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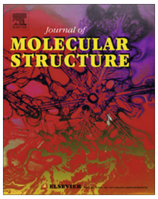

Synthesis and properties of synthetic fulvic acid derived from hematoxylin

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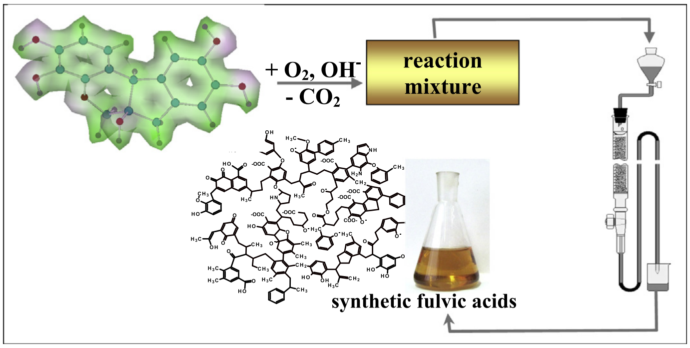
HIGHLIGHTS

- A new technique for producing the synthetic fulvic acids was developed.
- A complex study of the obtained synthetic fulvic acids was carried out.
- A computer simulation of the possible oxidation products of hematoxylin were made.

GRAPHICAL ABSTRACT



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ABSTRACT

A model fulvic acid (FA) was synthesized from a natural dye, hematoxylin, in a slow oxidative polymerization/condensation reaction catalysed by OH^- at pH ca. 12. The resulting dark-brown product, acidified to pH ca. 2, did not precipitate from the reaction solution. It was isolated and purified by cation-exchange resin. Its physicochemical and spectroscopic properties, as determined by means of elemental analysis, molecular weight analyses, Fourier transform infra red (FTIR) and ultraviolet–visible (UV–VIS) spectroscopy, X-ray diffraction and electron paramagnetic resonance (EPR) spectroscopy, showed a close resemblance to natural FA. The similarity and differences between synthetic fulvic acids derived from hematoxylin and the natural fulvic acids substances are discussed. Quantum-chemical calculations of the supposed primary oxidation products of hematoxylin are performed and compared with observations.

Introduction

Humic substances (HS) are ubiquitous natural materials occurring in huge amounts in soils, sediments and waters as the product of chemical and biological transformations of animal and plant residues [1]. According to the pH dependent aqueous solubility, these substances are classified, into three subcategories: humic acids (HA), which are insoluble at low pH; fulvic acid (FA) – the lower molecular weight members soluble at all pH, and humines – insoluble at all pH [2]. The increasing interest of chemists and soil scientists in the HS studies is caused by several reasons. First, the HS positively affect the growth and evolution of plants. Therefore, these substances and complex fertilizers on their base find growing use in agriculture. Secondly, the HS exhibit a pronounced pharmaceutical activity and can be used as highly active antioxidants, immunomodulators, biostimulants, bactericidal, antiviral, and antitumor preparations. Thirdly, due to their unique ability to bind

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heavy metals and radionuclides, HS can be used to solve environmental problems.

However, the analysis of the basic properties of HS indicates that HS extracted from natural sources, are characterized by variability of compositions and the dependence of their properties on the source and method of extraction. Their properties are difficult to standardize according to the requirements of specific technological process. This fact limits the use of drugs based on natural HS. Synthetic molecules used as FA models avoid this inconvenience because they are precisely produced under strictly controlled conditions [3]. In general, the synthetic polymer products with properties that simulate natural HS a have significant advantage over natural materials, which is caused by controllable and reproducible properties due to the possibility of variation and control of the synthesis conditions and the choice of a phenolic precursor. However, despite the almost centennial period of their research, only few models of synthetic HS have been developed. So far the majority of synthetic simulated HS have been obtained from dihydroxybenzenes and quinones [4–6], from phenols, and by coupling these phenolics with amino acids and proteins [1,7]. Essential disadvantages of the known methods of synthetic humic substances preparation are the long duration of process, difficulty to control the reaction conditions and purification of end-products. In addition, the main disadvantage of the known methods of preparation of synthetic HS is the use the phenolic compounds as the precursors. In addition to aromatic nuclei these precursors include the carboxyl or phenolic groups but they do not contain the saturated fragments, such as alcoholic hydroxyls that are always present in the structures of natural HS. This leads to a product with a structure, which does not fully corresponds to the functional composition and properties of natural HS. Therefore, an important task that emerges now is to develop new methods of preparation of synthetic HS and to the improve existing ones. The objective of this study is the development of the fast and simple preparation method of synthetic fulvic acid from hematoxylin (FAH). The similarity and differences between the synthetic FA and natural humic substances are examined. In addition, reproducibility of the basic parameters of synthetic humic substances is investigated.

