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Continuous microwave saturation of EPR spectra of melanin complexes at different temperatures

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Paramagnetic centers in DOPA-melanin and complexes of DOPA-melanin with netilmicin and Cu(II) were studied by the use of an X-band (9.3 GHz) electron paramagnetic resonance (EPR) spectroscopy. Measurements of continuous microwave saturation of EPR spectra at temperatures: 125 K, 175 K, 225 K, 275 K, were performed. Homogeneous broadening of all the examined EPR spectra was observed. EPR spectra of DOPA-melanin-Cu(II) complexes saturated at higher microwave powers than the others tested melanin samples. Fast spin-lattice relaxation exists in DOPA-melanin-Cu(II) complexes. Slow spin-lattice relaxation processes exist in melanin's paramagnetic centers of DOPA-melanin and its complexes with netilmicin, and its complexes with both netilimicin and Cu(II). EPR spectra of all the tested samples saturated at higher microwave powers with increasing of the measuring temperature. Faster spin-lattice relaxation processes occurs in DOPA-melanin and its complexes with netilmicin and Cu(II) at higher temperature.

Key Words: paramagnetic centers, DOPA-melanin, netilmicin, microwave saturation, EPR spectroscopy.

Introduction

Melanins are the paramagnetic biopolymers existing in human organism and in the plants [2, 4, 7, 9, 11-12, 16-18, 20]. Melanins react with drugs and metal ions [1, 4-6, 8-10, 13-14, 18, 22].

Dia- and paramagnetic metal ions affect differently the amounts of complexes, formed by melanin and the individual drugs [4-5, 8]. It was shown by spectroscopic studies that free radicals of melanins play an important role in binding of drugs to these polymers [1, 4-6, 8, 13]. The interactions of melanins with drugs are the very important problem in pharmacy and medicine, because of the prolongation of interactions of the pharmaceuticals on the tissues [4]. The melanins – drugs interactions are examined by the modern methods of medical physics [1, 4-6, 8, 13]. One of them is electron paramagnetic resonance (EPR) spectroscopy, which is the experimental method for examination of the samples containing free radicals such as the melanins [1-2, 4-18, 20, 22].

In this work the complexes of the model eumelanin (DOPA-melanin) with one of the aminoglycoside antibiotics - netilmicin were tested by the specific technical conditions of measurements of the EPR spectra. The influence of paramagnetic copper (II) ions on the interactions of melanin with this antibiotic was studied. The EPR spectra were continuously saturated by microwaves, and their saturation for the melanin, and melanin complexes with netilmicin and Cu(II), were compared. The microwave saturation of the EPR lines depends on the spin-lattice relaxation processes in the sample [19, 21]. The comparative analysis of the microwave saturation and spin-lattice relaxation processes of the individual melanin samples at different temperatures was done. This work improves the knowledge about physical properties of eumelanin and its complexes with drug at the presence of metal ions.

Material

DOPA-melanin, DOPA-melanin-netilmicin, DOPA-melanin-Cu(II), [DOPA-melanin-netilmicin]-Cu(II), and [DOPA-melanin-Cu(II)]-netilmicin complexes were examined. DOPA-melanin was obtained according to Binn's method [3]. Concentrations of complexing agents: netilmicin and Cu(II), were 1×10^{-3} M.

Netilmicin is an aminoglycoside antibiotic which is used in the treatment of serious infections, e. g. sepsis [23]. The chemical structures of eumelanin and netilmicin are presented in the Figure 1 a and b, respectively.

