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ORIGINAL PAPER

Effect of microwave power on EPR spectra of DOPA-melanin-netilmicin complexes with different drug concentrations – a study at temperatures in the range of 125-275 K

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Abstract. Electron paramagnetic resonance (EPR) spectra of the model eumelanin – DOPA-melanin complexes with netilmicin measured with a microwave power in the range 0.3–200 mW were examined. The influence of microwave power on amplitudes (A) and linewidths (ΔB_{pp}) of the spectra was determined. The EPR spectra were recorded by a BRUKER spectrometer at temperatures 125, 175, 225 and 275 K. Continuous microwave saturation of EPR lines was used to compare the acceleration of spin-lattice relaxation processes in melanin samples. Changes of the parameters of the EPR spectra of DOPA-melanin-netilmicin complexes, with the microwave power for the samples differing in netilmicin concentrations, were observed. The effect of temperature on the changes of the EPR spectra of the analyzed melanin complexes with the microwave power was determined. The spin-lattice relaxation is faster at higher temperatures for all the tested melanin complexes.

Key words: DOPA-melanin • electron paramagnetic resonance (EPR) spectroscopy • free radicals • netilmicin

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Introduction

Free radicals in the human organism play an important role during living processes [1]. Free radicals are formed in tissues in natural biological conditions, at pathological stages and also as a result of pharmaceutical interactions or physical factors [1, 7, 9, 26]. The extraordinary high contents of free radicals ($\sim 10^{17}$–10^{21} spin/g) characterize melanin biopolymers [5, 8, 10, 13, 16–18, 22, 24, 25]. Melanin exists in skin, inner ear, hair, eyes and *Substantia nigra* [15, 23, 28]. Melanin reveals the susceptibility for interactions with ligands, especially drugs and metal ions. *o*-Semiquinone free radicals of melanin take part in binding of ligands and as the effect free radicals concentrations change in this polymer [2, 5, 6, 14, 19, 30]. The complexation of drug molecules by melanin changes spin-lattice interactions in the polymer [20, 21, 31, 32]. EPR lines of melanin complexes with drugs and both drugs and metal ions saturate at different microwave powers [14, 32]. These microwave changes of spectroscopic lines reflect the structural modification in melanin complexes with drugs.

The aim of this work was to study the effect of microwave power on EPR spectra of DOPA-melanin complexes with netilmicin. We focused on microwave power and temperature effects on the EPR lines of free radicals. The influence of concentration of netilmicin and the measuring temperature on the correlations between the parameters of EPR lines was tested. The model DOPA-melanin was chosen to studies, because

of eumelanin mainly exists in the organisms [4, 11–13, 23]. Netilmicin is a popular aminoglycoside antibiotic, which is characterized by high practical applications [33]. This antibiotic is used against serious infections as sepsis, infections of the respiratory and urinary tracts. Netilmicin is a drug of the lowest toxicity of this aminoglycoside antibiotics [33].

In this work, the microwave correlations between EPR parameters of DOPA-melanin-netilmicin complexes with different drug concentrations were examined to obtain the confirmation of the free radicals – netilmicin interactions. This spectroscopic examination is important to our knowledge about the free radicals in eumelanin complexes with drugs.

Experimental details

DOPA-melanin and its complexes with netilmicin were studied. DOPA-melanin was obtained according to the Binns method [3]. Chemical structure of netilmicin is shown in Fig. 1 [33].

The following concentrations of netilmicin were used during the preparation of the complexes with DOPA-melanin [M]: 1×10^{-4}, 5×10^{-4}, 5×10^{-3} and 1×10^{-2}. The complexes of DOPA-melanin with netilmicin were prepared by melanin incubation with drug solutions in phosphate buffer at pH 7.0.

The electron paramagnetic resonance spectra of DOPA-melanin and DOPA-melanin-netilmicin complexes were measured by an X-band (9.3 GHz) EPR spectrometer of BRUKER. EPR spectra recorded with a microwave power in the range 0.3–200 mW were examined. The influence of microwave power on the amplitudes (A) and linewidths (ΔB_{pp}) of the spectra at the measured temperatures of 125, 175, 225 and 275 K was determined.

Continuous microwave saturation of EPR lines was used to compare the velocity of spin-lattice relaxation processes in melanin samples. Changes of the parameters of the EPR spectra of DOPA-melanin-netilmicin complexes with a microwave power for the samples with different drug concentrations were evaluated. The effect of microwave power on the EPR spectra of the analyzed melanin complexes at different temperatures was compared.

