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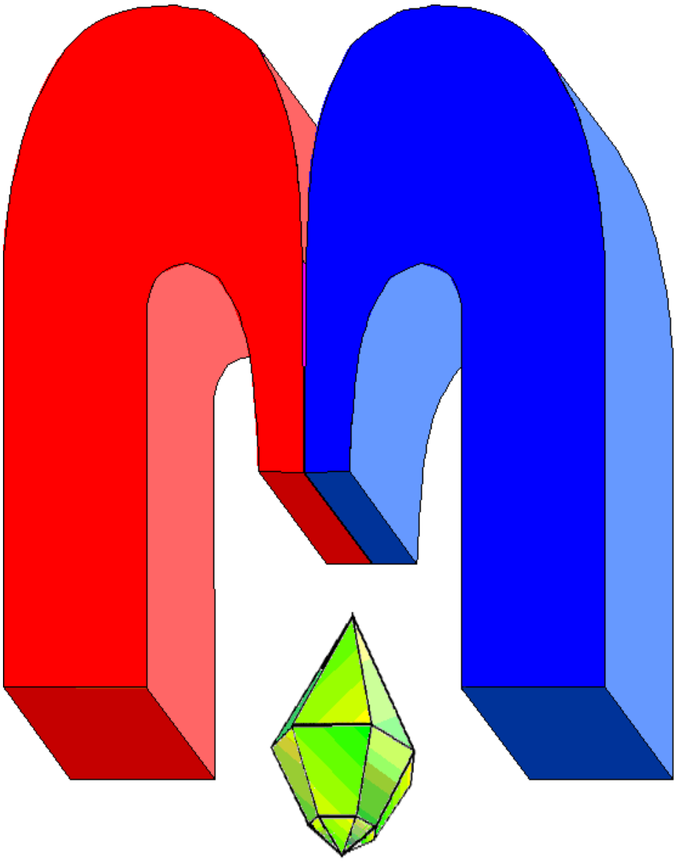

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[*] In Kazan University the Electron Paramagnetic Resonance (EPR) was discovered by Zavoisky E.K. in 1944.

Paramagnetic properties and antioxidant activity of metal-containing bionanocomposites based on humic substances

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Paramagnetic properties of novel bioactive nanocomposite materials based on humic substances and the precious metals (Ag, Au) are investigated by electron paramagnetic resonance and compared to their antioxidant properties. The possible mechanisms of antioxidant activity in the light of the free radical activation or inhibition of oxidation processes are analyzed.

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1. Introduction

In recent years, the search for new biogenic antioxidants that combine low toxicity and ability to effectively inhibit free radical processes in living organisms is gathering momentum [1-4]. Among such antioxidants, the compounds bearing the phenol moiety continue to attract a special research interest for many years [4].

Humic substances (HS) are considered to be very promising antioxidants. They represent complex polymeric organic compounds of aromatic and carbohydrate nature. Also, HS comprise various oxygen-containing groups, including phenolic hydroxyls. It is known that HS possess diverse biological activity [5, 6]. In particular, they are natural polymer antioxidants that are capable of neutralizing the free radicals formed in the inflammatory locations, and are inductors of destabilizing and breaking the cell membrane [7]. Previously, we have demonstrated the possibility of successful application of HS derived from biogenic sediments for the synthesis of water-soluble nanocomposites [8, 9]. Thus, the obtained silver-containing nanocomposites are expected to combine specific properties of HS (antioxidant activity and water solubility) with those of gold and silver nanoparticles, such as antimicrobial properties. A comparative study of the antioxidant activity of the resulting nanocomposites as well as the initial HS shows that all the samples *in vitro* inhibit the peroxidation model processes of the living cell membrane lipids [10].

Despite the advances in the studies of bioantioxidant properties of natural compounds, many issues related to the relationships between the structural organization and antioxidant properties of the substances still remain poorly understood. Here we report the results of comparative analysis of the paramagnetic characteristics and antioxidant properties of the obtained metal-containing nanocomposites with the original matrixes. Also, the general regularities of the change in their oxidising properties are elucidated.

2. Experimental

All the target HS-based nanocomposites were characterized by a complex of modern spectral and physicochemical methods [8, 9]. Silver (HS-Ag) and gold (HS-Au) nanocomposites were synthesized by the reaction of silver and gold ions with the corresponding humic substances, extracted from medical muds (HSm) of Mongolian Lake Gurvan Nuur, shales (HSs) of Shine Hudag and brown coals

Table 1. Metal content and spin concentration of HS and nanocomposites on its base.