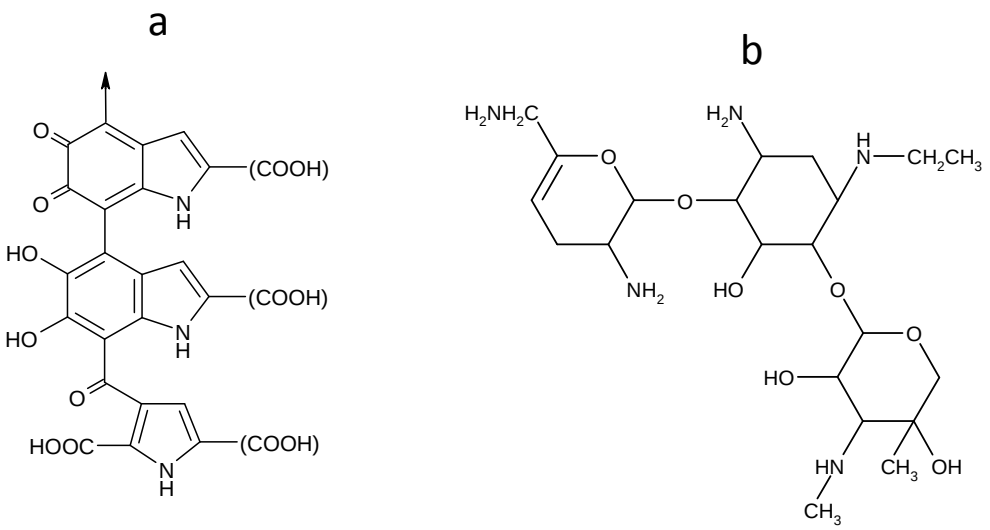

Fig. 1. Chemical structures of eumelanin [20] (a) and netilmicin [23] (b)

Methods

Measurements were done by the use of an X-band (9.3 GHz) EPR spectrometer of BRUKER. The first derivative EPR spectra were measured with microwave power in the wide range of 0.3-200 mW. Changes of amplitude (A) and linewidth (ΔB_{pp}) of the EPR spectra with increasing of microwave power were determined. The spectra were obtained at the following temperatures: 125 K, 175 K, 225 K, and 275 K. The microwave saturation of the EPR spectra at the measuring temperatures was compared.

Results

EPR spectra of DOPA-melanin, DOPA-melanin-netilmicin complexes and the complexes of DOPA-melanin with Cu(II) and both Cu(II) and netilmicin differ in shape. Single asymmetrical EPR lines were measured for DOPA-melanin and DOPA-melanin-netilmicin complexes. EPR spectra of DOPA-melanin-Cu(II), [DOPA-melanin-netilmicin]-Cu(II), and [DOPA-melanin-Cu(II)]-netilmicin complexes were superposition of two EPR lines of o-semiquinone free radicals of melanin and copper ions. The exemplary spectra of DOPA-melanin and DOPA-melanin-Cu(II) complexes are presented in Figures 2 a and 2 b, respectively. EPR spectra of the studied

samples changes with increasing of microwave power. Microwave saturation of EPR spectra of free radicals was tested.

