Chapter 5

Application of Electron Paramagnetic Resonance Spectroscopy to Examine Free Radicals in Melanin Polymers and the Human *Melanoma Malignum* Cells

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Additional information is available at the end of the chapter

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Abstract

The studies of free radicals in melanin and the human *melanoma malignum* cells by an X-band (9.3 GHz) electron paramagnetic resonance (EPR) spectroscopy were presented. The original results were compared with those published earlier. The aim of this work was application of the advanced spectral analysis to determine free radical properties in melanin biopolymers obtained from different melanotic tumor cells and free radicals existing in the human melanoma cells. Magnetic spin-lattice interactions in melanin samples were tested. The evolution of lineshape of tumor cells with increasing of microwave power was determined to confirm their complex free radical system. The useful shape parameters were proposed. The shape of melanotic tumor cells was analyzed. EPR spectra of free radicals in the melanin isolated from different tumor cells measured in the wide range of microwave power were analyzed. The melanins were obtained from the control tumor cells and the cells cultured with the several antitumor substances. The usefulness of the electron paramagnetic resonance spectroscopy was confirmed.

Keywords: melanin, human *melanoma malignum* cells, free radicals, paramagnetic centers, electron paramagnetic resonance spectroscopy

1. Introduction

Free radicals of natural melanin and the melanin in the human *melanoma malignum* cells were studied. Eumelanin and pheomelanin biopolymers are known [1–7]. Chemical structures of eumelanin and pheomelanin were shown in **Figure 1** [1]. Sulfur atoms exist in pheomelanin, but

they were not found in eumelanin [1]. Eumelanin mainly exists in the human organism [1, 2]. Melanin was found in skin [3, 4], hair [5, 6], eye [7], and liver cells [8].

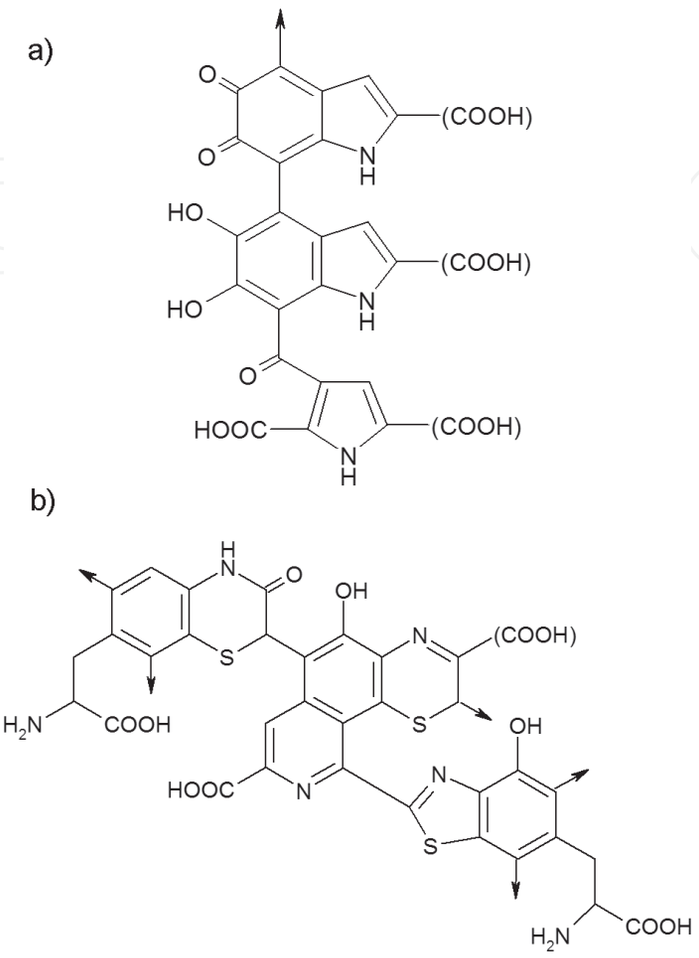

Figure 1. Chemical structure of eumelanin (a) and pheomelanin (b) [1].

Eumelanin was found in the melanotic tumor cells [9, 10]. Melanotic tumor cells are studied by NMR [11, 12], FTIR [13, 14], and HPLC [15, 16] methods. In this work we were interested in EPR studies of melanin from different tumor cells. Melanin polymers are known from paramagnetic character and o-semiquinone free radicals with spin S = 1/2 [17–40]. Unpaired electrons of free radicals obey the electron paramagnetic resonance (EPR) effect [17–40]. o-Semiquinone free radicals absorb microwaves in the magnetic field. This absorption is the base of electron paramagnetic resonance (EPR) spectroscopy [41–43]. Free radicals in eumelanin [17–19, 26, 40] and pheomelanin [22, 31, 38–40] are responsible for the EPR spectra, which differ in the shape. Typical EPR spectra of the model eumelanin DOPA-melanin and pheomelanin are shown in **Figure 2**. Comparison of the lineshape of these EPR spectra indicates that eumelanin reveals the simple single line (**Figure 2a**) and EPR line of pheomelanin reveals the complex shape with the unresolved hyperfine structure (**Figure 2b**). The lineshapes of the EPR spectra of DOPA-melanin [17–19, 26, 34–37] and pheomelanin [22, 31, 38–40] were presented in a lot of papers. EPR spectra were measured for free radicals in melanotic tumor cells [44–52].