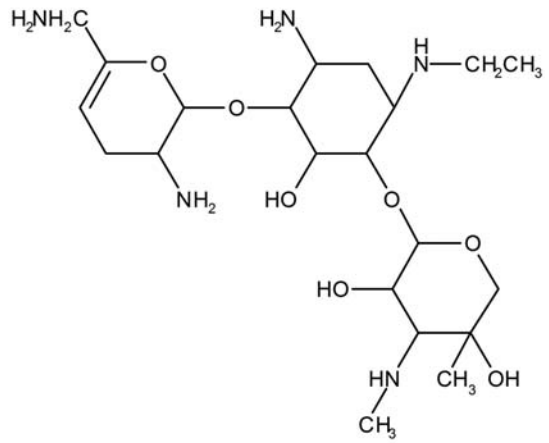

Fig. 1. Chemical structure of netilmicin [33].

Results and discussion

EPR spectra of DOPA-melanin complexes with netilmicin change with microwave power and the measured temperature. The influence of microwave power (M) on the amplitudes (A) and linewidths (ΔB_{pp}) of the EPR lines of the reference sample – DOPA-melanin at temperatures 125, 175, 225 and 275 K is presented in Fig. 2.

Amplitudes (A) increase with increasing microwave power, reach a maximum and then decrease with increasing microwave power (Fig. 2a). Linewidths (ΔB_{pp}) of the EPR lines of DOPA-melanin at all the tested temperatures increase with increasing microwave power (Fig. 2b). These correlations are characteristic of homogeneously broadened EPR lines [27, 29]. The EPR lines of DOPA-melanin saturate at low microwave powers (Fig. 2a), so it can be seen that slow spin-lattice relaxation processes exist in this polymer. The microwave saturation of the EPR lines of DOPA-melanin increases with increasing measured temperature (Fig. 2a). This means that faster spin-lattice interactions appear at a higher temperature. Similar dependences were observed by as earlier [31, 32].

The results of the studies of DOPA-melanin complexes with netilmicin are presented in Figs. 3–7.

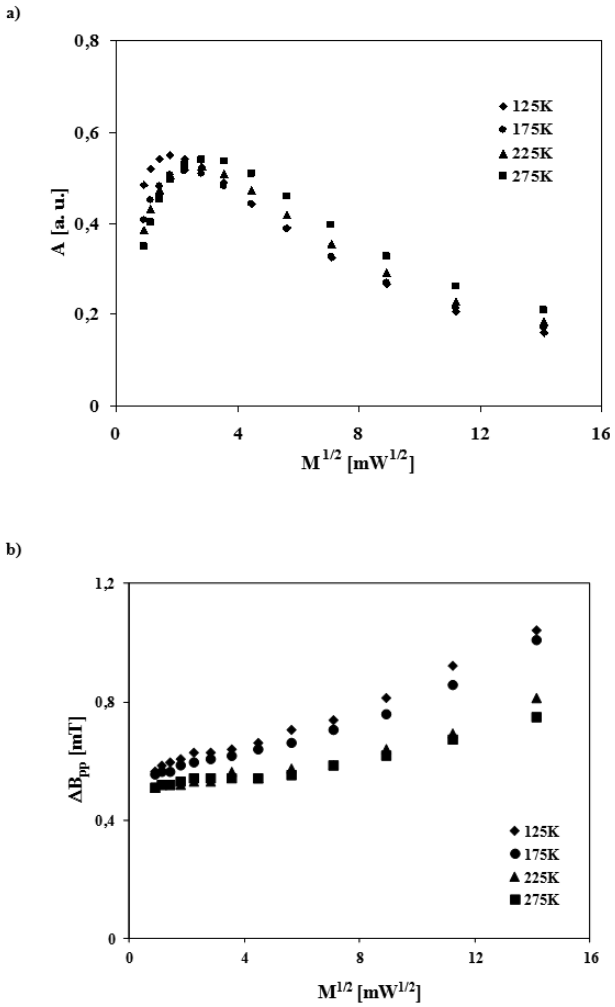

Fig. 2. Influence of microwave power (M) on (a) amplitudes (A) and (b) linewidths (ΔB_{pp}) of EPR spectra of DOPA-melanin at temperatures 125, 175, 225 and 275 K.