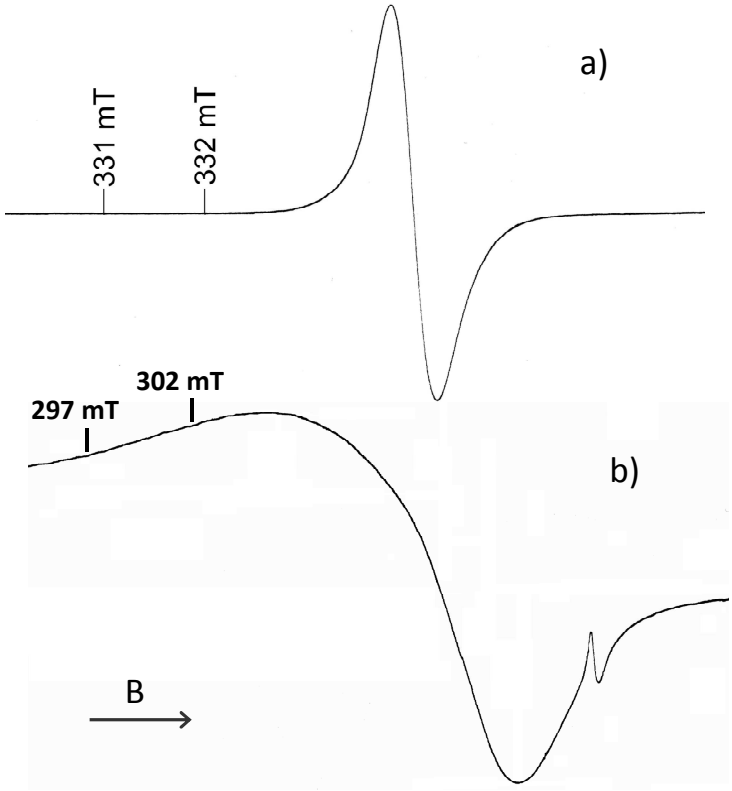

Fig. 2. EPR spectra of DOPA-melanin-netilmicin (a) and DOPA-melanin-Cu(II) (b) complexes measured with microwave power of 0.3 mW at temperature of 225 K.

Influence of microwave power (M) on amplitudes (A) and linewidths (ΔB_{pp}) of EPR lines of free radicals of DOPA-melanin, DOPA-melanin-netilmicin, and DOPA-melanin-Cu(II) complexes at the tested temperatures (125 K, 175 K, 225 K, and 275 K) are compared in Figure 3 a-c. Amplitudes (A) of EPR lines of DOPA-melanin and DOPA-melanin-netilmicin complexes increase with increasing of microwave power, reach the maximum and decreases with the continued increase of microwave power (Fig. 3 a-b). The amplitude (A) of the EPR spectra of DOPA-melanin-Cu(II) complexes does not decrease at the higher microwave powers (Fig. 3 c). The values of microwave power accompanied by the maximal amplitudes (A) increase with the increasing of the measuring temperature (Fig. 3 a-c). Broadening of the EPR lines with the increasing of microwave power was observed for the mentioned above samples (Fig. 3 a-c).

