

HUMIC ACIDS FROM OXIDIZED COALS: CAPILLARY ZONE ELECTROPHORESIS AND MASS-SPECTROMETRY STUDY

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ABSTRACT

Humic acids from open-air and laboratory oxidized bituminous coals (under various temperatures) were prepared and characterized. For the comparison, lignite and oxidized lignite (oxihumulite) were examined as well. All samples were analyzed by capillary zone electrophoresis (CZE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). For the CZE separation of humic acids (HAs), the borate-Tris-EDTA (pH=8.3) and borate- β -cyclodextrine (pH=9.6) electrolytes were used.

The samples of humic acids were prepared by their dissolution in sodium hydroxide solutions. All CZE electropherograms are typical by a huge non-resolved peak (hump) which contains a lot of migrating humic acids. The ageing effect of HAs, brought about their self-assembling processes, was observed as a prolongation of hump migration times in both electrolyte systems. However, after 30 days a very good reproducibility of electropherograms was obtained. In the borate-Tris-EDTA electrolyte the migration times of HAs relate to their metal (Pb) binding capacities. The broadening of the CZE humps of HAs, isolated from laboratory oxidized bituminous coal, corresponds to their thermal decomposition during coal oxidation. Their shorter migration times were registered in the more alkaline borate- β -cyclodextrine electrolyte probably due to the further dissociation of HAs hydroxyl groups. In this electrolyte, the electrophoretic mobilities of HAs, isolated from lignite and oxidized lignite, were significantly retarded likely due to the formation of inclusion complexes with β -cyclodextrine.

By using the mass spectra of HAs isolated from laboratory and naturally oxidized coal it was found that the intensities of mass spectra lines are dependant on the temperature and amount of oxygen. The higher degree of this alteration, the lower intensities of lines from 680 to 920 m/z and the higher intensities of lines from 250 to 450 m/z were observed.

On the basis of obtained results electrophoretic and mass spectra measurements were found to be useful for the study of changes in chemical structure of HAs owing to their oxidation and thermal treatment.

KEYWORDS: Humic acids, oxidized coal, capillary electrophoresis, mass spectrometry

1. INTRODUCTION

Humic acids are complex polyfunctional macromolecules. Their spectroscopic study is often complicated by overlapping of spectral bands and therefore their separation is usually necessary. For this reason chromatographic techniques were applied, e.g., high performance liquid chromatography as a reverse phase chromatography (Preuße et al., 2000), size exclusion chromatography (Tatár et al., 2002), and high-performance immobilized metal ion affinity chromatography (Wu et al., 2002) and so forth.

Also electrophoretic techniques, such as capillary isotachopheresis (Kopáček et al., 1991; Kaniánský et al., 1999), isoelectric focusing (Schmitt et al., 1997), and capillary zone electrophoresis have been used for the humic acids separation for a long time. Namely CZE has been developed into a very efficient and sensitive method and is preferred by many authors (Schmitt-Kopplin et al., 1998;

Shirshova and Österberg, 1998; Fetsch and Havel, 1998; Fetsch et al., 1998; Gajdošová et al., 2003). Recently, a comprehensive overview about CZE of natural organic matter including humic substances has been published by Schmitt-Kopplin and Junkers (2003).

Other approach to the humic compounds study is the separation of their fragments. Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and Curie-point pyrolysis-gas chromatography-combustion-isotope ratio mass spectrometry (Py-GC-C-IRMS) were applied for the investigation of humic acids as well (Río et al., 1994; Schulten and Leinweber, 1996; Schulten and Gleixner, 1999; Smeulders et al., 2000). The humic acids in sewage sludge were investigated using thermally assisted hydrolysis and methylation coupled to gas chromatography-mass spectrometry (THM-GC-MS) (Reveillé et al., 2003).

Mass spectrometry is now an important method for the research of humic substances. Temperature programmed pyrolysis in the ion-source of a mass spectrometer (Py-FIMS) was firstly used for the study of humic substances and soil organic matter by Shulten (1995). However, pyrolysis results in the loss of some structural information due to compound decomposition. Using the soft ionization techniques, such as electrospray ionization (ESI) (Kujawinski et al., 2002; Stenson et al., 2002; Stenson et al., 2003), matrix-assisted laser desorption/ionization (MALDI) (Fievre et al., 1997), and MALDI time-of-flight (Remmler et al., 1995; Gajdošová et al., 2003), this problem is largely reduced although fragmentation remains in the case of laser desorption methods including MALDI.