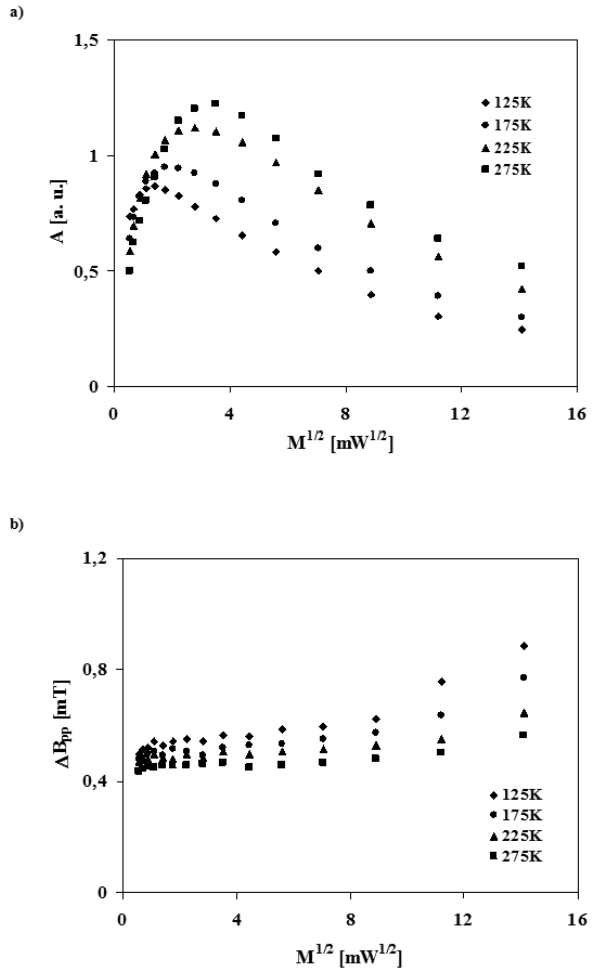

Fig. 3. Influence of microwave power (M) on (a) amplitudes (A) and (b) linewidths (ΔB_{pp}) of EPR spectra of DOPA--melanin-netilmicin complexes at temperatures 125, 175, 225 and 275 K. The concentration of netilmicin was 1×10^{-4} M.

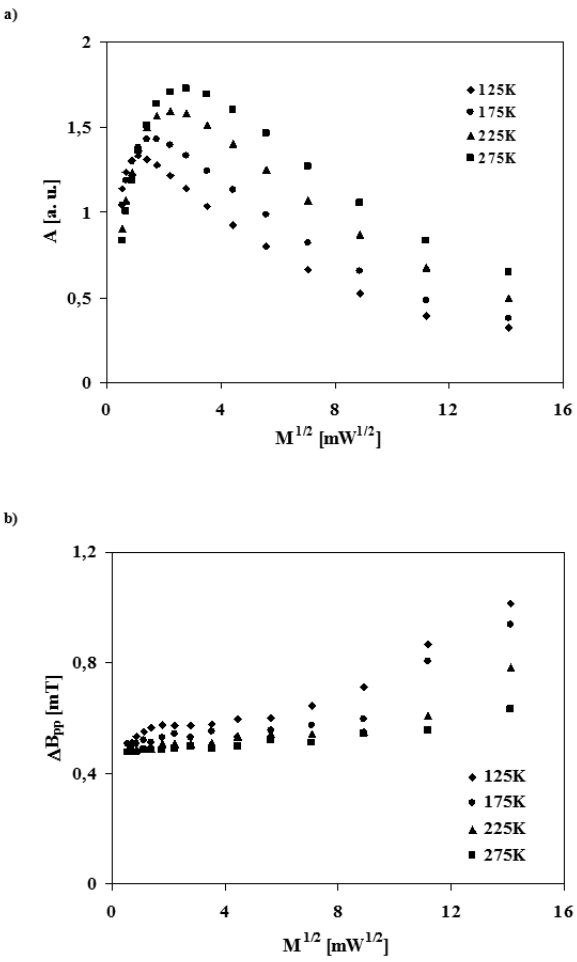

Fig. 4. Influence of microwave power (M) on (a) amplitudes (A) and (b) linewidths (ΔB_{pp}) of EPR spectra of DOPA--melanin-netilmicin complexes at temperatures 125, 175, 225 and 275 K. The concentration of netilmicin was 5×10^{-4} M.

The influence of microwave power (M) on the amplitudes (A) and linewidths (ΔB_{pp}) of the EPR lines of DOPA-melanin-netilmicin complexes at temperatures 125, 175, 225 and 275 K for concentrations of netilmicin of 1×10^{-4} M, 5×10^{-4} M, 5×10^{-3} M and 1×10^{-2} M is shown in Figs. 3–6, respectively. The same character of the changes as was observed for DOPA-melanin (Fig. 2) was obtained for DOPA-melanin-netilmicin complexes. The EPR spectra of these complexes are homogeneously broadened (Figs. 3–6).

