Optimization of Ionization Conditions for Analysis of Humic Substances from Natural Waters Using Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FTICR MS)[1]

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Abstract—An approach to optimizing conditions of acquiring Electrospray Ionization Fourier Transform Ion Cyclotron Resonance mass spectra (ESI FTICR MS) was suggested. This method was employed to study the influence of cone voltage and the composition of the solution on peak intensity in ESI FTICR mass spectra of natural water humic substances. A presence of water in methanol solution was shown to reduce signal intensity significantly. The highest integral intensity of peaks selected was reached on 110 mg/l sample solution in acetonitrile and cone voltage 3.0 kV. The number of peaks to which molecular formulas were assigned, including doubly-charged peaks, was increased using these conditions.

INTRODUCTION

Humic substances (HS) represent high-molecular compounds with nonregular structure and variable composition, formed in result of postmortal transformations from organic residues of vegetable and animal origin [1]. The interest to the study of HS is determinated by their detoxic properties relative to anthropogenic wastes and also the possibility to accumulate microelements at the available form to vegetables [2]. HS as the products of stochastic synthesis have nonstoichometric elemental composition, high heterogeneity and high polydispersity of molecular masses. As a result HS occupy one of the highest stages by complexity at the hierarchy of organic compounds surpassing fuels, lignins, tannins, chitosans, etc.

Considerable advantage in understanding of the molecular structure of HS has been achieved recently due to applying of Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICR MS) method [3]. High resolution power reaching more than one hundred thousands at the range of masses < 1000 Da and low mass measurement errors < 0.5 ppm make it possible to determine chemical formulas of organic compounds by the mass value [4] even for complex mixtures with high quantity of different by chemical composition substances with identical nominal mass

[5−8]. This determines a perspective of applying FTICR MS for analysis of HS on molecular level, especially in combination with the "soft" ionization method—electrospray (ESI) [7−10]. ESI ionization possesses the advantage that in conditions of low voltages applying to the capillary (to 40 V) there is no fragmentation of HS. However, high heterogeneity of HS (there is about five thousands compounds with different chemical formulas identified in ESI FTICR mass spectrum of the fulvic acids specimen [8]) results in very small contribution from ions of every substance to the signal emerging at the detector and as a result signal to noise ratio for majority of peaks is low. This complicates determination of ions with high masses and moduluses of charge larger than one at the background of more intensive peaks of low-mass, single-charged ions and disallows to conclude about the molecular-mass distribution of HS using the data from ESI FTICR MS. To improve signal to noise ratio during mass spectra measurement of HS averaging of signal during the long time (to 22000 scans [9]) should be done. Improvement of ionization efficiency also can promote increasing of signal to noise ratio and probability—for detection of ions which are in the low quantity in solution. Choice of the solvent, concentration of analysed substance, ESI voltage and capillary voltage most significantly influence on efficiency of ESI ionization. The conditions listed above significantly differ in works of various authors [7, 9, 12], who

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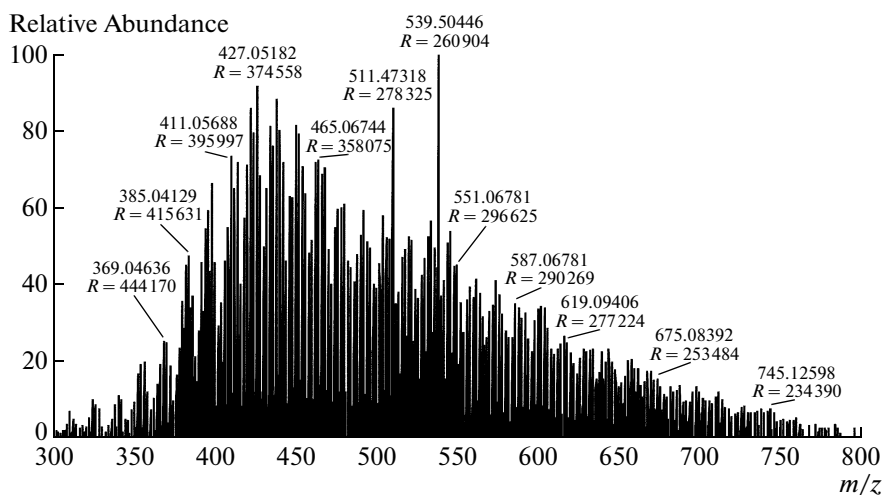[1] The article was translated by the authors.