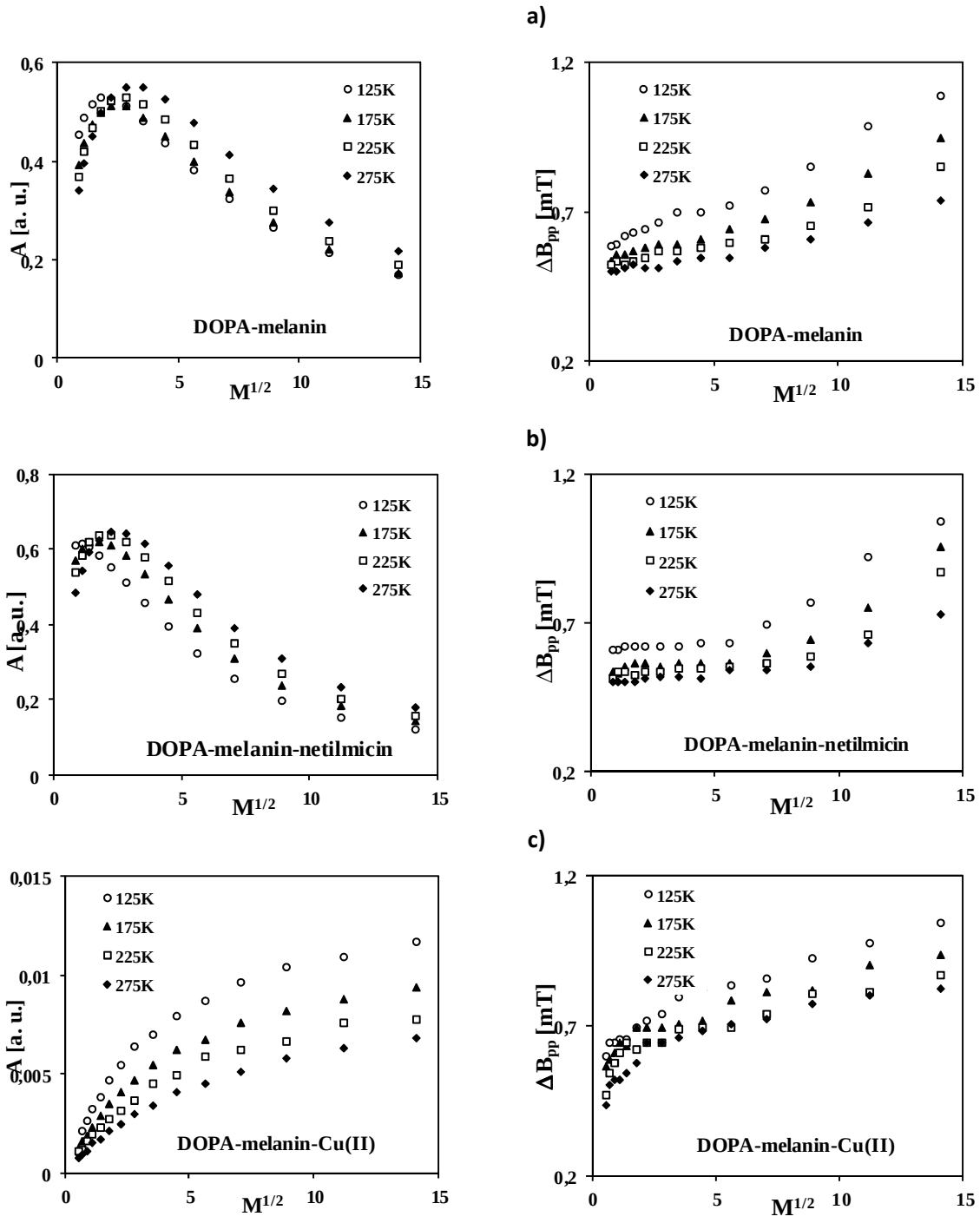

Fig. 3. Influence of microwave power (M) on amplitudes (A) and linewidths (ΔB_{pp}) of EPR spectra of DOPA-melanin (a), DOPA-melanin-netilmicin (b), and DOPA-melanin-Cu(II) (c) complexes at temperatures 125 K, 175 K, 225 K, and 275 K.

Influence of microwave power (M) on amplitudes (A) and linewidths (ΔB_{pp}) of EPR spectra of [DOPA-melanin-netilmicin]-Cu(II) and [DOPA-melanin-Cu(II)]-netilmicin complexes are shown in Figure 4 a-b, respectively.