Recently, HAs from naturally and laboratory oxidized bituminous coal and those from lignite and oxidized lignite were isolated and studied. The coal occurrence and its characteristics as well as characteristics of isolated HAs (elemental composition, pK_{app} values, binding capacity of heavy metals, ^{13}C CP/MAS NMR, and infrared spectra) were described (Kurková et al., 2004). In this paper, the CZE and MALDI-TOF experiments were carried out with the same HAs samples.

The aim of this work was to confirm our previous results (Kurková et al., 2004) and verify hypotheses that bituminous coal, deeply deposited in the proximity of the red bed body (RBB), was oxidized at about ambient temperature in the atmosphere poor in oxygen. The low partial pressure of oxygen was caused by dilution of atmospheric oxygen with combustion products, such as CO , CO_2 , and H_2O , originated from coal oxidation (Klika et al., 2004).

2. MATERIALS AND METHODS

2.1. COAL AND HAS SAMPLES

The non-altered bituminous coal with a negligible content of humic acids (sample 1) was used for laboratory oxidation by air at 150, 200, 250, and 300 °C forming oxidized coal samples 1-1, 1-2, 1-3, and 1-4, respectively. From these samples the humic acids were isolated: HA1-1 (150 °C), HA1-2 (200 °C), HA1-3 (250°C), and HA1-4 (300 °C). HAs isolated from natural oxidized bituminous coal are HA2 and HA3 from coal samples 2 and 3, respectively. HAs from lignite are HA4 from non-oxidized lignite (sample 4) and HA5 from oxidized lignite (sample 5) (Table 1). All samples were prepared in sodium hydroxide solutions at a laboratory temperature according to the already published procedure (Kurková et al., 2004).

2.2. CHEMICALS

All reagents were of analytical purity grade. Sodium hydroxide, 1,1,1-Tris-hydroxymethyl-amino-

methan (Tris) and ethylenediaminetetraacetic acid (EDTA) were obtained from Lachema (Brno, Czech Republic). Boric acid was purchased from Merck (Darmstadt, Germany). Mesityloxide, used as an EOF marker, was purchased from Fluka (Buchs, Switzerland). Water distilled from a quartz apparatus of Heraeus Quarzschmelze (Hanau, Germany) and deionized by a mixed-bed ion-exchanger was used for preparation of all solutions.

2.3. PROCEDURES AND APPARATUS

Electrophoretic separations were carried out on a Beckman System 5500 - Model P/ACE (Palo Alto, CA, USA) equipped with a diode array detector (DAD), automatic injector, fluid-cooled column cartridge and System Gold Data station. A Fused silica capillary tube of 47 cm (40.3 cm length to the detector) x 75 μm I.D. was used. The normal polarity mode of the CZE system (cathodic pole at the side of detection) was applied. Between runs, the capillary was washed out for 1 min. with 0.1 M NaOH, 5 min. with deionised water and 3 min. with the background electrolyte (BGE). All HAs solutions were filtered through the 0.2 μm filters from Sigma (USA). Electroosmotic flow (EOF) was determined by 0.1 % mesityl oxide under the same conditions as the HAs separations were performed. At the end of the working day, the capillary was washed out for 3 min. with 0.1 M NaOH followed by 5 min. washing with deionised water.

The borate-Tris-EDTA buffer consists of 90 mM Tris with 90 mM boric acid and 1 mM EDTA. The experiments were run under the following separation conditions: pH=8.3, 20 °C temperature, 20 kV separation voltage, 20 s hydrodynamic injections, and wavelength 210 nm. The second BGE contained 50 mM $Na_2B_4O_7$ with 15 mM β -cyclodextrine (β -CD). The optimal separation conditions were found to be pH=9.6, temperature 40 °C, separation voltage 15 kV, 20 s hydrodynamic injection and wavelength of 210 nm. The HAs solutions were prepared by dissolution of the HA samples in 900 μl of 0.1 M NaOH and filling up by deionised water in 25 ml volumetric flask. The concentrations of the HAs solutions were of 100 mg/l.

The mass spectra were performed in the laser desorption ionization (LDI) mode and measured by Compact MALDI III mass spectrometer (Kratos Manchester, U.K.) in linear positive mode. The instrument was equipped with a nitrogen laser (wavelength 337 nm, pulse duration $\tau = 10$ s, pulse energy 200 μJ). The laser energy ranged from 130 to 180 units and 100 laser shots were used for each sample. Insulin was used for calibration. The HAs concentrations, used for the measuring, were of 1000 mg/l and mass spectra were measured for 4 days, 1 month (30 days), and 5 months after their dissolution in the NaOH solutions.