Fig. 1. Mass-spectrum of HS from natural water 1R101N. Solvent—acetonitrile, concentration 120 mg/l, ESI voltage 3.2 kV.

analysed HS of natural waters by ESI FTICR MS, however there are no works with optimisation procedure described. The goal of the current work was to optimize conditions for registration a mass spectra of HS to reach high efficiency of ionization, improvement of signal to noise ratio and decrease time for the analysis.

EXPERIMENT

As the object of the analysis the standard sample (1R101N) of dissolved organic matter from International humic society (IHSS) isolated from river waters (Suwannee River) was used. The sample consists of nonfractionated mixture of humic and fulvic acids. For preparation of the sample for analysis weighed amount of the sample (1 mg) was dissolved in 1 ml of distilled water ("Milli-Q"). Prepared solution was immediately diluted to concentration 20–200 μg/ml, using as a solvent water-methanol mixture (1:3 by volume), and also methanol or acetonitrile.

For registration of ESI FTICR mass spectra hybrid mass spectrometer "LTQ FT" (Thermo Electron Corp.", Germany) equipped with superconductive magnet with intensity of magnet strength 7 T and electrospray ion source "Ion Max" ("Thermo Electron Corp.", Germany)* was used. Analyzed solution was sprayed with rate 1 μl/min in absence of drying gas in negative ions mode. The detection was performed in wideband scanning mode in the mass range z 400–800 Da with resolving power R = 400 000 at m/z = 400. Recording of spectrum was performed by averaging of the results of 50 scans. External ions accumulation was done in the mode with automatic gain control (AGC). AGC was tuned to accumulate 10^6 charges in the ion trap which were then transferred in the ICR trap. The choice of optimal spray voltage was performed manually for the each composition of the solution. The opti-

mization of capillary voltage and parameters of ion optics was done automatically by intensity of peak with m/z = 499.1 using the program "Tune Plus" ("Thermo Electron Corp.", Bremen, Germany). Mass spectrometer was calibrated using of standard calibration mixture LTQ FT. For handling of ICR mass spectra the software "Xcalibur 1.4" ("Thermo Electron Corp.", Bremen, Germany) was used. Further spectra processing for assignment of molecular formulas to peaks was done using original software "FIRAN" after internal recalibration was performed using the program "Recalibrate Offline" from the package "FT Tools" ("Thermo Electron Corp.", Bremen, Germany).

RESULTS AND DISCUSSION

The typical mass spectra of HS contain thousands of peaks [8, 12], the most intensive ones correspond to single-charged ions and are in the range of masses 300–800 Da [8, 12]. Spectra obtained in the this work contain up to 9618 peaks in this range (Fig. 1). It is worth noting especially the high density of peaks: at each nominal weight several peaks are observed [8, 9, 12] corresponding to ions with different elemental composition (Fig. 2).