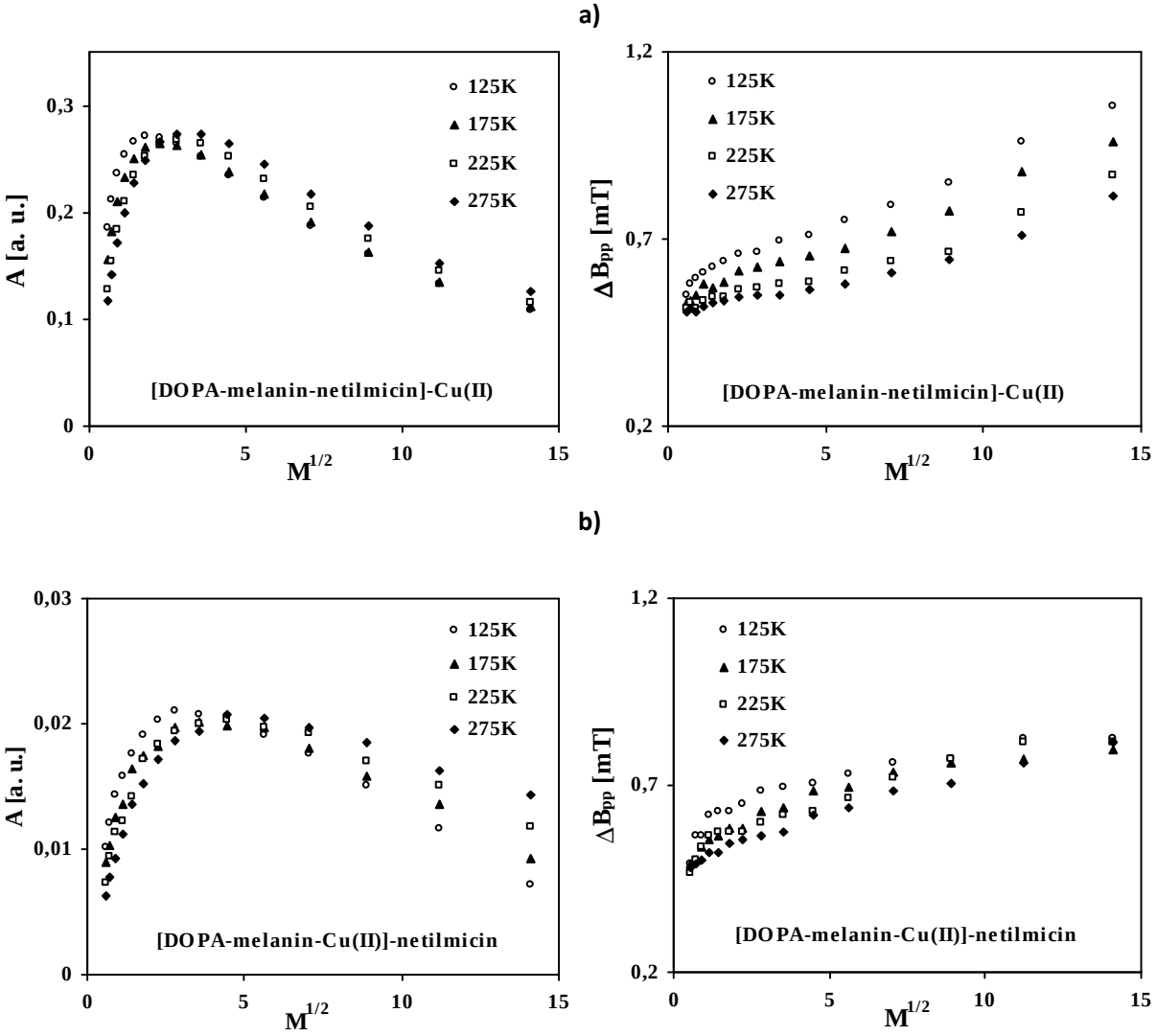

Fig. 4. Influence of microwave power (M) on amplitudes (A) and linewidths (ΔB_{pp}) of EPR spectra of [DOPA-melanin-netilmicin]-Cu(II) (a) and [DOPA-melanin-Cu(II)]-netilmicin (b) complexes at temperatures 125 K, 175 K, 225 K, and 275 K

In Figure 4 data for the measurements at temperatures 125 K, 175 K, 225 K, and 275 K are compared. The amplitudes (A) of the EPR spectra of DOPA-melanin complexes containing both netilmicin and Cu(II) decrease at the higher microwave powers at all the testing temperatures (Fig. 4 a-b). At the higher temperatures their EPR lines saturate at the higher

microwave powers (Fig. 4 a-b). Linewidths (ΔB_{pp}) of the EPR spectra of [DOPA-melanin-netilmicin]-Cu(II) and [DOPA-melanin-Cu(II)]-netilmicin complexes increase with the increasing of microwave power at all the tested temperatures (Fig. 4 a-b).

Discussion

Free radicals exist in all the tested melanin samples revealing the strong EPR spectra (Fig. 2). The free radicals in DOPA-melanin take a part in netilmicin binding to this polymer. The amounts of free radicals in DOPA-melanin and in its complexes with netilmicin and Cu(II) are different, and as the result the amplitudes (A) of their EPR lines are different (Fig. 3-4). The higher amplitudes (A) of EPR lines are observed for DOPA-melanin complexes with netilmicin (Fig. 3 b). The lower amplitude (A) of EPR lines characterizes DOPA-melanin complexes with Cu(II) (Fig. 3 c). The effect of quenching of free radicals EPR signals by paramagnetic copper ions was observed earlier [5, 8, 10-11, 14]. This quenching is also observed for DOPA-melanin complexes with netilmicin (Fig. 4 a-b).

