

Modification of Humic Substances for Development of Materials for Environmental Technologies

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Abstract—Humic substances are high molecular weight refractory polycationites formed during decay of living organic matter and through biosynthesis of low molecular weight organic substances (metabolites or decay products of living organisms). Presence of many functional groups in the structure of humic substances determines their ability to interact with metal ions forming stable complexes and influencing metal ion speciation in the environment and mobility, behaviour and speciation forms in the environment. Presently humic substances are a product of industrial scale and quantities in amounts of hundreds of tons are produced. The aim of this study is to analyse derivatization possibilities of humic substances. To achieve this aim derivatization of humic substances using acylation (at first introduction of acetylgroups, but also changing length acyl chains are considered) are used. Also alkylation is used. Mild oxidation can help to obtain modified products with reduced molecular weight. Another approach includes introduction of new functional groups and structures. To achieve this aim, conjugates with short peptides, amines and sugar derivatives using coupling with water-soluble carbodiimides are obtained. As basic characteristics elemental analysis as well as functional analysis have been used, supported with Fourier transform infrared (FTIR), ¹³C nuclear magnetic resonance spectrometry and other methods. Derivatives of humic substances containing sulpho, amino, and hydroxylgroups and thiolgroups were synthesized and their properties were analyzed in respect to their their elemental composition; functional group content changes in spectral characteristics. The derivatives of humic substances showed significant differences in the number and in ability to interact with the metal ions, which were reflected in their complexation properties towards metal ions. FTIR spectra gave evidence of the presence of metal ions, strongly bound and protected in inner sphere complexes. The obtained derivatives of humic substances can be used for remediation of environment contaminated with heavy metal ions.

Keywords—humic substances, modification, functional groups, pollutants, remediation.

INTRODUCTION

Humic substances (HS) are high molecular weight refractory high-molecular substances with numerous acidic functional groups formed as a result of decay of living organic matter as well as due to synthesis from

low molecular weight organic substances (metabolites or decay products of living organisms) [1]. Humic substances are considered as refractory and inert, but they do have numerous functional groups which influence their behaviour in the environment. Humic substances do contain carboxylgroups, phenolic and carbohydrate hydroxylgroups, aminogroups, quinonic groups and reactive positions in aromatic structures [2]. Presence of many functional groups in the structure of humic substances determines their ability to interact with metal ions as well as organic substances forming stable complexes and influencing speciation forms in the environment and mobility, behaviour in the environment [3]. Considering the complex forming properties of humic substances, they are suggested for use in remediation of environments contaminated with metals [4, 5]. In many studies high complex forming capacities of humic substances in respect to metal ions are demonstrated [5-7]. At the same time humic substances are industrially produced in quantities of tons at first for applications in agriculture and thus can be considered as low-cost natural materials. Considering presence and high concentrations of many functional groups in structure of humic substances their derivatization can be done to increase concentrations of existing functional groups as well as introducing new groups. However use of many derivatization reagents for modification of humic substances is of limited use, considering low solubility and presence of labile structures in their molecules. Another problem is relatively low reactivity of main complex forming groups in the structure of humic substances (-COOH, -OH and other) [6, 7]. Thus a need for search of new derivatization methods for modification of humic substances is a challenge to obtain derivatives of humic substances as well as for development of environmental technologies. Several studies have been done to obtain derivatives of HS for their structural studies [6 – 8], however the use of expensive and toxic chemicals limit application possibilities of suggested modification methods. The real number of methods suggested for derivatization of humic substances is highly limited and their derivatization in general has been used mostly for their structural analysis.

The aim of the present study is to study derivatization

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possibilities of humic substances to obtain derivatives of humic substances with increased complex forming ability in respect to metal ions.

MATERIALS AND METHODS

A. Materials

Analytical quality reagents (Merck Co., Sigma – Aldrich Co., Fluka Chemie AG) were used without purification. For preparation of solutions, high purity water Millipore Elix 3 (Millipore Co.) 10 – 15 M Ω was used throughout the study.

