поведение гуминовых кислот при их нагревании. ИК-спектроскопией показано, что гуминовые кислоты гумизоля похожи на гуминовые кислоты донных осадков Балтийского моря.