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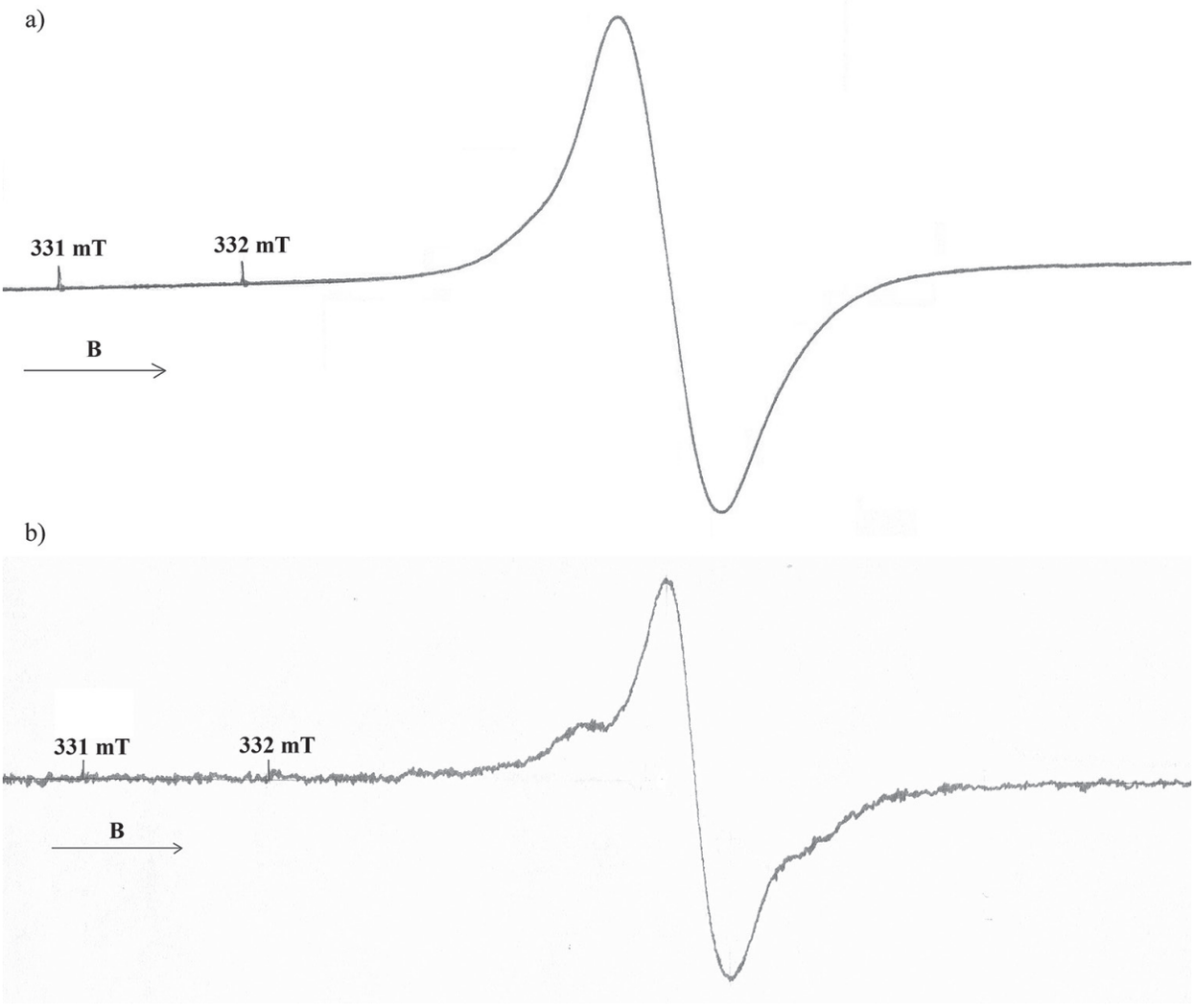

Figure 2. The EPR spectra of eumelanin (a) and pheomelanin (b). The measurements were done with the low microwave power with attenuation of 7 dB (microwave power of 14 mW). The melanin samples were studied in paper [40].

The drugs and substances with the antitumor interactions are still developed and searched [53, 54]. Melanin by free radicals interacts with drugs [55–57]. The effect of the antitumor substances on free radicals in the human tumor melanotic cells was shown by the use of the EPR spectroscopy [58–60].

The aim of this work was application of the advanced spectral analysis to determine free radical properties in melanin biopolymers obtained from the melanotic tumor cells and free radicals exiting in human melanoma cells. Free radicals in the original melanin samples and samples treated by the several antitumor substances were studied. The physical method of free radical detection based on paramagnetic character of melanins was used. EPR spectra of the tested natural melanins were compared with those of the model synthetic melanin polymers.

The innovatory lineshape analysis and the influence of microwave power on the complex EPR spectra were performed. The results were useful in medicinal therapy of the melanotic tumor cells. Both our published quantitative results [58–60] were cited, and the original spectral

unpublished results were presented. The novelty in the present work, relative to our earlier papers [58–60], was the proposition of the spectral parameters to examine of the multicomponent EPR spectra as the sum of lines resulted from different types of free radicals existing in the melanotic A-2058 cells. The changes of these parameters with increasing of microwave power for the EPR spectra of the control cells and the cells cultured with valproic acid (VPA), 5,7-dimethoxycoumarin (DMC), and both valproic acid and 5,7-dimethoxycoumarin were presented.

2. Experimental

2.1. The tested antitumor substances

The influence of the following substances on human *melanoma malignum* cells, valproic acid (VPA) ($C_8H_{16}O_2$), 5,7-dimethoxycoumarin (DMC), and both VPA and DMC, was examined. Chemical structures of the tested substances are shown in **Figure 3** [61]. VPA and DMC were used as the potential antitumor substances [61].