Samples	HS		silver nanocomposites			gold nanocomposites		
	Fe, %	*N*, spin/g	Ag, %	Fe, %	*N*, spin/g	Au, %	Fe, %	*N*, spin/g
HSm	0.7	3.4×10^{18}	9.8	1.0	3.6×10^{18}	7.1	0.5	1.7×10^{18}
HSs	–	4.4×10^{17}	10.5	0.1	9.9×10^{17}	6.8	1.1	8.4×10^{17}
HSc	–	1.6×10^{19}	10.8	1.5	1.3×10^{19}	8.6	0.6	1.4×10^{19}

(HSc) of Baga Nuur field, in the aqueous-alkaline medium for 20 min at 90°C according to previously published protocols [8, 9]. The formation of Ag^0 and Au^0 nanoparticles was proved by the appearance of intensive plasmon absorption in the visible spectral region (Ag 408-432 nm, Au 510-540 nm) due to collective excitation of conduction electrons of gold or silver.

The metal percentage of nanocomposites has been determined by atomic absorption analysis using HITACHI TM 3000, detector SDD XFlash 430-H. The metal mass percentages of the nanocomposites on the basis of HS matrix are presented in tab. 1.

The X-ray diffraction (XRD) analysis has been made on powder diffractometer D8 ADVANCE (Cu radiation). The estimated size of metal nanoparticles was determined by XRD analysis, their linear dimensions were calculated by the Scherrer formula [11]. XRD analysis of nanocomposites based on HSm, HSc and HSs showed that the average region size of the coherent scattering (L, nm) of silver nanoparticles in the obtained samples varied in the range of 6-14 nm. The average size of the coherent scattering of gold nanoparticles in the nanocomposites obtained was 10-17 nm (XRD data).

The antioxidant activity was determined by the measurement of the inhibitory effect of a potential antioxidant on the in vitro model of Fe^{2+} ascorbate induced oxidation of polyoxyethylene sorbitan monooleate (Tween-80) to malondialdehyde with air oxygen [10]. The measurement precision of antioxidant activity was ±1.0%.

Continuous wave electron paramagnetic resonance (EPR) spectra were recorded with FT X-band Brüker ELEXSYS E-580 spectrometer (X-wave range 9.7 GHz). The EPR-spectra were recorded under the following conditions: amplitude modulation 1.0 G, modulation frequency 100 kHz, time constant 0.02 s, conversion time 0.06 s, microwave power 0.6325 mW, field range 6000 G / centre field 3480 G and field range 100 G / centre field 3443 G, averaged scans 10, receiver gain 30 dB at room temperature. The concentrations of paramagnetic centers were calculated by the known method [12] with the use of diphenylpicrylhydrazyl as a standard (tab. 1).

3. Result and Discussion

The initial HS possess paramagnetism and give in the EPR spectra the main high intensity signal with *g*-factor of 2.005 and width of about 5 G (fig. 1). Narrow symmetrical lines of the samples correspond to the concentration of unpaired spins of 10^{17}-10^{19} spin/g, see tab. 1. Paramagnetic properties of HS are caused by polyaromatic strongly condensed nature of the conjugated bonds system. The molecular structure of the investigated substances is characterized by the presence of aromatic and aliphatic components, especially the so-called "core" of the macromolecule. Furthermore, in the HS structure, the aromatic moieties predominate over the chained aliphatic ones. Ratios (degree of benzenoid) H/C for the selected HS products vary in the range of 0.067-0.083 and shows a high content of aromatic structures in their composition. The presence of a well-developed system of conjugated bonds in HS structure is consistent with high concentration of paramagnetic centers. The intensive signal is often present in such natural macromolecules, due to including free radicals in these compounds (co-called macroradicals) [13, 14]. The most intense signal is shown in the spectrum of substances extracted from brown coal, due to the natural complicated structure of these compounds (with a high degree of unpaired electrons delocalization) and high spin concentration [15].