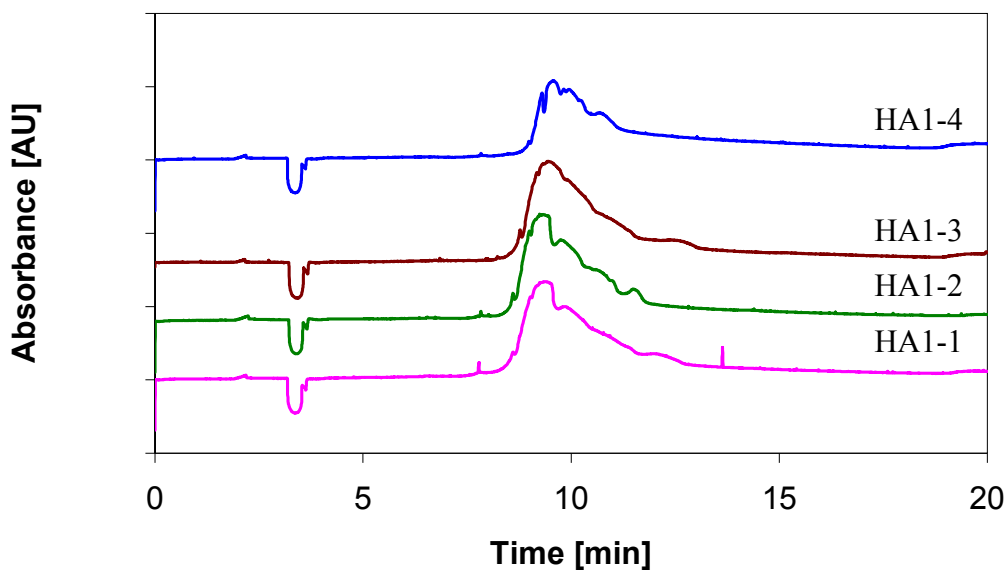


Fig. 1 Electropherograms of HA1-1, HA1-2, HA1-3, and HA1-4 samples measured in the borate-Tris-EDTA electrolyte

3. RESULTS AND DISCUSSION

3.1. CAPILLARY ZONE ELECTROPHORESIS

For the CZE separations the acetate, borate, and phosphate background electrolyte systems are mostly used (Schmitt-Kopplin et al., 2003). Two different BGEs were used for the study of HAs: the borate-Tris-EDTA and borate- β -cyclodextrine buffers (Pokorná et al., 2000). When using borate buffers, attention must be paid to peaks that are artifacts of borate ions interaction with 1,2- and 1,3- diols presented in the humic acids (Schmitt-Kopplin et al., 1998). But for rapid screening and characterization of HAs the borate electrolytes are suitable.

In general, the electropherograms of HAs are typical by migration of major humps which contain a lot of unresolved humic species. These humps can be described by mean electrophoretic mobilities, which are given by charge-to-mass ratio governed by the ionization of acid groups, and by molecular weight. The humps mutually differ in their migration times and shapes. From this it follows the electropherograms could be used for fingerprinting of HAs from various types of coal.

3.1.1. BORATE-TRIS-EDTA ELECTROLYTE

The stability of HAs was tested by their dissolution in the NaOH solutions during various time periods. The used BGE consist of borate, Tris, and EDTA (pH=8.3). With increase of dissolving time the HAs humps tend to be broader and slower probably because of the HAs structures changes. The longer migration times indicate the formation of high-

molecular-weight molecules. HAs were found to be stable after 7 days from their dissolution. It is well known that HAs are self-assembling systems which are able to modify their structure because of their flexible nature. It can be observed also as the changes of A_{465}/A_{665} ratio (Gajdošová et al., 2000), where A_{465} and A_{665} are the absorbance of HAs at 465 and 665 nm, respectively. All next electropherograms were measured a month after the HAs dissolution in NaOH. Reproducibility of electrophoretic measurements, tested using the stable HA1-3 solution, was found to be very good.

The electropherograms of HAs, isolated from laboratory oxidized coal at various temperatures, are shown in Fig. 1. Except HA1-4, all other electropherograms are similar. The humps are unsymmetrical and partially broken. The hump symmetry corresponds to the distribution of migrating species in their unresolved zone. It is obvious that the faster fragments with the lower molecular weights prevail in these humps. The hump of HA1-4 is wider and lower likely as a result of the HAs partial decomposition at high temperature of 300 °C.