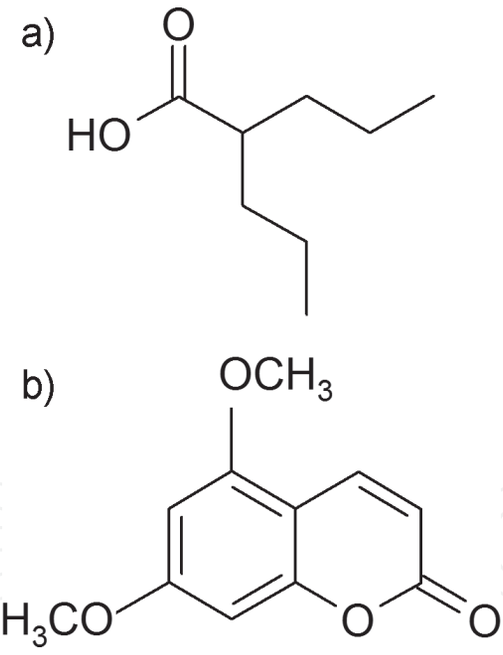

Figure 3. Chemical structures of valproic acid (VPA) (a) and 5,7-dimethoxycoumarin (DMC) (b) [61].

2.2. The tested human *melanoma malignum* cells

The three types of the human malignant melanoma cell lines, A-2058, A-375, and G-361, were used in this study. The cells were also cultured with the antitumor substances: valproic acid (VPA), 5,7-dimethoxycoumarin (DMC), and both VPA and DMC. In our EPR studies, the measurements were performed for the same number of cells.

The A-2058, A-375, and G-361 cells were obtained from LGC Promochem (Łomianki, Poland). A-2058 cells and A-375 cells were grown in the Minimum Essential Medium Eagle (Sigma-Aldrich). G-361 cells were grown in McCoy's medium (Sigma-Aldrich). These media were supplemented by the following components: 10% fetal bovine serum (FBS, PAA), 100 U/ml penicillin (Sigma-Aldrich), 100 μg/mL streptomycin (Sigma-Aldrich), and 10 mM HEPES (Sigma-Aldrich). The cells were incubated at temperature 37°C with the use of 5% CO_2. The incubation details were described in [58, 60].

The human malignant melanoma cell lines were incubated with 1 mM VPA, 10 μM DMC, and their combination for 4 days (A-2058) or 7 days (A-375 and G-361). EPR spectra of free radicals in the A-2058 cells and in melanin isolated from A-375, and G-361 cells were analyzed.

2.3. Isolation of melanin biopolymers from the melanotic cells

Melanin was isolated from the human *melanoma malignum* cells: A-375 and G-361. The enzymatic isolation procedure was described in detail in papers [62, 63]. The cells were lysed by incubation with 1% Triton X-100 (Sigma-Aldrich) for 1 hour at room temperature. The melanin was obtained by centrifugation of the lysates of the control cells, and the cells were cultured with VPA, DMC, and both VPA and DMC. The concentrations of VPA and DMC were 1 mM and 10 μM, respectively. The remaining pellets were washed with phosphate buffer, resuspended in Tris-HCl buffer (50 mM, pH 7.4), and incubated for 3 h at temperature 37°C. This Tris-HCl buffer contained sodium dodecyl sulfate (5 mg/ml) and proteinase K (0.33 mg/ml, Sigma-Aldrich). Melanin as the insoluble pigments was successively washed with 0.9% NaCl, methanol, and hexane, dried to a constant weight at temperature 37°C, and stored in a glass desiccator over P_2O_5.

2.4. The model eumelanin

The model eumelanin as DOPA-melanin was obtained by tyrosinase-catalyzed oxidation of 3,4-dihydroxyphenylalanine. The precursor (3,4-dihydroxyphenylalanine) was obtained from Sigma-Aldrich firm. The precursor was dissolved in 50 mM sodium phosphate buffer (pH 6.8). The final concentration was 2 mM. The reaction mixture after addition of tyrosinase (100 U/ml) was incubated for 48°C at temperature of 37°C. DOPA-melanin was obtained from the mixture by centrifugation (5000 × g, 15 min). The samples were washed by deionized water. Tyrosine was removed from melanin sample by treatment with SDS and methanol and NaCl. Finally, the sample was rewashed with deionized water and dried to a constant weight at temperature 37°C. This procedure was described in detail in [59, 60].

2.5. EPR measurements

2.5.1. EPR detection system

Free radicals in melanin biopolymers existing in different types of tumor cells and model synthetic melanin were examined by the use of electron paramagnetic resonance (EPR) spectroscopy. EPR spectra of melanin isolated from the cells and EPR spectra of the whole melanotic cells were tested. The first-derivative spectra were measured by an X-band (9.3 GHz) EPR spectrometer produced by Radiopan (Poznań, Poland) and the numerical data acquisition system—the Rapid Scan Unit of Jagmar (Kraków, Poland) (**Figure 4**).