The amplitudes (A) easy reach maximum at low microwave powers (Figs. 3–6), so the slow spin-lattice relaxation processes exist in the DOPA-melanin complexes with netilmicin. Similarly to DOPA-melanin (Fig. 2a), the microwave saturation of the EPR lines of DOPA--melanin-netilmicin complexes appear at low microwave powers (Figs. 3–6). The slow spin-lattice relaxation processes characterize these melanin complexes.

Microwave changes of the EPR lines (Figs. 3–6) indicate that at higher temperatures the spin-lattice relaxation processes in the analyzed complexes are faster. The EPR lines of DOPA-melanin-netilmicin complexes for all netilmicin concentrations saturate at higher microwave power at the higher measured temperatures (Figs. 3–6). Similar effects were observed also for DOPA-melanin complexes with other drugs, as kanamycin [14].

The microwave saturation of the EPR lines of DOPA--melanin complexes with netilmicin depend on the drug concentration in the polymer. The changes of amplitude (A) of EPR spectra of DOPA-melanin-netilmicin complexes, for the concentrations of netilmicin: 1×10^{-4} M, 5×10^{-4} M, 5×10^{-3} M and 1×10^{-2} M, with microwave power (M) at a temperature of 125 K are compared in Fig. 7a. Similar correlations at temperature 275 K are compared in Fig. 7b.

The EPR lines of DOPA-melanin-netilmicin complexes with the used drug concentrations saturate at similar microwave powers (Fig. 7). This effect was observed at all the measured temperatures. It can be then concluded that spin-lattice relaxation processes in DOPA-melanin--netilmicin complexes reveal the same acceleration, independently of the drug content in melanin polymer.

The performed electron paramagnetic resonance (EPR) examination of DOPA-melanin complexes with netilmicin of different drug concentrations confirmed the role of free radicals of melanin in the formation of melanin-netilmicin complexes. The effect of the measured temperature on microwave saturation of EPR lines of DOPA-melanin complexes with netilmicin was brought to light. The dependence of this effect on netilmicin concentration in the melanin samples was discussed.

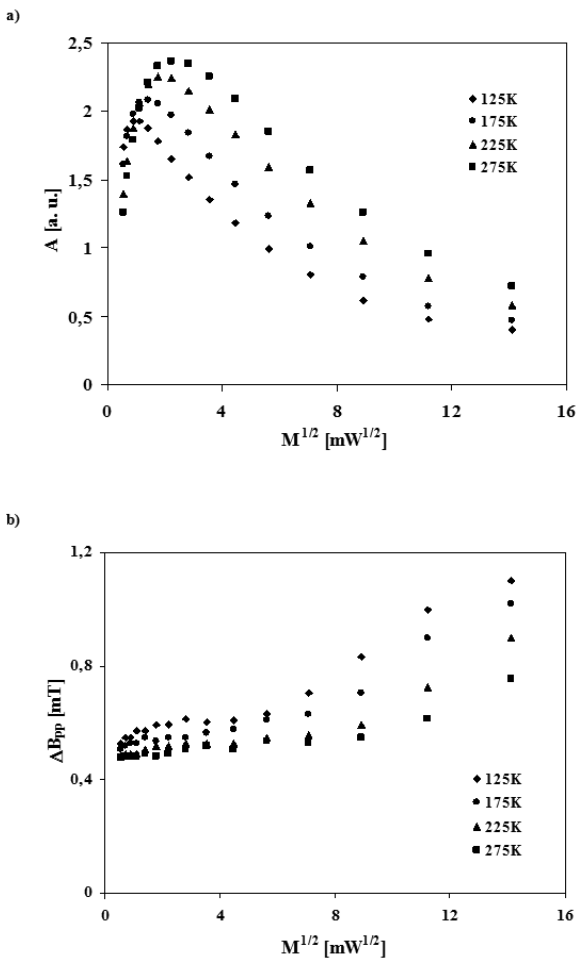

Fig. 5. Influence of microwave power (M) on (a) amplitudes (A) and (b) linewidths (ΔB_{pp}) of EPR spectra of DOPA--melanin-netilmicin complexes at temperatures 125, 175, 225 and 275 K. The concentration of netilmicin was 5×10^{-3} M.