Experimental

Chemicals

All of the reagents used in this study were of a purity adequate for the analysis (analytical grade) and purchased directly from Merck. All aqueous solutions were prepared with deionized water (18.2 MΩ/cm resistivity) obtained from a Milli-Q system (Millipore).

Preparation of the synthetic FA from hematoxylin

Hematoxylin (3.2 g), weighed in polyethylene vessel, was placed in Kjeldal flask with the volume of 1 l. 80 mL of 1 M NaOH solution and 20 mL of distilled water were added cautiously. Air was displaced from the system by a weak stream of pure oxygen. After the hermetic seal of the equipment, the flask was mechanically stirred. As a result, the mixing of the reagents and dissolution occurred and initiation of the oxidation process started. The amount of consumed oxygen at the moment of the measurement was calculated according the following formula:

$$n_{O_2} = \frac{(P_{atm} - P_{H_2O}) \cdot V \cdot 273.15}{760 \cdot (T + 273.15) \cdot V_m}, \qquad (1)$$

where n_{O_2} – the number of moles of O_2 consumed; P_{atm} – atmospheric pressure, mm Hg; P_{H_2O} – water vapour pressure at the synthesis temperature, mm Hg; V – volume of absorbed oxygen, ml; V_m – molar volume of gas under normal conditions (22,415 mmol/ml); T – temperature, °C;

The process was considered to be finished when oxygen consumption was completed. After completion of the oxidation, the reaction mixture was passed through a column filled with the cation-exchange resin (KU-2-8) in the H-form. Collection of the eluent began at occurrence of the brown-colored solution; collection was stopped when the color tuned into light red. The volume of the received fraction was practically equal to the volume of the reaction mixture introduced into the column. For removal of carbonates the solution was heated and evaporated to a final volume of 100 mL.

Elemental analysis

Elemental analysis of synthetic FA were carried out using a Perkin Elmer 2400 Series II CHNS/O Analyzer (Perkin Elmer, Inc. Boston, MA, USA). Samples were dried under vacuum at 60 °C to a constant weight before assaying for elemental composition. The percent of oxygen was calculated by subtracting the sum of the C and H percents from 100%. The H:C and O:C atomic ratios were also calculated.

Acidic functional groups

Total acidity and carboxylic group contents were determined using the $Ba(OH)_2$ and $Ca(CH_3COO)_2$ methods, respectively [8]. The phenolic hydroxyl group content was calculated by subtracting the carboxylic group content from the total acidity.

FTIR spectra

The FT-IR spectra of the samples were recorded by the Perkin-Elmer spectrometer (SpectrumGX) with a resolution of 2 cm^{-1} over a scan range 4000–500 cm^{-1} using KBr pellet method. Relevant spectral regions were assigned according to the previously reported assignments [9,10].

XRD analyses

The X-ray powder diffraction (XRD) analyses were performed by a DRON-2 X-ray diffractometer (LOMO, Russia) with Fe Kα radiation (λ = 1.9360 Å) at a scanning speed of 0.01°/s over the 2θ range of 20–120°.

EPR analyses

EPR measurements were carried out using a Bruker ESP 300 E spectrometer operating in the X-band and equipped with 100 kHz field modulation. EPR spectra of synthetic FA were measured using a 2 mm quartz tube. In order to calculate the spin content of the sample, a spin standard $CuCl_2 \cdot H_2O$ was used.

Molecular weights

The molecular weights of the synthetic FA were determined by size exclusion chromatography. 100 mg/L solutions of synthetic FAH in 0.03 M phosphate buffer were prepared. A sample (2 mL) was injected into a column (25 × 200 mm) filled with the gel Toyopearl-50HW (S). The rate of solvent elution was of 1 mL/min and the detection was spectrophotometric at λ = 254 nm. Polystyrene sulfonic acid sodium salts (Fluka, molecular weights of 208, 1400, 4300, 6800, 17,000, 32,000, 49,000) were used as standard materials for calibrating the molecular weights. The weight average (M_w) and number average (M_n) molecular weights were