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EPR STUDY OF PHOTOONDUCED AND O_2 -DEPENDENT CHANGES IN GALLIC ACID-DERIVED MODEL HUMIC SUBSTANCES

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Paramagnetic properties of model humic substances (HS) synthesized from 3,4,5-trihydroxybenzoic acid (gallic acid) were studied by means of EPR spectroscopy. A singlet EPR signal without any fine structure was observed for powder sample with spin density 6×10^{18} spin/g, witdh 6.45 Gs and g=2.002. Results of the microwave power saturation pointed to a high dipolar relaxation enhancement. In aqueous solutions a „dark" EPR signal depends on atmosphere and pH. The shapes of EPR spectra are very similar to those of melanins and humic acids indicating on the semiquinone as the possible source of the signal. Light induced (wavelengths 290-600 nm) spectra and kinetics of the EPR signal amplitude appeared to be O_2, pH and wavelengths dependent suggesting slow oxidative degradation of paramagnetic centers in the HS. The relevance of these data to environmental photophysical and photochemical processes associated with the multiplicity conservation and spin-spin interaction of HS and O_2 are shortly discussed.

INTRODUCTION

Humic substances (HS) are the most complex macromolecular substances resulting from the decomposition of dead plant residues. These dark paramagnetic weak anionic polyelectrolytes occur in soils, peat, coals and surface waters. Both the dark color and paramagnetism of HS result from an extended π-electron system and the donor-acceptor moieties, while the weak acid character originates from COOH and phenolic OH groups (Tan, 2003). There is a close relationship between certain fractions of HS (humic acids, HA) and melanins. A spongy-like supramolecular structure of HS ensures sorption of low-molecular compounds, e,g, xenobiotics. Sorption of O_2 is of particular ecological significance because: (1) A photoinduced transfer of energy from electronically excited HS* to O_2 with the formation of $^1O_2$ and O--2 can initiate a chain of oxidative degradation of HS or environmental xenobiotics (Aguer, Richard & Andreux, 1999) and (2) A magnetic coupling between π-electrons and sorbed O_2 exists (Slawinska, Slawinski & Sarna, 1975a, b). A high content of unpaired spins in HS is an important factor as the terrestrial and aquatic organic matter is far from thermal equilibrium with atmospheric O_2. The main factor which slows down an unavoidable oxidation reactions of organic matter is the different multiplicity of the reaction's substrates, the singlet of organic compounds and triplet of O_2. Therefore, paramagnetism of HS is an important yet underestimated factor relevant to oxidative degradations of organic matter, thus to the C-cycle and greenhouse effect, photosensitized detoxification and physiological activity of HS (Drozd, Gonet, Senesi & Weber, 1977). Results of EPR studies of natural HS are ambiguous because of the heterogeneity, complex unknown structure and paramagnetic impurities (Jezierski & Bylinska, 1997). Therefore we have synthesized a simple model of HA from one of the typical polyphenolic precursors of natural HA, 3,4,5-trihydroxybenzoic acid (gallic acid, GA) and studied its interactions with UV, visible light and oxygen by the EPR spectroscopy.

MATERIALS AND METHODS

3,4,5-trihydroxybenzoic acid was of analytical grade from Sigma-Aldrich (Germany). Other chemicals of analytical purity were from POCH (Poland). Autooxidation of GA solution in aerated 0,1 M Na2CO3 (pH≈10) at 23°C lasted 37 days. The resulting dark reaction mixture was acidified and treated according to the ISHS protocol for humic acids. The yield of the black amorphous humus acid (HAG) was 24.5% of the initial GA. The HAG was analyzed using elemental analysis, FTIR, UV-VIS, 1H and 13C NMR and fluorescence

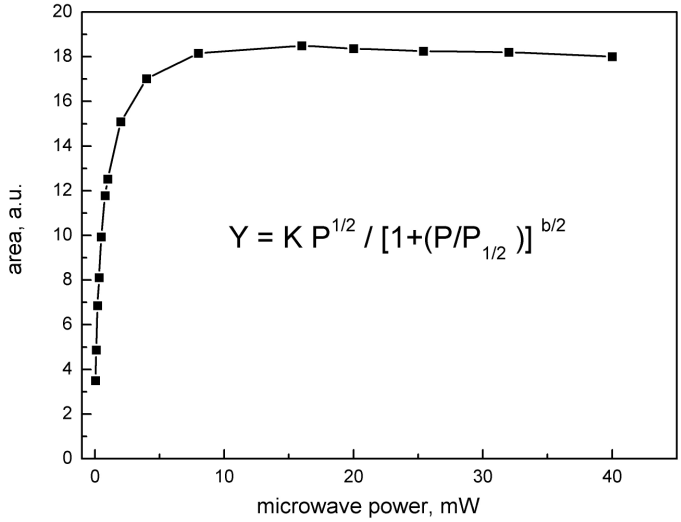

Fig.1. Saturation curve of the solid HAG sample showing the relation between first-derivative area versus increasing microwave power. Parameters are given in the methods.

excitation/ emission spectroscopy. Results of these analyses are to be published elsewhere.