The electropherograms of HAs, isolated from open-air altered bituminous coals (HA2 and HA3), from lignite (HA4), and open-air oxidized lignite (HA5), are displayed in Fig. 2. The mean migration times of HA2 and HA3 are similar with HAs isolated from bituminous coal HA1 oxidized in laboratory (Fig. 1). However, there are significant differences between retention times of the bituminous coal HAs (HA2, HA3) and the lignite ones (HA4, HA5). The HA4 and HA5 humps are broader and lower in

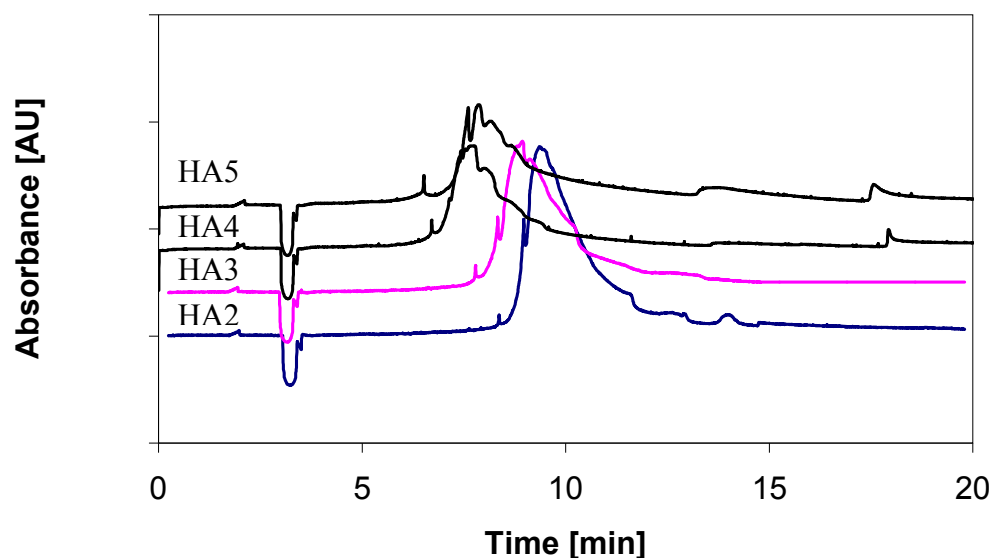


Fig. 2 Electropherograms of HA2, HA3, HA4, and HA5 samples measured in the borate-Tris-EDTA electrolyte

Table 1 List of HAs samples

HA samples	Coal mine/locality	Coal and type/condition of its oxidation	Pb-binding capacity	pK_{app}
HA1-1	Lazy	Bituminous coal oxidized at 150 °C by air for 14 days, (sample 1-1)	0.50	4.61±0.05
HA1-2	Lazy	Bituminous coal 1 oxidized at 200 °C by air for 7 days, (sample 1-2)	0.62	4.27±0.04
HA1-3	Lazy	Bituminous coal 1 oxidized at 250 °C by air for 4 days, (sample 1-3)	0.70	4.08±0.04
HA1-4	Lazy	Bituminous coal 1 oxidized at 300 °C by air for 4 days, (sample 1-4)	0.82	3.66±0.02
HA2	Lazy	Oxidized bituminous coal from vicinity of red beds bodies deposited under the earth surface (sample 2)	0.30	4.85±0.05
HA3	Landek Hill	Oxidized (weathered) coal from outcrop of bituminous coal seam (sample 3)	0.53	4.47±0.05
HA4	Mír Mikulčice	Lignite (sample 4)	0.61	3.35±0.02
HA5	Bilina	Weathered lignite (oxihumolite, sample 5)	0.76	3.80±0.03

Note: Bituminous coal was sampled in Upper Silesian Coal Basin, lignite in North-Bohemian Lignite Coal Basin, (both basins in the Czech Republic), pK_{app} (the apparent dissociation constant) and Pb-binding capacity (mmol/g) were adopted from Kurkova et al. (2004).

contrast with those of HA2 and HA3. The electrophoretic migration of HA2 and HA3 is slower than that of HA4 and HA5. The humic acids HA4 and HA5 likely possess higher charge because of the higher amount of ionized acid groups ($-COOH$ and $-OH$). This suggestion agrees with the values of metal (Pb) binding capacity (Table 1). Metal binding capacity seems to be more suitable for the description of acid

group ionization than pK_{app} values (up to 4.6) which are mainly associated with the dissociation of carboxylic groups.