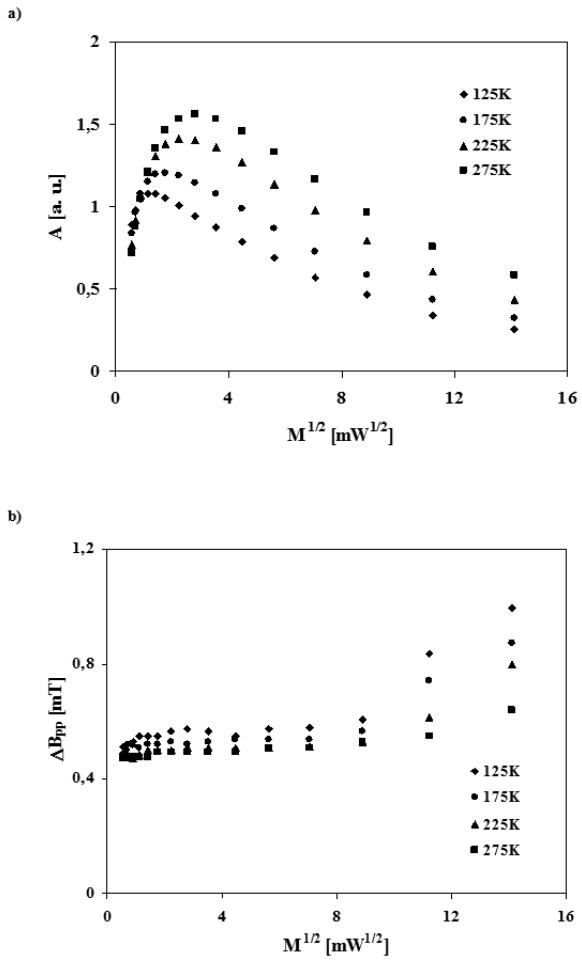

Fig. 6. Influence of microwave power (M) on (a) amplitudes (A) and (b) linewidths (ΔB_{pp}) of EPR spectra of DOPA--melanin-netilmicin complexes at temperatures 125, 175, 225 and 275 K. The concentration of netilmicin was 1×10^{-2} M.

Conclusions

EPR studies of DOPA-melanin complexes with netilmicin pointed out that:

1. Slow spin-lattice relaxation processes exist in DOPA--melanin complexes with netilmicin independently of drug concentration.
2. The faster spin-lattice relaxation processes exist in DOPA-melanin-netilmicin complexes at higher temperatures.
3. The EPR lines of DOPA-melanin-netilmicin complexes with different drug concentrations saturate at a similar microwave power at each of the tested temperatures. Acceleration of spin-lattice relaxation

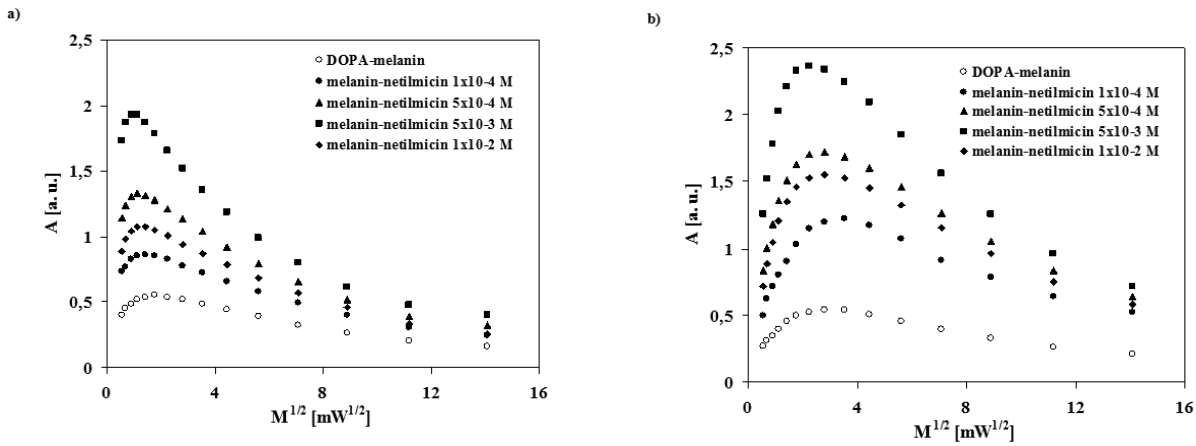

Fig. 7. Comparison of the effect of netilmicin concentrations on changes of amplitude (A) of EPR spectra of DOPA-melanin--netilmicin complexes with microwave power (M) at temperatures (a) 125 K and (b) 275 K. The concentrations of netilmicin were 1×10^{-4} M, 5×10^{-4} M, 5×10^{-3} M and 1×10^{-2} M.

does not depend on the netilmicin concentrations in melanin polymer.
4. The continuous microwave saturation indicates that the EPR lines of DOPA-melanin complexes with netilmicin are homogeneously broadened.

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