EPR spectra of HAG-powder were measured in a 2 mm quartz tube, EPR measurements of aqueous samples HAG 2mg/ml in 0.1 M Na2CO3 at pH 6.6 and 10.8 in a flat quartz cell and in situ irradiation were performed using a Bruker ESP 300E spectrometer operating at X-band with 100 kHz field modulation. The spin content in the sample of solid HAG was determined using a DPPH standard. Samples were irradiated with polychromatic light 290-600 nm using a high-pressure xenon lamp Photomax 150 W Oriel, equipped with quartz lenses and pass cutoff filters at 390 nm, 340 nm and 290 nm. Irradiations were carried out at room temperature in aerated and deaerated samples by purging the sample with argon for 40 min. The lamp output at the optical cell for specific wavelengths was monitored with an International Light IL1700 radiometer using calibration factors (in W/m^2) derived by the manufacturer. Detailed description is given elsewhere (Polewski, Slawinska, Slawinski & Pawlak, 2005).

RESULTS AND DISCUSSION

EPR spectra of solid HAG sample

The spectrum is broad without any fine structure, ΔH_{pp} = 6.45 Gs, g = 2.0018 and the calculated spin density is 6×10^{18} spin/g. This stable free radical is most likely a semoquinone type and seems to be universal with HS and melanins. The power (P) saturation curve $y = f(P)$ obtained in progressive saturation measurements for double-integrated intensity (y) of the in-phase first harmonic absorption spectra is given in Fig. 1. From these data and from fitting procedure to equation (1) which is the

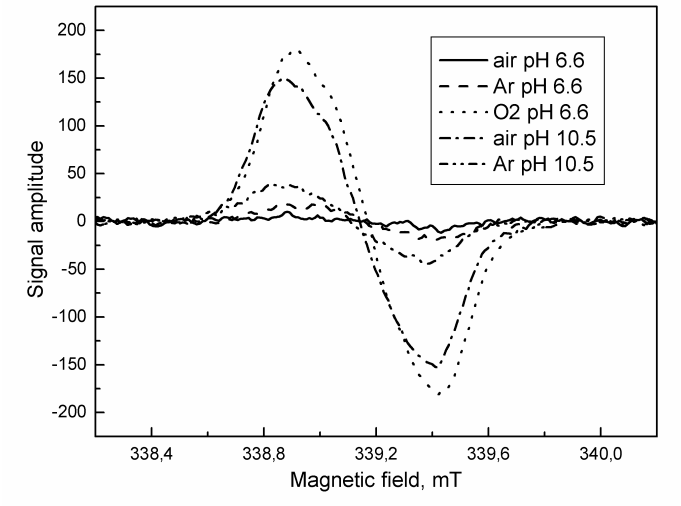

Fig. 2. EPR spectra of aqueous non-irradiated "dark" sample of 2 mg ml-1HAG in 0.1 M Na2CO3 in air, argon and oxygen atmosphere at pH 6.6 and 10.5. The assignment of the curves is given on the legend.