The remarkable difference observable between the migration velocity of HA2 and HA3 can be explained by the weaker oxidation of HA2 in atmosphere poor in oxygen while the HA3 was formed by weathering on earth surface. Therefore the

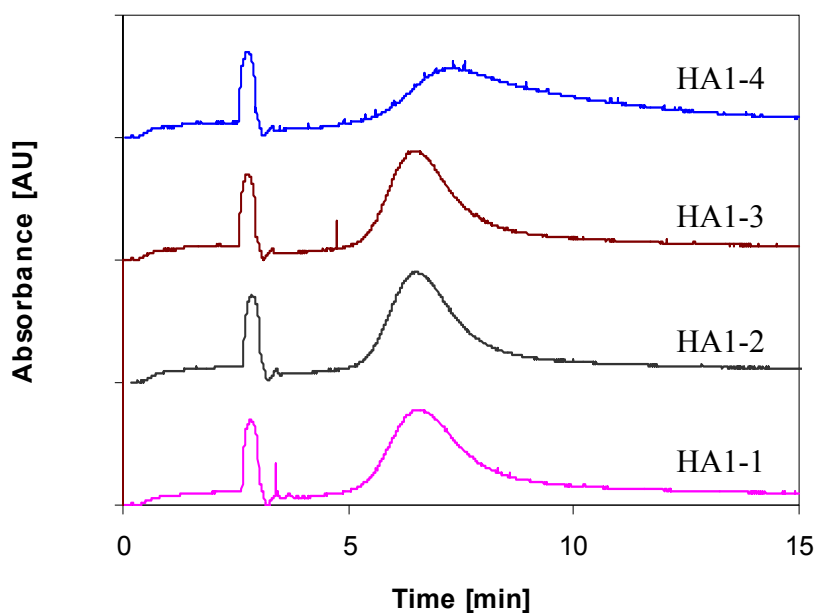


Fig. 3 Electropherograms of HA1-1, HA1-2, HA1-3, and HA1-4 measured samples in the borate- β -CD electrolyte

content of dissociated acid groups of HA3 humic acids, described by the higher Pb-capacity, results in their higher charge and subsequently higher electrophoretic mobilities.

3.1.2. BORATE- β -CYCLODEXTRINE ELECTROLYTE

In general, the humps, examined in this system of borate and β -cyclodextrine (pH=9.6), are more symmetrical than those in the former BGE. The electropherograms in Figs. 3 and 4 were also recorded a month after HAs dissolution in NaOH. Except HA1-4, the hump shapes of other HAs (Fig. 3) are similar to those separated in the borate-Tris-EDTA electrolyte (Fig. 1). The significant peak broadening of HA1-4 sample corresponds to its thermal destruction at 300 °C during coal oxidation. The smaller and faster compounds are strongly complexed with β -CD and thus retarded. It agrees with suggestion that the hump asymmetry in the borate-Tris-EDTA buffer relates to low-molecular-mass fractions. Formation of inclusion complexes between HAs and β -CD was described by Gajdošová et al. (2003). However, this effect makes the humps symmetrical and leads to a loss of information about the distribution of migrating HAs. Generally, the shorter migration times are ascribed to dissociation of hydroxyl groups at the higher pH of this BGE. The tests of the time stability and repeatability were performed with the similar good results as in the former electrolyte.

If using β -CD, the peak broadening of HA4 and HA5 (Fig. 4) is also more evident than in previous borate-Tris-EDTA electrolyte. The electropherograms of HA2 and HA3 show many individual peaks on the hump shoulders corresponding to various molecular fractions. This phenomenon was also observed by, e.g., Pokorná et al. (2000) and was explained by formation of the inclusion complexes of β -CD and HAs fractions. The highest amount of the fractions was observed in HA3 sample. However, these fractions were not found in the electropherograms of HA1-1 to HA1-4 likely because of their thermal decomposition.

The humps of HA4 and HA5 are slightly shifted towards longer migration times because of inclusion of these less condensed aromatic compounds into the β -CD structures. This suggestion agrees with the low values of aromaticity index about 50 measured in our previous work (Kurková et al.; 2004). It is known that inclusion mechanism of CDs is based not only on the penetration of a separand inside the cavity but also on Van der Waals interactions and strong hydrogen bonding between host and guest molecules as demonstrated by e.g., Li and Purdy (1992). The stronger retardation of HA4 and HA5 indicates their higher content of still not dissociated hydroxyl groups which interact with β -CD ones. It is in consistency with the CZE experiments in borate-Tris-EDTA electrolyte (Fig. 2).