B. Studied humic acids (HA) and their characterization

Humic acids were isolated from a raised bog peat (Dzelves Bog, peat decomposition degree 10 %, humic acid/fulvic acid ratio: 6.8). Peat HA properties: C 45.53 %; H 5.60 %; N 0.47 %; S 0.81 %; ash 1.29 %. Other determined parameters: concentration of carboxylic groups 4.2 mmol/g; total acidity 9.31 mmol/g; phenolic hydroxylgroups 5.11 mmol/g.

Elemental composition. Carbon, hydrogen, nitrogen and sulphur concentrations in the humic acid samples (elemental analysis of C, H, N, S) were determined by combustion-gas chromatography technique, using an Elemental Analyzer Model EA-1108 (Carlo Erba Instruments). Ash content was measured after heating 50 mg of each peat sample at 750 °C for 8 h. Elemental composition was corrected considering the ash content, and the oxygen amount was calculated as a difference.

Total acidity. An automatic titrator TitroLine easy (Schott-Geräte GmbH) was used for measuring total acidity of HA and their modification products. To estimate the total acidity [9, 10], 20 mg of humic acid or its modification product, were dispersed in 10 ml of 0.1 M Ba(OH)₂ solution, then shaken overnight under N₂ atmosphere, filtered and washed with water. The filtrate with the washing solution was potentiometrically titrated with 0.1 M HCl down to pH 8.4 under N₂ flow.

Infrared spectra. Fourier transform infra-red (FTIR) spectra were recorded in the 4000 to 500 cm⁻¹ wave number range using Perkin Elmer 400 IR spectrophotometer, in KBr pellets obtained by pressing mixtures of 1 mg samples and 400 mg KBr with precautions taken to avoid moisture uptake.

C. Modification of humic acids

Synthesis of derivatives of humic acids modified with thiolgroups

Solution of 10 g of humic acids in 100 ml of 5 % NaOH were added with 9.3 g (0.1 mol) of 2,3-epoxy-1-chloropropane and the suspension was stirred at 20 °C for 6 h. The weight of 4.8 g (0.02 mol) of Na₂S \times 9H₂O was added to the reaction mixture and additionally stirred at 60 °C for 8 h. The reaction mixture was acidified with 6 N HCl to pH 1. The precipitated thiol derivatives of humic acids were filtered off, the precipitates redissolved into 0.1 N HCl and precipitated with 6 N HCl. Precipitates were washed with water, acetone and dried. The weight

of 8.6 g of humic acids modified with sulphogroups were obtained with following elemental composition: C - 46.67 %; H - 5.33 %; N - 0.37 %; S - 3.67 %.

Synthesis of derivatives of humic acids modified with sulfoalkylgroups

To dispersion of 10 g of humic acids in 50 ml of formalin were added 10.41 g (0.1 mol) of NaHSO₃ and 10 % NaOH dropwise until pH 10 – 12. The reaction mixture was stirred at 80 °C for 6 h. The filtrate was acidified with 6 N HCl to pH 1 after filtration process. The precipitated sulfoalkylhumic acids were filtered off and washed with water, acetone and dried. The weight of 8.6 g of humic acids modified with sulphogroups was obtained with following elemental composition: C 42.91 %; H 5.21 %; N 0.26 %; S 4.76 %.

Synthesis of sulphopropylhumic acid

The weight of 10 g of humic acids were added into the solution of 100 ml of dimethylformamide 1,3-propanesultone (Table 1) and was stirred at 70 °C for 8 h. The reaction product was poured into 1 l of acetone, the precipitated modified humic acids were filtered off, washed with water, acetone and dried. Reaction conditions and properties of obtained products are summed up in the Table 1.