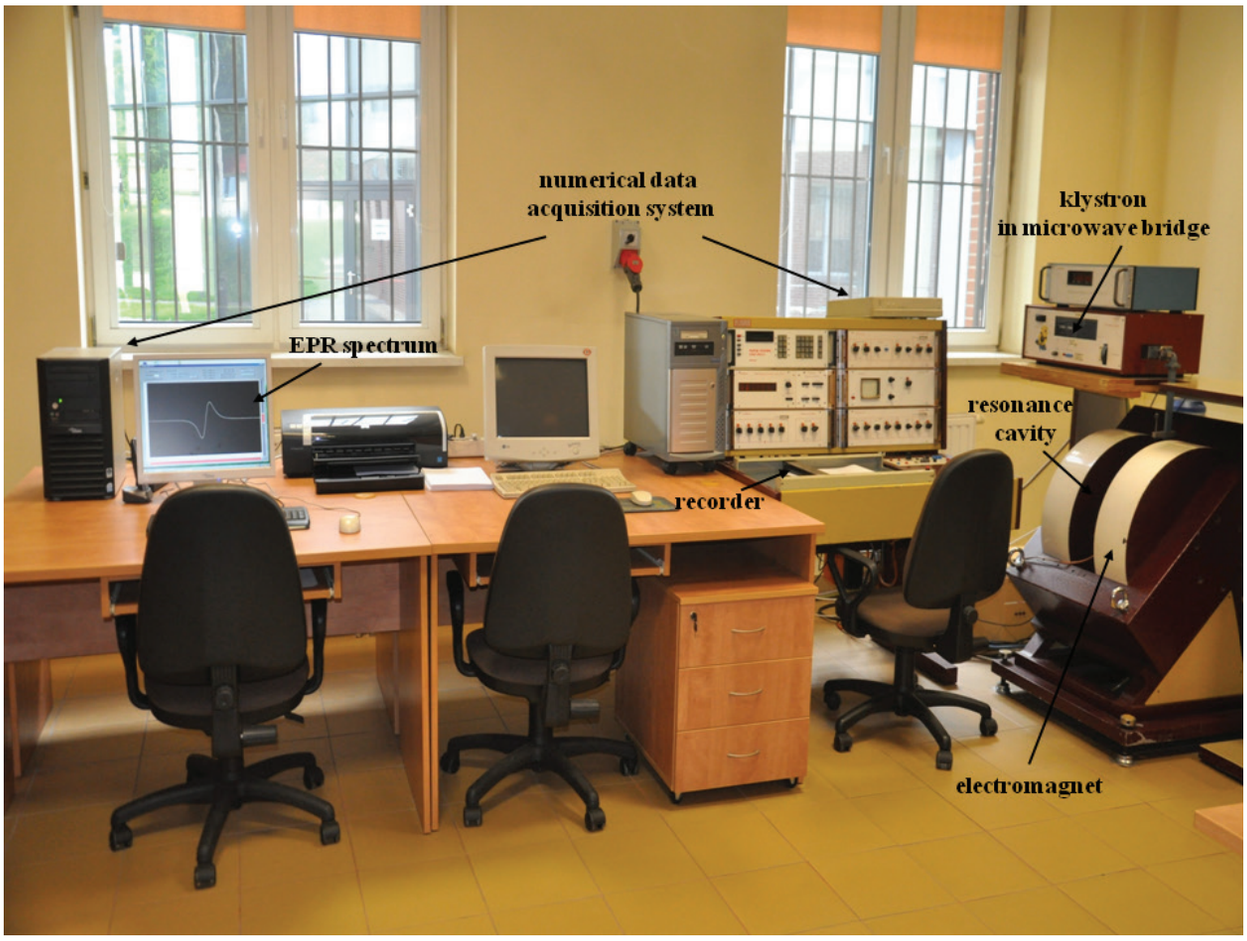

Figure 4. The X-band (9.3 GHz) electron paramagnetic resonance (EPR) spectrometer of Radiopan (Poznań, Poland) and the numerical data acquisition system—the Rapid Scan Unit of Jagmar (Kraków, Poland).

The cells or melanin samples in thin-walled glass tubes were located in the resonance cavity in magnetic field produced by electromagnet of the EPR spectrometer (**Figure 5**). In the magnetic field, the Zeeman splitting appeared [41, 42]. Free radicals absorb microwaves according to the electron paramagnetic resonance condition [41, 42]:

$$h\nu = \mu_B g B_r \tag{1}$$

where h, Planck constant; ν, microwave frequency; μ_B, Bohr magneton; g-factor; and B_r, resonance magnetic induction.

The absorption is proportional to the free radical concentrations in the samples. The detailed determination of the free radical concentrations in cells and melanin samples was described in [58–60].

For the measurements and spectral analysis, the professional spectroscopic programs of Jagmar (Kraków, Poland), LabVIEW 8.5 of National Instruments (USA) and Origin (USA) were used. The Silesian Medical University has the right to use these programs. The program to spectroscopic analysis was prepared by Jagmar firm specially to our EPR spectrometer. The other programs are widely available.