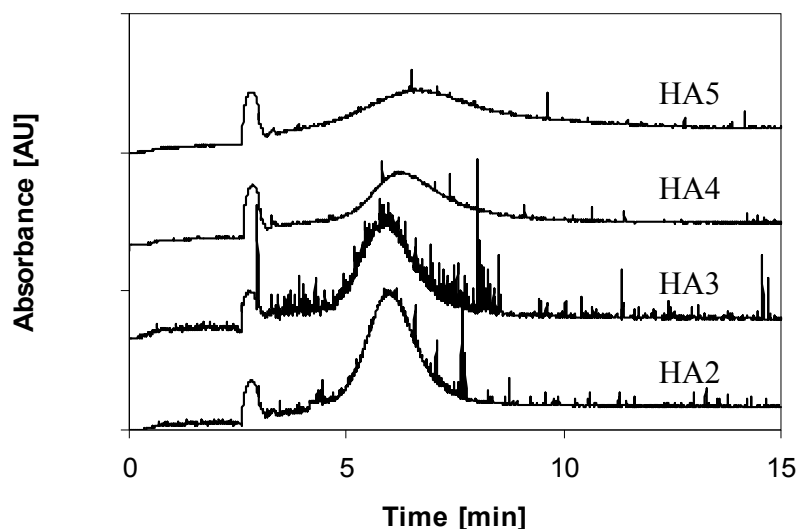


Fig. 4 Electropherograms of HA2, HA3, HA4m, and HA5 samples measured in the borate- β -CD electrolyte

3.2. MASS SPECTROMETRY

The mass spectra of HAs (Figs. 5, 6) show two important groups of peaks from 250 to 450 m/z and from 680 to 920 m/z . All findings described here were made in these regions. The most often differences in the HAs mass spectra were recognised for 14 m/z (related to $-\text{CH}_2$), 16 m/z (related to $-\text{O}-$), 17 m/z (related to $-\text{OH}$), 28 m/z (related to $-\text{CH}_2-\text{CH}_2-$ or $>\text{C}=\text{O}$), 44 m/z (related to $-\text{COO}$ or $-\text{CH}_2-\text{O}-\text{CH}_2$) and even for 45 m/z that can be attributed to $-\text{COOH}$ group (Gajdošová et al., 2000).

The ageing effect of HAs in alkaline solutions was investigated. The HAs spectra were measured 4 days, 1 month, and 5 months after their dissolution. With increasing ageing time, the peak intensities decrease in the range 250 to 450 m/z and increase in the range 680 to 920 m/z . It indicates that smaller molecules partially hydrolyse and sequentially form new and larger structures. This self-assembling effect was also observed when the time stability of HAs was tested by CZE. The retention times were slightly prolonged and the humps became wider likely as a result of the molecular size increase.

The reproducibility of the HAs mass spectra was examined by using the HA1-3 solutions. The repeated records were run a month after its preparation. The peaks show good reproducibility of the m/z values and that is why other mass spectra were measured at the samples prepared in this manner.

From Fig. 5 it is obvious that the mass spectra intensities depends on the oxidation temperatures. At the temperatures of 250 to 300 °C (the samples HA1-3

and HA1-4, respectively) the intensities within 680 to 920 m/z are very small while they are very high in the interval 250 and 450 m/z . At the temperatures of 150 and 200 °C (the samples HA1-1 and HA1-2) there are intensive lines at higher m/z , specially in the range 680 to 920 m/z . It indicates that the humic acids start to decompose into smaller fragments at the temperatures above 200 °C. This finding is in consistency with the peak broadening observed during the CZE separations, mainly in the case of HA1-4 (Figs. 1 and 3).

The mass spectra of humic acids from natural coals are demonstrated in Fig. 6. The intensities in the range 680 to 920 m/z are significantly higher for the non-oxidized (HA4) or less oxidized (HA2) samples in comparison with their more oxidized analogues HA5 and HA3, respectively. In addition, HA4 shows the highest intensities of all examined humic acids. Lignite, from which HA4 was isolated, is the only non-oxidized sample. These findings are in agreement with the spectra of HAs isolated from the artificially oxidized coal in laboratory (Fig. 5).

The higher intensities between 680 and 920 m/z in the HA2 mass spectrum confirm the lower oxidative degradation than in the case of HA3 (Fig. 6). We can suppose that the sample HA2, isolated from coal in the vicinity of the RBB (deposited under the earth surface), was formed under the lower partial pressure of oxygen than the HA3 one from coal weathered on the earth surface. We can also suppose that the temperature, at which the bituminous coal was oxidized (forming HA2), was not much higher than ambient temperature.