Modification of humic acid with amino groups

Twenty g of humic acid (Sigma-Aldrich Co) were dissolved in 60 ml of 0.5 N NaOH, after which 9.25 g (0.1 mol) of 1-chloro-2,3-epoxypropane were added. The mixture was then stirred at 60 °C for 2 hrs, after which 50 ml of ammonium hydroxide were added. Next, the mixture was stirred at 60 °C for 8 hrs, after which it was acidified by adding concentrated HCl until it had a pH of 1. Acidification resulted in precipitation of the modified HAs, which were then removed by filtration, washed with H₂O and dried to give 14.85 g of modified HA.

Modification of humic acid with hydroxyl groups

Ten g of humic acid (Sigma-Aldrich Co) were dissolved in 50 ml of 0.5 N NaOH, after which 30.0 g (0.5 mol) of 2,3-epoxypropane were added. The reaction mixture was then stirred at 80 °C for 24 hrs. Next, the pH of the reaction mixture was reduced to 1 by the addition of concentrated HCl. Acidification resulted in precipitation of the modified HAs, which were then removed by filtration, washed with H₂O and dried, to give 8.99 g of modified humic acid.

D. Humic acids-metal complexation.

Study of the interaction between metal ions and humic acids using ion selective electrode

Metal-humic binding were analyzed with Consort Cu²⁺ ion selective electrode (ISE) (pHoenix Electrode Co.). ISE was filled with 10 % KNO₃ solution and conditioned with 5 M NaNO₃. Calibration curve in the 6.3 \times 10⁻⁴ to 3.17 \times 10² M Cu²⁺ concentration range was determined. 10 ml aliquots of HA solution (10 mg/l) were mixed with 40 ml aliquots Cu(NO₃)₂ \times 3H₂O solution with final Cu²⁺ concentration 100 mg/l and hold for 24 h. In the filtered solutions the unbound Cu²⁺ concentration was measured

with ISE (all measurements were done in triplicate).

Conditional stability constants [16] were determined for complexation reactions which can be written as equation (Scatchard equation):

$$K_o = \frac{CuHA}{[Cu] \cdot (HA - CuHA)}$$

where: Cu – total number of moles of Cu²⁺;

HA – the total number of moles of humic acid in solution;

CuHA – number of moles, which are linked in complexes.

RESULTS AND DISCUSSION

Humic substances have significant quantities of reactive structures [12, 13] in their molecules and thus provide possibility to obtain derivatives of humic substances with significantly different properties in respect to natural ones. To increase complex forming capacities of humic substances, modification of HS with sulphur containing groups (sulpho- and thiol groups, as well as amino groups) - strong complex forming groups. The aim of modification is to increase ability of obtained modified humic substances to bind metal ions.

The suggested modification approaches are based on reactions of hydroxylgroups in the structures of humic substances with reactive modifiers: 1) with 1,3-propanesultone to obtain sulphopropylhumic acid; 2) etherification with sulfoalkylgroup by treatment with 2,3-epoxy-1-chloropropane and following thiolysis of epoxygroup to obtain humic acid modified with thiolgroups; 3) treatment with 2,3-epoxy-1-chloropropane and following aminolysis of epoxygroup to obtain humic acid modified with aminogroups; 4) treatment with 2,3-epoxy-1-chloropropane and following hydrolysis of epoxygroup to obtain humic acid modified with hydroxylgroups...

Selection of experimental conditions are based on cellulose modification reactions, considering experience of humic acid modification conditions in previous studies [6, 7, 11]. Selected derivatization conditions allow to obtain sulphopropylhumic acid (III) with differing derivatization degree (Table 1).