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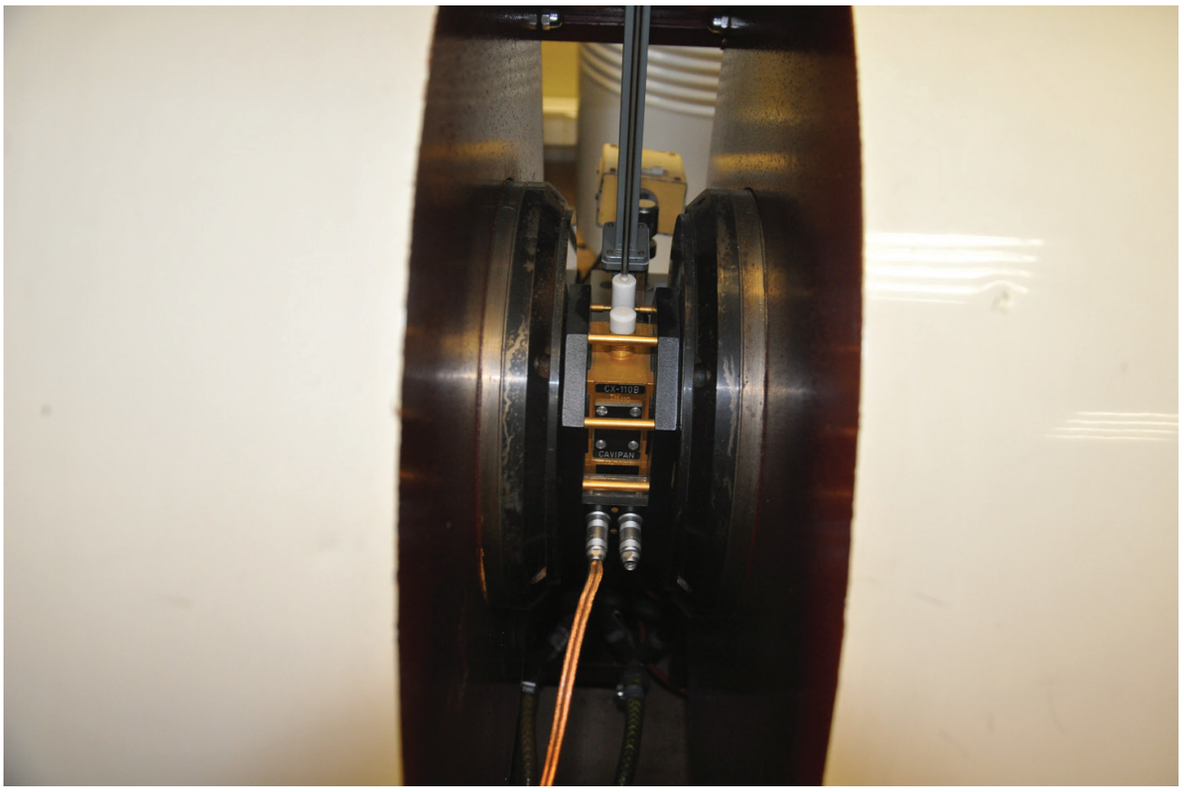

Figure 5. The resonance cavity of the X-band (9.3 GHz) EPR spectrometer of Radiopan (Poznań, Poland).

2.5.2. The parameters of the EPR measurements

The EPR spectra were measured with the magnetic modulation of 100 kHz. Microwave frequency (ν) from the X-band (9.3 GHz) was obtained by MCM 101 detector of EPRAD (Poznań, Poland). The magnetic induction (B) in the range 332–338 mT was measured by NMR magnetometer of EPRAD (Poznań, Poland).

The maximal microwave power produced by klystron in microwave bridge of the EPR spectrometer was 70 mW. The measurements of the EPR spectra were done in the range of microwave power from 2.2 mW (attenuation of 15 dB) to 70 mW (attenuation of 0 dB). The microwave power was regulated by attenuation according to the formula [41, 42]:

$$\text{attenuation (dB)} = 10 \log M_o/M \tag{2}$$

where M is the microwave power used for detection of the EPR spectrum and M_o is the maximal microwave power (70 mW).

2.5.3. Analysis of the EPR spectra

The influence of microwave power in the range of 2.2–70 mW on the lineshape parameters of the EPR spectra of the tested samples was determined. The model first-derivative EPR spectrum with the values, A_1, A_2, B_1, and B_2, was shown in **Figure 6**. The lineshape parameters were obtained as A_1/A_2, A_1-A_2, B_1/B_2, and B_1-B_2.

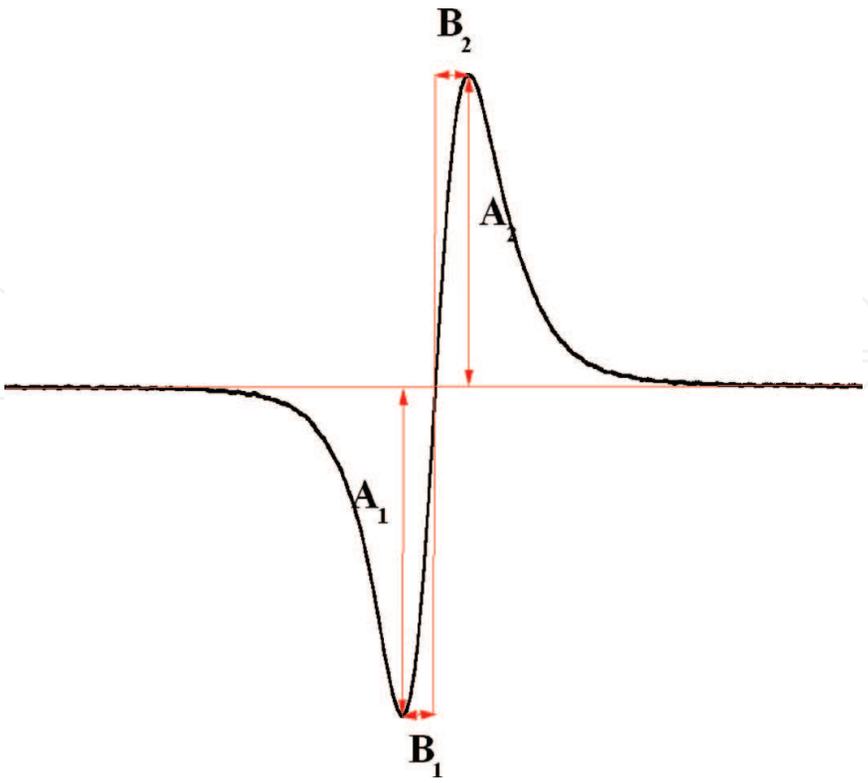

Figure 6. The exemplary model first-derivative EPR spectrum with the shape parameters: A_1, A_2, B_1, and B_2.