Absolute and relative intensity of peaks depend on majority of factors. In the current work influence of the solvent being used, spray voltage and concentration of the sample was studied. The summary intensity of six high-intensive peaks at three nominal mass values was suggested to be used as a criterion for the optimization. Criteria for these peaks selection were the next: (1) the peak must possess high relative intensity to be able to be registered at even not optimal ionization conditions; (2) the choice of three different nominal mass values gives higher representativeness within the analyzed mass range; (3) the choice of the couple
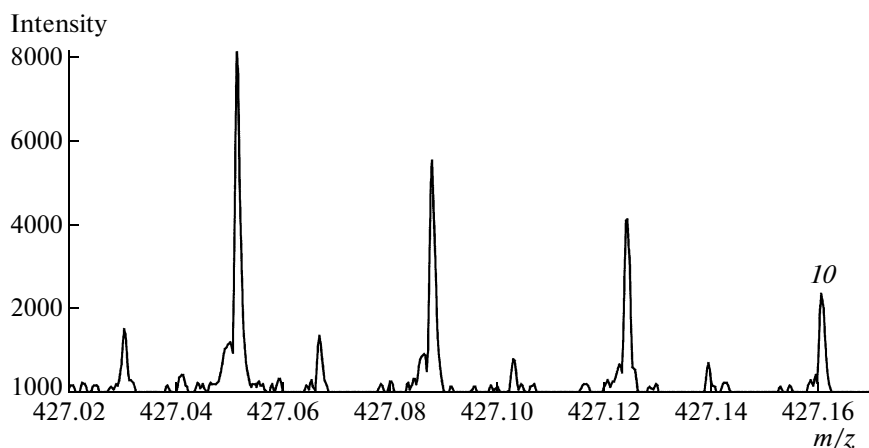
Fig. 2. Segment of mass spectrum of HS from natural water 1R101N in the mass range 427.02000–427.17000 a.m.u. Solvent—acetonitrile, concentration 120 mg/l, ESI voltage 3.2?kV.

of the most intensive peaks at the each nominal mass allows to decrease random error compartment to the results being obtained. The peaks which were selected using listed above criteria are presented in the table.

Spray voltage and solvent influence. Considerable increasing of the signal intensity was observed while increasing of ESI voltage in the range 2.0–3.2 kV. However increasing of the voltage above definite critical value led to the slump of the signal (Fig. 3) and appearance of corona discharge. Appearance of corona discharge was registered as visually (using the camera translating zoom image of the nebulizer tip to the monitor) also by sharp increasing of measured ion current value (from 0.12–0.25 to 0.5–2.0 Å) in absence of a discharge.

Observed critical values of spray voltage depended on the solvent and increased in the series of water-methanol mixture < methanol < acetonitrile. In the same order the summary intensity of chosen peaks increased for the equal ESI voltage. Maximum inten-
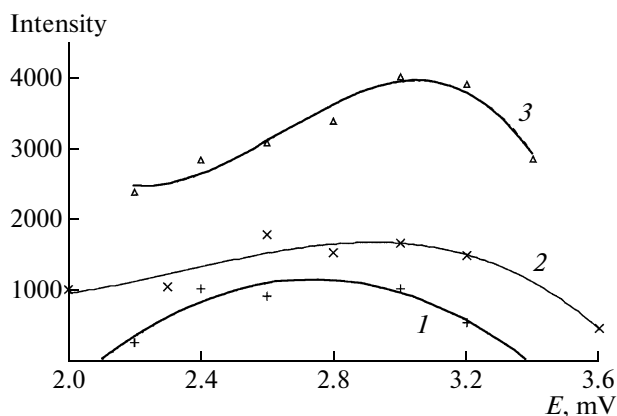
sity was observed for the samples dissolved in acetonitrile. Thereby acetonitrile has shown its worth as the optimal solvent for obtaining ESI FTICR mass spectra of humic substances from nature waters. By-turn methanol was found to be more preferable than water-methanol mixture used by other authors [7, 9, 12].

Influence of the sample concentration. The range of the concentrations of analyzed sample is very wide: from 16 [12] to 1250 [9] mg/l. In current work the influence of analyzed sample concentration in aceto-nitrile solution was studied at the electrospray voltage 3 kV at the range of concentrations 20–200 mg/l. Electrospray become unstable if the sample concentration in the solution exceeded 200 mg/ml. This fact was registered by force of the camera translating zoom image of the nebulizer tip to the monitor. It was found that the summary intensity of the chosen peaks increased evenly in more than four times if the concentration of the sample was increased from 20 to 110 mg/l. Further growth of concentration leads to insignificant even decreasing of summary intensity of chosen peaks. Thus at the concentration of the sample 200 mg/l summary intensity of chosen peaks was about 80% of the maximum summary intensity observed for 110 mg/l.