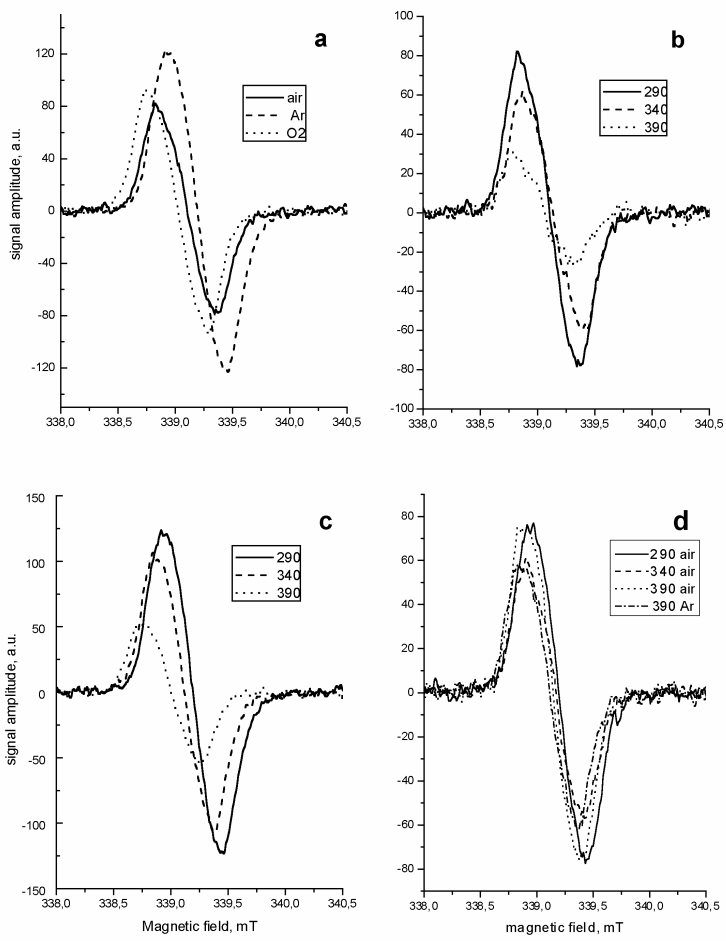

Fig 3. First-derivative EPR spectra of aqueous HAG samples irradiated at different conditions: a) samples at pH 6.6 irradiated with λ > 290 nm in the presence of air, Ar and oxygen; b) samples at pH 6.6 in the presence of air irradiated with λ > 290 nm, >340 nm and >390 nm; c) samples at pH 6.6 in the presence of Ar irradiated with λ > 290 nm, >340 nm and >390 nm; d) samples at pH 10.5 irradiated with λ > 290 nm, >340 nm and >390 nm in the presence of air and Ar.

empirical expression used to fit saturation data (Galli, Innes, Hirsh & Brudvig, 1996) it is possible to obtain inhomogeneity parameter b.

$$Y = \frac{KP^{1/2}}{\left(1+\frac{P}{P_{1/2}}\right)^{b/2}} \quad (1)$$

where K is a constant, $P_{1/2}$ is the microwave power at half-saturation and b is the inhomogeneity parameter. The applied least square procedure to data presented in Fig.1 gave *b* value equal to 1 what suggest a high dipolar-relaxation enhancement occurs in dried HAG.

EPR spectra of aqueous HAG samples

A nonirradiated „dark" sample of 2 mg/ml exhibits a pH- and O_2-dependent EPR signal (Fig. 2). In aerated solutions the EPR signal amplitude increases as the pH of the sample increases. In the O_2-free „dark" samples the EPR signal at pH = 6.6 is barely seen whereas at pH = 10.5 its amplitude is comparable to the sample of „dark" aerated solutions at pH = 6.6. About 10-fold increase of the EPR signal is observed in „dark" oxygenated solution at pH = 6.6. The oxygenation of samples at pH = 10.5 leads to a fast colorization and a transient increase of the EPR signal and then following decrease of these parameters. The shapes of the all EPR spectra recorded for the dark samples are very similar and also the signal amplitude is mainly connected with presence of O_2 in the sample rather than with increasing pH what suggests that the signal recorded from the all samples originates from the same free radical. However, at higher pH of aerated solutions the EPR signal also increases that suggests that ionization state of the HAG components is another factor which influences the number of free radicals in the solution. It is known that at pH = 10.5 practically all active groups are ionized (ionization constants of GA are: pK_1 = 4.4, pK_2 = 8.5, pK_3 = 10 and pK_4 = 11.3). Anionic forms are more susceptible to autooxidation than neutral ones. Processes of oxidation proceed with participation of reactive oxygen species including

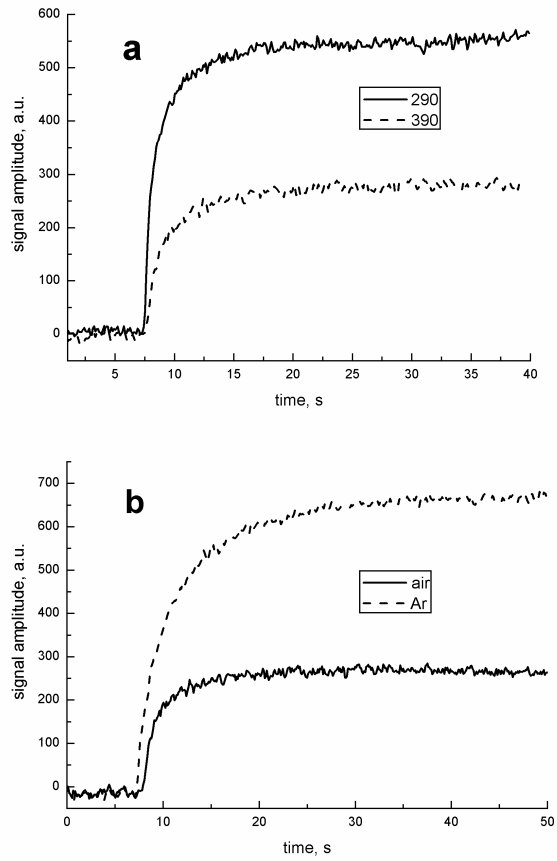

Fig. 4. The initial kinetics of the EPR amplitude change of aqueous samples at pH 6.6 of HAG: a) during irradiation with λ > 290 nm and >390 nm in the presence of air; b) irradiated with λ > 390 nm in the presence of air and Ar. The amplitude of the unirradiated sample is taken as a zero level.