The evolution of the proposed lineshape parameter with increasing of microwave power gives information about complex free radical system in the biological samples.

The influence of microwave power on the integral intensities (*I*) of the EPR spectra was determined. Integral intensity (*I*) is proportional to the concentration of free radicals in the sample [41–43]. Integral intensity (*I*) of the EPR spectrum is the area under the absorption line [41–43]. Because the EPR spectra were measured as the first derivative of absorption, the spectral lines were double integrated to calculate the integral intensity. The first integration gives the absorption spectra. The second absorption gives the area under the absorption line.

The changes of integral intensity (*I*) of the EPR line with increasing of microwave power bring to light the spin-lattice interactions in the samples [41, 42]. Integral intensity (*I*) of the homogeneous broadened lines increased with increasing of microwave power, and after the reaching the maximal values, it decreased with the continuing increase of microwave power of the measurement [41, 42]. The faster spin-lattice relaxation caused microwave saturation of the EPR line at the higher microwave powers [41, 42].

3. Results and discussion

3.1. EPR spectra of free radicals in the human *melanoma malignum* A-2058 cells

Free radicals with the strong EPR lines of g-factor near 2 were found in A-2058 human melanoma cells [58]. The EPR spectra of the A-2058 cells recorded with the attenuation of micro-

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wave power of 7 dB were presented in **Figure 7**. The other spectra of these samples were presented in paper [58]. The EPR spectra are the broad nonsymmetrical lines (**Figure 7**). The broadening of the EPR lines of A-2058 cells is caused by dipolar interactions between free radicals. In this study we concentrated on the spin-lattice interactions in A-20058 cells and on their complex system of free radicals.

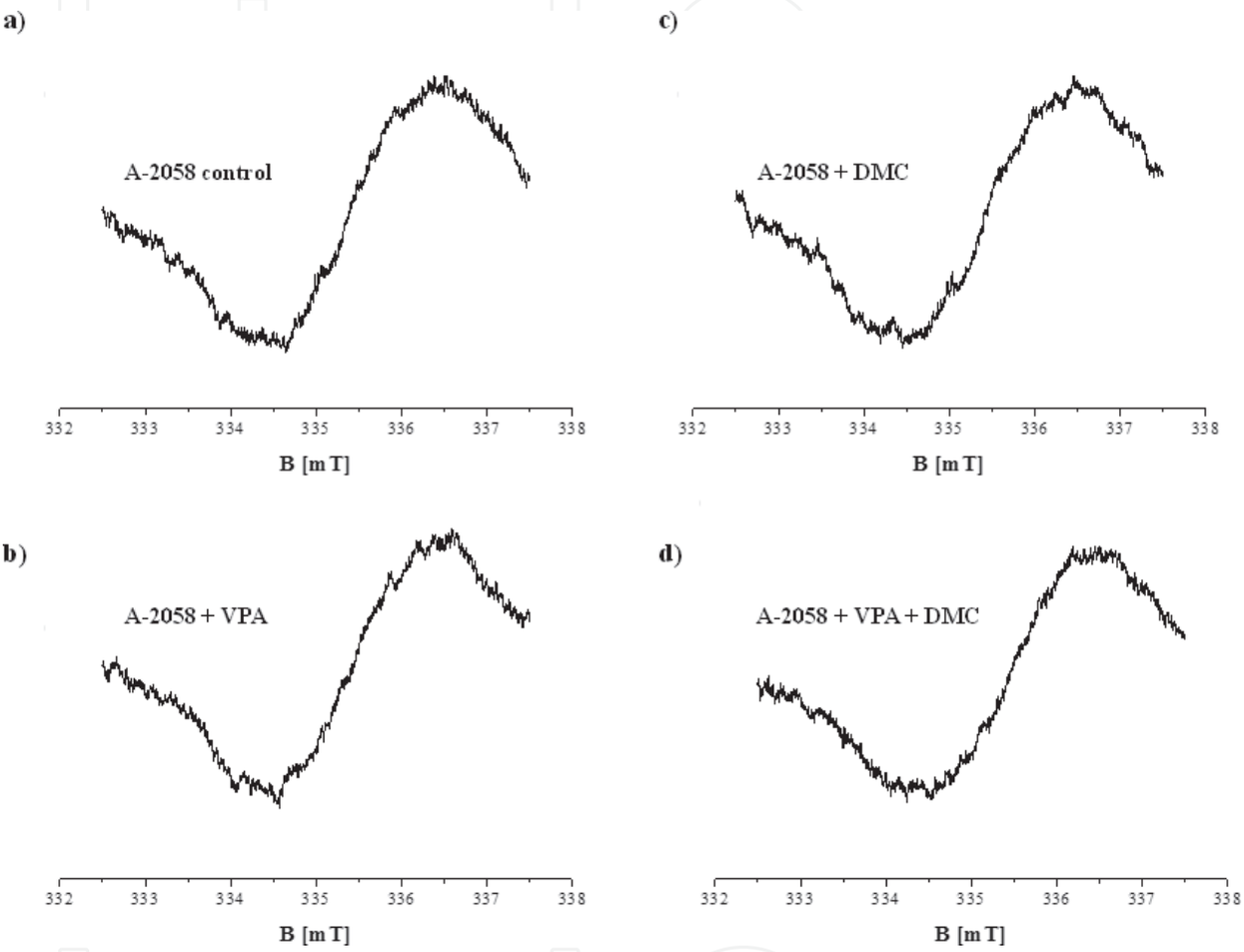

Figure 7. The EPR spectra of the human *melanoma malignum* control A-2058 cells (a) and the A-2058 cells cultured with VPA (b), DMC (c), and both VPA and DMC (d). The EPR spectra were measured with the attenuation of microwave power of 7 dB. *B*, magnetic induction.