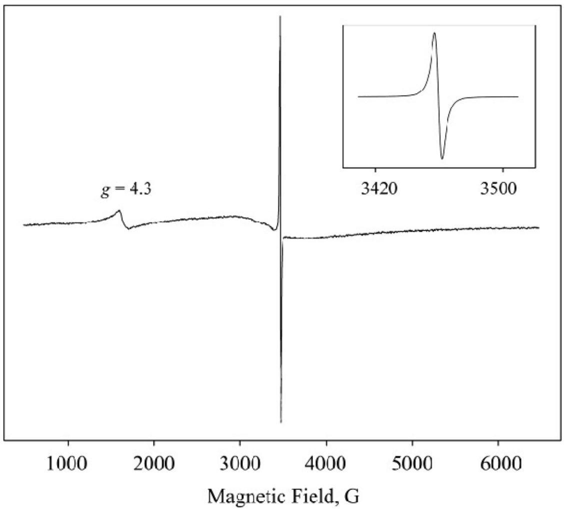

Figure 1. A typical EPR spectrum of started HS (measured at room temperature).

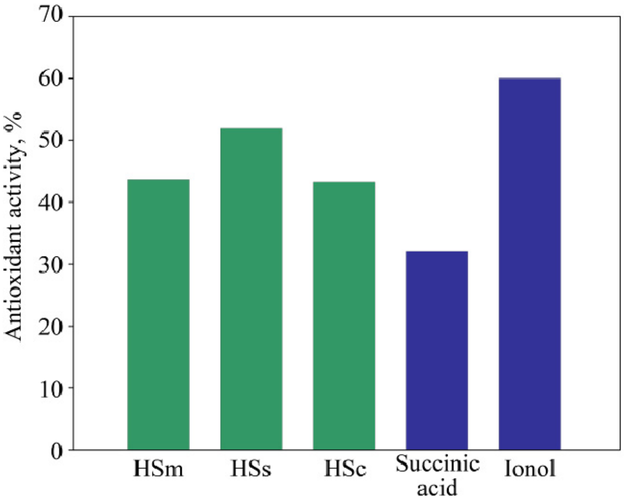

Figure 2. Antioxidant activity of the started HS in comparison with the activity of known antioxidants.

The study of the antioxidant activity of the starting HS has revealed that all samples *in vitro* have a higher inhibitory effect on oxidative processes within 42-52% (fig. 2), the amount of antioxidant activity being greater than that for the known antioxidant succinic acid. The diagram shows that the greatest inhibition of the peroxidation products accumulation (52%) is observed when HSs is used as an antioxidant, HSm possesses lesser antioxidant activity (43.6%) and HSc exerts the lowest (42.3%) the inhibitory effect. Different antioxidant ability of the substances is probably due to the difference between elemental and functional composition of the macromolecules. High characteristics of antioxidant activity and high spin concentrations allow to trace their change in the obtained metal-containing bionanocomposites.

In the EPR spectra of the silver- and gold-containing nanocomposites HS-Ag and HS-Au, intense main signal is also observed (fig. 3), as in the original matrixes. The narrow line for metal-containing samples broaden to 6.5 G, g-factor increases (in range of 2.0060-2.0065) and deviates from the values of a free electron g_e that indicates the contribution of the spin-orbit interaction of the electrons with a heavy nucleus. It can be concluded that the narrow line is a superposition of signals, caused by free radicals of the original matrix and the zero-valent metal nanoparticles, which is consistent with the EPR data for the conductivity electrons of zero-valent metal particles in nanoscale systems [16-18]. It is assumed that the main contribution to the reduction of metal cations to the zero-valent state is made by the HS aldehyde groups (with different aliphatic and aromatic substituents) and the phenolic hydroxyls. In addition, the nanocomposites are characterized by a high degree of oxidation as compared to the initial HS samples, which also testifies to their oxidation in the process of metal reduction.

The diagram shows that the greatest inhibition of the oxidation products accumulation is observed in the case of HS-Ag nanocomposites (22-52%) and HS-Au nanocomposites (44-52%) (fig. 4). The antioxidant properties of silver composites either decrease or remain almost unchanged (HSs-Ag). The smallest inhibitory effect (22%) is found for silver-nanocomposites having a maximum content of silver in 10.8% for HSc-Ag. For gold composites, there is a slight increase (or a very weak decrease in the case HSs-Au), i.e. the value of antioxidant activity slightly differs from that of the original matrices. It can be assumed that the antioxidant activity of nanocomposites based on HS, like many other bioantioxidants, is caused by the presence of phenolic groups. The observed decrease in antioxidant activity of nanocomposites with maximum silver content can be attributed to a decrease in the concentration of the phenolic groups of the obtained nanocomposite due to their one-electron oxidation by silver cations in the synthesis process.