Not only the amount of free radicals, but also the magnetic interactions in DOPA-melanin are dependent on netilmicin and copper(II) ions. In this work, the spin-lattice interactions were tested. The absence of microwave saturation of EPR lines of DOPA-melanin-Cu(II) complexes at the used microwave power (Fig. 3 c) points out that fast spin-lattice relaxation exists in this sample. Continuous microwave saturation of EPR lines indicates that slow relaxing free radicals exist in organic substance of DOPA-melanin (Fig. 3 a), DOPA-melanin-netilmicin complexes (Fig. 3 b), and DOPA-melanin complexes with both netilmicin and Cu(II) (Fig. 4 a-b). Spin-lattice relaxation time decreases with increasing of power of microwave saturation of EPR spectra [19]. Taking to account this correlation it can be seen that fastening of spin-lattice relaxation processes in melanin structure occurs at higher temperatures, because the lines saturate at higher microwave powers (Fig. 3 a-b, Fig. 4 a-b). The correlation between amplitudes of EPR lines and microwave power were similar for DOPA-melanin and its complexes with netilmicin (Fig. 3 a-b, Fig. 4 a-b). Only the weak differences in spin-lattice relaxation processes upon netilmicin are observed in DOPA-melanin complexes. Correlation between amplitudes of EPR lines and microwave power are similar for DOPA-melanin and its complexes with netilmicin (Fig. 3 a-b, Fig. 4 a-b).

The fastening of spin-lattice relaxation with the increasing of the measuring temperature is observed (Fig. 3 a-c, 4 a-b). The fast spin-lattice relaxation processes characterize the fast

transitions of unpaired electrons between the excited energy level and the ground energy level [21].

The observed decrease of amplitudes (A) of EPR lines at higher microwave powers (Fig. 3 a-b, Fig. 4 a-b) and broadening of EPR lines with increasing of microwave power (Fig. 3 a-c, Fig. 4 a-b) are characteristic for homogeneously broadened EPR lines [19, 21]. The homogeneous broadening of EPR lines reveals the samples with the free radicals interacting in their whole structures, and isolated spin-packet do not occur then [21]. This study confirmed (Fig. 3 a-c, Fig. 4 a-b) described at literature [5, 8, 10-11, 14] effect of quenching of EPR spectra by Cu(II), which was observed and described earlier [5, 8, 10-11, 14].

The performed studies indicate that the electron paramagnetic resonance spectroscopy is the useful method in medical physics. This work is the example of application of EPR to examination of physical properties of melanin as the important biopolymer in human organism. The EPR spectroscopy may be used to interactions of melanin biopolymer with drugs. Continuous microwave saturation of EPR spectra is the important tool to examine magnetic interactions in biopolymers and their complexes with drugs and metal ions.

Conclusions

Electron paramagnetic resonance (EPR) studies of DOPA-melanin and its complexes with netilmicin and Cu(II) at temperatures in the range 125-275 K pointed out that:

1. Fast spin lattice-relaxation processes exist in DOPA-melanin-Cu(II) complexes, their EPR lines do not saturate at microwave powers of 0.3-200 mW.
2. Slow spin-lattice relaxation processes exist in DOPA-melanin, DOPA-melanin-netilmicin, [DOPA-melanin-netilmicin]-Cu(II), and [DOPA-melanin-Cu(II)]-netilmicin complexes, which EPR lines saturate at all the tested temperatures.
3. Continuous microwave saturation indicates that the EPR lines of DOPA-melanin and DOPA-melanin complexes with netilmicin and Cu(II) are homogeneously broadened.

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