The influence of the antitumor substances, VPA, DMC, and both VPA and DMC, on spin-lattice interactions in A-2058 human melanoma cells was determined. The influence of microwave power (M/M_o) on integral intensities (I) of the A-2058 cells for the control cells, and cells cultured with VPA, DMC, and both VPA and DMC, is compared in **Figure 8**.

Integral intensities (I) of the control A-2058 cells, the A-2058 cells cultured with VPA, and the A-2058 cells cultured with DMC increased with increasing microwave power (M/M_o). VPA and DMC did not change the character of changes of integral intensities (I) of A-2058 cells with microwave power (**Figure 8**). The absence of microwave saturation of the EPR lines of the control A-2058 cells, the A-2058 cells treated by VPA or treated by DMC in the microwave power up to 70 mW, indicated the fast spin-lattice relaxation processes existed in these cells.

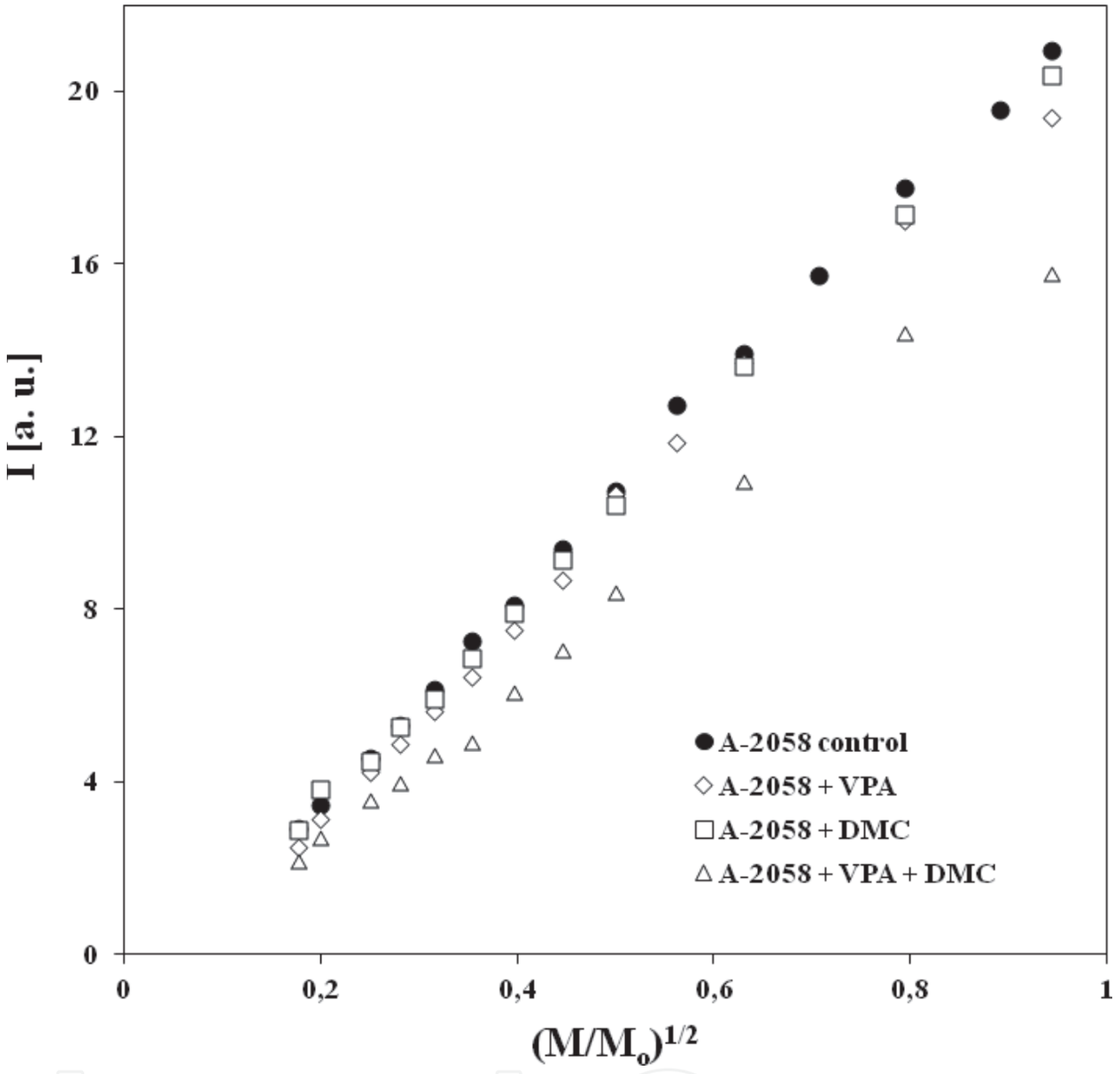

Figure 8. The influence of microwave power (M/M_o) on integral intensities (I) of the EPR spectra of the human *melanoma malignum* control A-2058 cells (●) and the A-2058 cells cultured with VPA (◊), DMC (□), and both VPA and DMC (Δ). M is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

The other situation was observed for the human malignant melanoma cell line A-2058 cultured with both VPA and DMC. The integral intensity (I) of the EPR lines of A-2058 cells treated with VPA and DMC together increased with increasing of microwave power (M/M_o) and it started saturating (**Figure 8**). The decrease of the integral intensity (I) was not observed, but the approaching to the maximum was visible (**Figure 8**). It means that the relatively slower spin-lattice relaxation processes existed in A-2058 cells cultured with both VPA and DMC, compared to the control cells, and the cells treated only with VPA or only with DMC. As one can see, the strongest effect on magnetic interactions in A-2058 cells was caused by the VPA and DMC used together in the cell culture.