Analysis of HS sample from natural waters in optimized conditions. The ESI FTICR mass spectrum of HS sample from natural waters 1R101N was registered in optimized conditions with averaging of the results of 1000 scans to improve signal to noise ratio. This spectrum was subjected to further processing to determine molecular formulas of the substances contained in the sample composition. Obtained data were compared with the results of processing of the spectrum which we obtained at the control conditions analogous to ones cited in the work [12] (water-methanol mixture 30:70, concentration 20 mg/l, ESI voltage 2.2 kV, other conditions were identical to ones used in current work). The number of formulas determined by us increased

Six intensive peaks from mass spectrum of HS from natural water 1R101N, which were used for evaluation of experimental conditions influence on the measured mass spectrum

Mass	Formula	Relative intensity, % (Fig. 1)
399.05690	$C_{16}H_{15}O_{12}^-$	66.11
399.09329	$C_{17}H_{19}O_{11}^-$	37.29
427.05181	$C_{17}H_{15}O_{13}^-$	91.46
427.08820	$C_{18}H_{19}O_{12}^-$	61.84
451.05181	$C_{19}H_{15}O_{13}^-$	83.04
451.08820	$C_{20}H_{19}O_{12}^-$	55.02

Fig. 3. Dependence of summary intensity of reference peaks of HS sample from natural waters (1R101N) from the solvent and electrospray potential. Sample concentration 50 mg/l. +—water-methanol mixture 1:3; ×—methanol; △—acetonitrile.

from 1731 in control conditions to 2703 in optimized conditions. This is connected with detection of ions including doubly-charged ones whose intensity is usually low (in optimized conditions 300 peaks with the charge state −2 were found and identified while in conditions used in the work [12]—only 12 peaks).

REFERENCES

1. Orlov, D.S., *Humic Acids from Soil and General Theory of Humification*, Moscow, 1990.

2. Chaney, K. and Swift, R.S., *J. Soil Sci.,* 1984, vol. 35, p. 223.

3. Marshall, A.G., Hendrickson, C.L., and Jackson, G.S., Mass Spectrometry Reviews, 1998, vol. 17, pp.1−35.

4. Meija, J., *Anal. and Bioanal. Chem.,* 2006, vol. 385, pp. 486−499.

5. Hughey, C.A., Rodgers, R.P., Marshall, A.G., Qian, K., and Robbins, W.K., *Org. Geochem.,* 2002, vol. 33, pp. 743−759.

6. Rodgers, R. and Marshall, A., *Asphaltenes, Heavy Oils and Petroleomics*, New York: Springer, 2007, pp. 63−93.

7. Stenson, A.C., Landing, W.M., Marshall, A.G., and Cooper, W.T., *Anal. Chem.,* 2002, vol. 74, pp. 4397−4409.

8. Stenson, A.C., Marshall, A.G., and Cooper, W.T., *Anal. Chem.,* 2003, vol. 75, pp. 1275−1284.

9. Kujawinski, E.B., Hatcher, P.G., and Freitas, M.A., *Anal. Chem.*, 2002, vol. 74, pp. 413−419.

10. Kujawinski, E.B. and Behn, M.D., *Anal. Chem.*, 2006, vol. 78, pp. 4363−4373.

11. These, A. and Reemtsma, T., *Anal. Chem.,* 2003, vol. 75, pp. 6275−6281.

12. Kim, S., Kramer, R.W., and Hatcher, P.G., *Anal. Chem.,* 2003, vol. 75, pp. 5336−5344.

13. Kunenkov, E.V., Kononikhin, A.S., Perminova, I.V., Gaspar, A., Schmitt-Kopplin, P., Hertkorn, N., Popov, I.A., Garmash, A.V., and Nikolaev, E.N., *Anal.?Chem.*, 2009, vol. 81, no. 24, p. 10 106.

14. Brown, T.L. and Rice, J.A., *Anal. Chem.,* 2000, vol. 72, pp. 384−390.

15. Fievre, A., Solouki, T., Marshall, A.G., and Cooper, W.T., *Energy Fuels*, 1997, vol. 11, pp. 554−560.