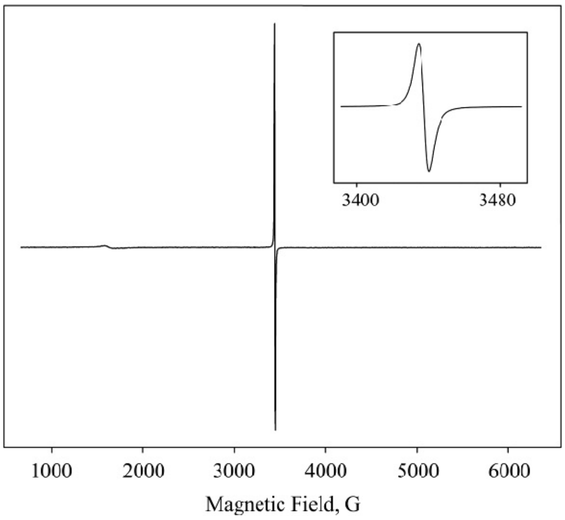

Figure 3. A typical EPR spectrum of gold-containing HS-based nanocomposites (measured at room temperature).

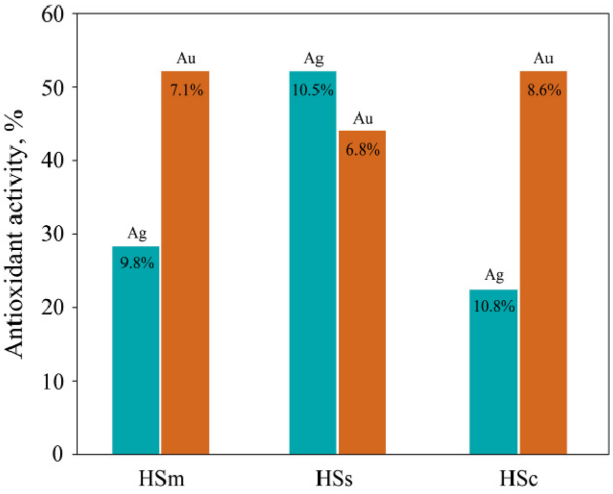

Figure 4. Antioxidant activity of the metal-containing HS-based nanocomposites (metal contents specified in the figure).

Note the changes of the spin concentration also are not unequivocally (tab. 1). So free radicals together with the phenolic groups can also act as a reducing agent of silver and gold ions owing to interaction of metal ions with an electron-donor centers during the formation of nanosystems. This can be explained by the fact that the introduction of a larger amount of metal in the matrix leads to a greater decreasing of the spin concentration in the composite, which is consistent with the hypothesis on the interaction of metal ions with free radicals. Reduction process occur due to different types of free radicals in such complicated macromolecule structures (HSs and HSm are more oxidized unlike substances HSc of deeper decomposition), which explains the dissimilar expenditure of free radicals. Note that the reduction of Au^{3+} ions to zero-valent state requires the use of $3\bar{e}$ that should cause substantially greater decrease of spin concentration in the HS-Au, as compared to HS-Ag compounds. However, not so high difference in concentrations could be explained by the different content of metals in the nanocomposites. Moreover, one should consider the contribution in a spin concentration from the zero-valent metal nanoparticles. It is important to note that the complexities of the changes in the antioxidant properties can be attributed to other processes, so the antioxidant properties of phenolic compounds are determined not only by their anti-radical action, they are also able to interact with transition metals and can thus contribute to the formation of free radicals. As a result, reduction of metal ions can activate the processes of hydroperoxides decompositions (Fenton reaction type) [4], and the phenolic group may act as prooxidants. Thus, by interacting with the transition metal ions, they can both inhibit and activate free radical oxidation processes. Moreover, iron, presented in the humic substances in amount 0.1-1.5%, can act as an additional initiator of free radical peroxidation process [10].

4. Summary

Bionanocomposites based on HS of Mongolian deposits preserve their paramagnetic properties, at least for one year, and represent biologically active substances containing a lot of stable nanoparticles of zero-valent silver and gold (6-14 nm). Attempts to correlate antioxidant activity and spin concentration of HS samples have shown its ambiguity, that makes impossible to approach the evaluation of an antioxidant activity based on EPR characteristics in such complicated substances. In addition, all obtained nanocomposites exhibit high antioxidant activity comparable with that of the known antioxidants, ionol and succinic acid. Thus, the HS-based bionanocomposites find new applications in the synthesis of novel pharmaceuticals with antioxidant properties, in the manufacture of composite materials for the healing with antimicrobial properties of HS-Ag and softening the effects of pollution on living organism.

Acknowledgments

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