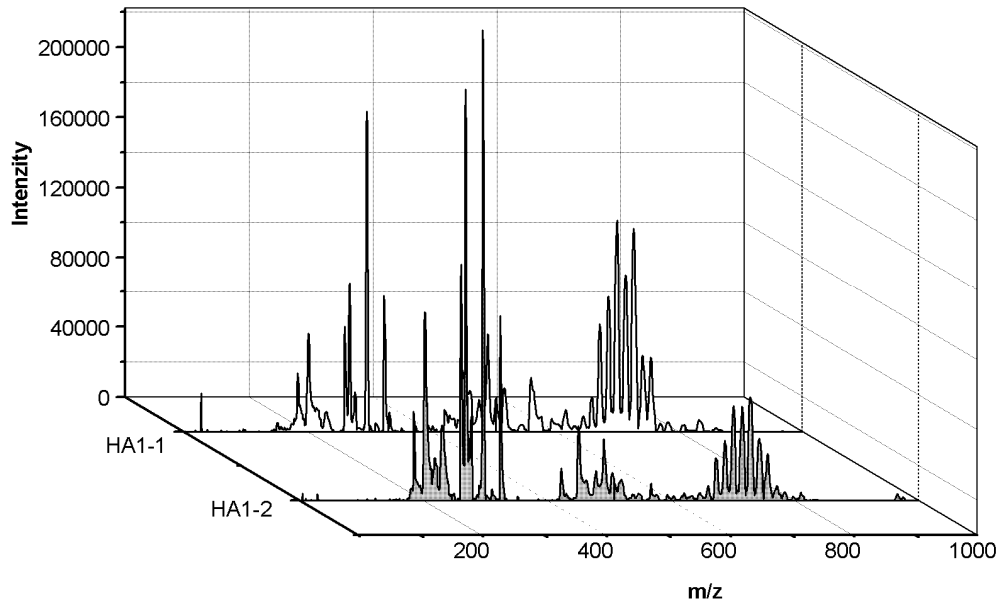
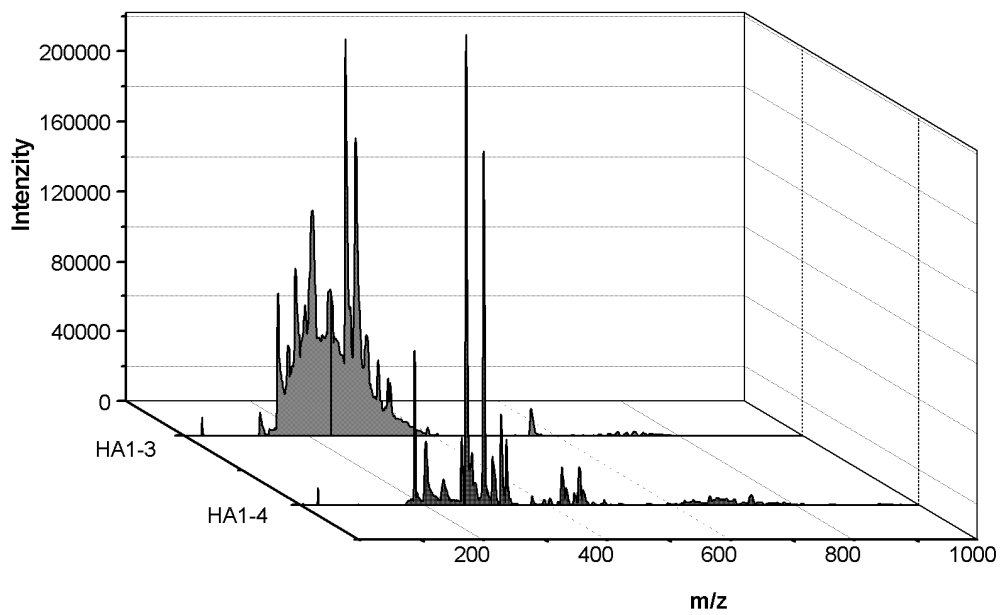
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Fig. 5 Mass spectra of HAs samples isolated from laboratory oxidized bituminous coals
A-Mass spectra of HA1-1 and HA1-2
B-Mass spectra of HA1-3 and HA1-4