TABLE 1
CONDITIONS* OF SYNTHESIS AND YIELDS OF SULPHOPROPYLDERIVATIVES OF HS

Amount of 1,3-propanesultone	T C	T hrs	S, %
0.1 mol	80	8	8.34
0.2 mol	80	8	8.66
0.1 mol	20	25	8.55
0.05 mol	80	8	8.15
0.01 mol	80	8	3.42
0.05 mol	40	8	2.18
0.05 mol	20	8	1.38
0.05 mol	80	4	1.22

*Conditions of the synthesis in details are described in Materials and Methods

Yields of humic acids modified with sulfoalkylgroup,

thiolgroups and aminogroups are less variable and rather limited by experimental conditions, but also allows obtain correspondingly substituted substances. During derivatization process the molecular mass of humic substances is reduced. On one hand it can be explained by destruction of humic macromolecules during derivatization process, but in the same time disintegration of hydrogen bonds of HA subunits [12] allow to use obtained derivatives for structural studies of HA.

Obtained sulphur, amino-, hydroxylgroups containing derivatives of humic substances are characterized using weight gain during synthesis, elemental analysis as well as FTIR spectra.

In FTIR spectra sorption maximum characterizing for C-S (780 cm⁻¹), S-S (466 cm⁻¹), organic sulfates (1388 cm⁻¹) and other bonds can be seen and for humic substances modified with aminogroups similar presence of groups can be observed.

NMR spectra shows similar changes in the structure of obtained compounds and, if compared with NMR spectra of modified model, compounds allow to evaluate the sites of substitution in the molecules of obtained derivatives.

Thus, the combination of functional and structural analysis allows to describe obtained derivatives of HA. The obtained derivatives of HA do have significantly differing hydrophobicity as indicated by their K_{PEGW} values [14] and, for example hydrophobicity of aminoderivatives is significantly higher than those of original compounds.

Derivatization of humic substances with sulphogroups, aminogroups and hydroxylgroups significantly influence their hydrophobicity as evident from changes in their polyethyleneglycol-water distribution coefficient (Table 2).

TABLE 2
CHARACTERISTICS OF MODIFIED HUMIC ACIDS

	Total acidity, mEq g	K _{PEGW}	M _n , Kda	logK
Peat HA	4.4	17.6	22.5	4.89
Sulpho propyl HA	6.8	21.4	20.6	8.52
Sulphoalkyl HA	4.6	28.4	12.5	6.66
Thiol HA	4.4	21.8	14.8	7.97
Amino HA	4.4	32.5	12.5	5.23
Hydroxyl HA	4.5	33.7	11.9	4.92

Humic acids modified with sulpho, thiol, amino and hydroxylgroups have higher metal binding capacities and the formed complexes are more stable than with humic acids. Thus the obtained derivatives are prospective for use in contaminated environment remediation. To test it a well-known sequential extraction approach suggested by Tessier and others [15] and others were used. Accordingly to sequential extraction approach total metal amount in contaminated soil is divided as easily extractable metals (environmentally most dangerous fraction as far as metals present in this fraction are accessible to soil biota and plants), metals bound to carbonates, iron and manganese

oxides, organic matter and refractory fraction. The aim of remediation thus could be reduction of most easily available metal fraction [16].

The speciation analysis of copper present in contaminated soil after treatment with peat humic acids and modified humic acids demonstrates significant reduction of easily available copper forms in contaminated soils. Thus humic acids modified with sulpho- and thiol- groups are prospective agents for contaminated soil treatment. It could be expected that after mineralization of humic acids presence of sulpho- and thiol groups in humic acids could form stable sulphates or sulphides thus increasing stabilization effect of metal.

However considering high variety of modified humic substances they might find applications also in a wider array of environmental technologies, such as biomedicine, coloring industry and others.

CONCLUSIONS

Considering a large scale of production of humic substances, the obtaining of derivatives of humic substances, including HS with modified properties, are perspective and sustainable areas of use.

The derivatives of humic substances showed significant differences in the number and in ability to interact with the metal ions, which were reflected in their complexation properties towards metal ions.

The obtained derivatives of humic substances can be used for remediation of environmental contaminated with heavy metal ions. Modified humic acids with sulpho-, amino-, hydroxyl- and thiolgroups have higher metal binding capacities and the formed complexes are more stable, therefore the obtained derivatives are prospective for remediation of contaminated environment.

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