$^1O_2$*, OH• and ROO•. These findings are in good agreement with the hypothesis that generated free radicals originate from phenolic and/or quinoid moiety of HAG. This indicates on the semiquinone as the other possible source of the observed EPR signal from HAG solutions.

Upon irradiation of the HAG sample with light at different wavelengths (λ) the amplitude of EPR signal increases up to 20 times compared to the signal from the dark sample. The intensity depends on the pH, atmosphere and irradiation spectral range. Solutions irradiated with λ > 290 nm in the presence of air, O_2 and deaerated sample at pH = 6.6 gives spectra shown in Fig. 3a. The spectra are recorded after 20 min. of irradiation. It is seen that the spectrum of the oxygenated sample is shifted compared to that of deaerated one. The EPR signal of aerated sample is located between these two samples.

Figure 3 presents the EPR spectra of HAG samples at pH = 6.6 recorded in aerated (3b) and deaerated (3c) solutions during irradiation with λ > 290 nm, 340 nm and 390 nm. In both samples the intensity of signal decreases as the λ of irradiation light increases. The spectra of both samples irradiated at λ >340 nm and λ > 390 nm show the same position and shape, whereas those of the samples irradiated at λ > 290 nm are shifted about 1.5 Gs. Fig. 3 d shows spectra of the irradiated HAG solution at pH = 10.5. All spectra look very similar regarding their positions and shapes except those irradiated at λ >290 which are shifted compared to the others, although the shift is only 1.7 Gs. The resonance line is structureless with the peak-to- peak width 5.59 Gs and characterized by g-values ranging from 2.0044 for dark sample to 2.0048 in the irradiated sample. The deaerated sample practically does not change depending on the dark-light cycle; the *g*-value of its EPR signal oscillates between 2.0047 for dark sample and 2.0048 for irradiated sample. The fact that photoinduced and pH-generated EPR spectra are the same suggests that in both cases the signal originates from this same free radical.

Kinetics of the amplitude of EPR signals

The intensity of the EPR signal was found to change with increase of irradiation time depending on the irradiation wavelength, pH and sample atmosphere. The kinetics of the initial amplitudes of the photoinduced EPR signals is given in Fig. 4. Initial values of amplitude are higher than those recorded after 20 min. of irradiation. This lead to the conclusion that prolonged irradiation decreases

the free radical content in the HAG sample. In all cases the intensity of EPR signal in deaerated sample is higher than that in aerated. Also in deaerated samples irradiated with $\lambda > 390$ nm the time to reach plateau is longer than when irradiated with shorter λ. It can also be seen that the irradiation with $\lambda > 290$ nm generates more free radicals. This indicates that the main sources of the free radicals are aromatic structures that absorb in the range above 280 nm. Similar reasoning applies to thee irradiation with $\lambda > 340$ nm where the amplitude change is about 80% of that observed at 280 nm. This again indicates on aromatic structures like quinones which are known to absorb in this spectral range.

CONCLUSIONS

The humic acid-like preparation synthetized from GA reveals physicochemical, particularly paramagnetic properties similar to those of natural HS. Therefore it may be a suitable model for studying interactions of paramagnetic centers of HS with O2 at different pH. The rate r of the HS + O_2 reaction depends on the activation energy E_a, activation equilibrium constant $K^{\#}$, and the transmission coefficient K as follows:

$$r = K(kT / h)K^{\#} \exp(-E_a / RT) .$$

It is the K that efficiently affects the kinetics of O_2 with organic substances because it reflects the symmetry properties of reactants. When the substrates and products have the same multiplicity, K approximates unity; otherwise K may decrease even to 10^{-4} (Allen, 1990).

Results of our experiments confirm these theoretical considerations; the amplitude of EPR signal is almost constant in deaerated samples, while it slowly decreases during prolonged reaction with O_2. Light is a powerful factor which can: (1) change the multiplicity either of HA or O_2, and (2) produce more unpaired spins. These both effects accelerate photodegradation of HAG. Thus, applications of EPR techniques to study the fate of HS in aquatic and terrestrial ecosystems in the presence of prooxidants and solar radiation allow to better understand mechanisms of environment important processes. Our findings may be useful in envisaging the rate of long term processes of the slow degradation of organic matter caused by increasing contamination of the troposphere with ozone, $^1O_2$*, NO_x and UVB radiation.

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