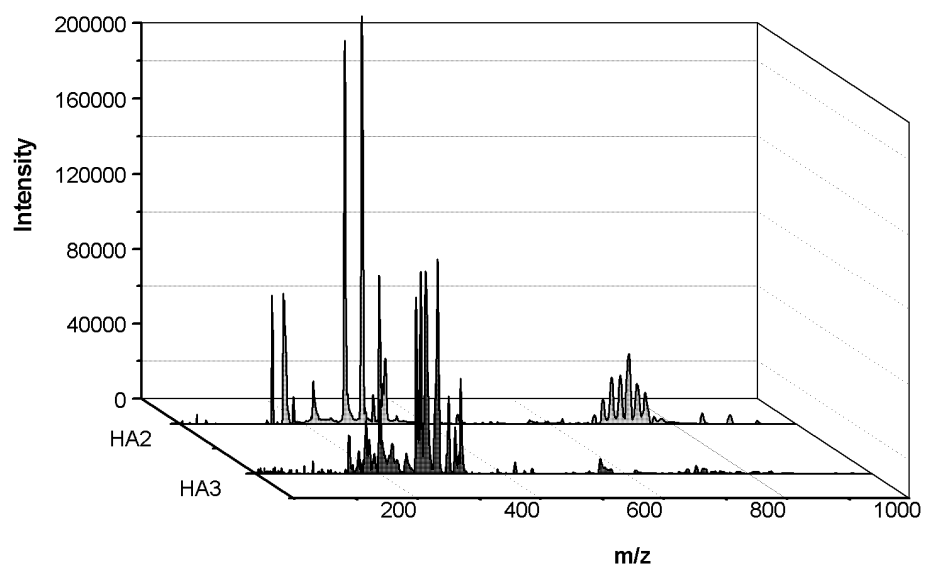
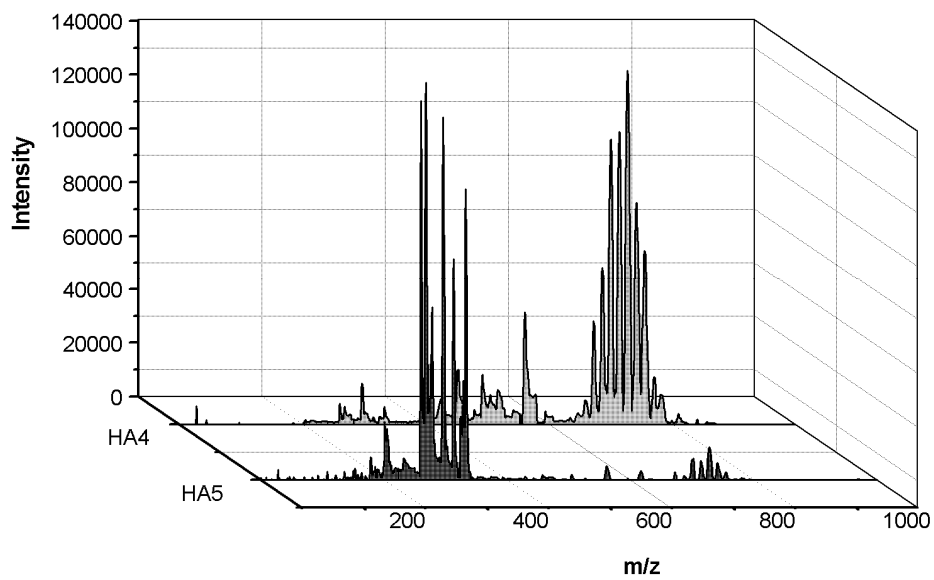
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Fig. 6 Mass spectra of HAs samples isolated from naturally oxidized bituminous coals and lignite
A-Mass spectra of HA2 and HA3
B-Mass spectra of HA4 and HA5

4. CONCLUSIONS

Humic acids, separated from the naturally oxidized bituminous coals (HA2, HA3), from lignite (HA4), and open-air oxidized lignite (HA5), were studied together with humic acids isolated from laboratory oxidized bituminous coal (HA1-1, HA1-2, HA1-3, HA1-4). For this purpose the CZE in the borate-Tris-EDTA and borate β -CD electrolyte and MALDI-TOF were tested. Both analytical techniques were found to be suitable for determination of the oxidation intensity of coals.

From the obtained results we can conclude that there are significant differences in electropherograms and mass spectra of HAs isolated from lignite/oxidized lignite and naturally/laboratory oxidized bituminous coal. Humic acids, isolated from deeply deposited oxidized coal, are supposed to be formed under low partial pressure of oxygen (lower than on the earth surface) and at the temperatures, which were close to ambient ones. This study completes and confirms our previous results published earlier (Kurkova et al., 2004).

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