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The lineshape of the EPR spectra of the control A-2058 cells, and the A-2058 cells cultured with VPA, DMC, and both VPA and DMC, changed with increasing of microwave power (M/M_o). The changes of the analyzed lineshape parameters, A_1/A_2, A_1-A_2, B_1/B_2, and B_1-B_2, for the control A-2058 cells, and A-2058 cells cultured with VPA, DMC, and both VPA and DMC, with the increasing microwave power (M/M_o), were presented in **Figures 9–12**, respectively.

All the tested lineshape parameters (A_1/A_2, A_1-A_2, B_1/B_2, and B_1-B_2) for the control A-2058 cells and for the A-2058 cells cultured with the antitumor substances (VPA, DMC, and both VPA and DMC) were not constant, and their changes with microwave power were observed (**Figures 9–12**). The strongest changes of the parameters A_1-A_2 (**Figure 10**) and B_1-B_2 (**Figure 12**) were obtained. The changes of the spectral shape parameters with microwave power were not regular (**Figures 9–12**). These nonregular changes of the spectral shape parameters with microwave power confirmed the existence of several types of free radical in the tested A-2058 cells, both in the control cells and in the cells treated with the used antitumor substances.

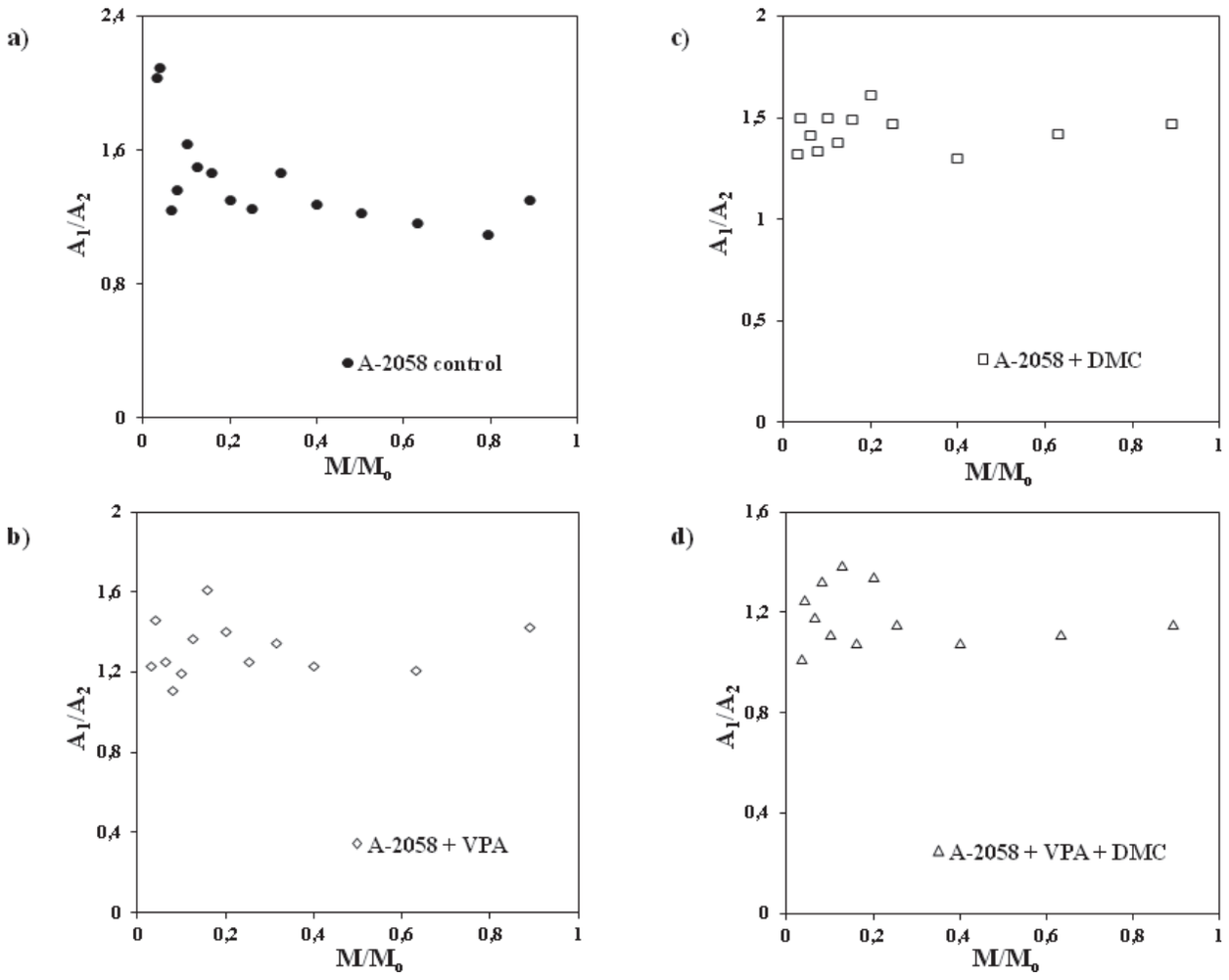

Figure 9. The influence of microwave power (M/M_o) on the lineshape parameter A_1/A_2, for the control A-2058 cells (●) (a) and A-2058 cells cultured with VPA (◊) (b), DMC (□) (c), and both VPA and DMC (Δ) (d). M is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

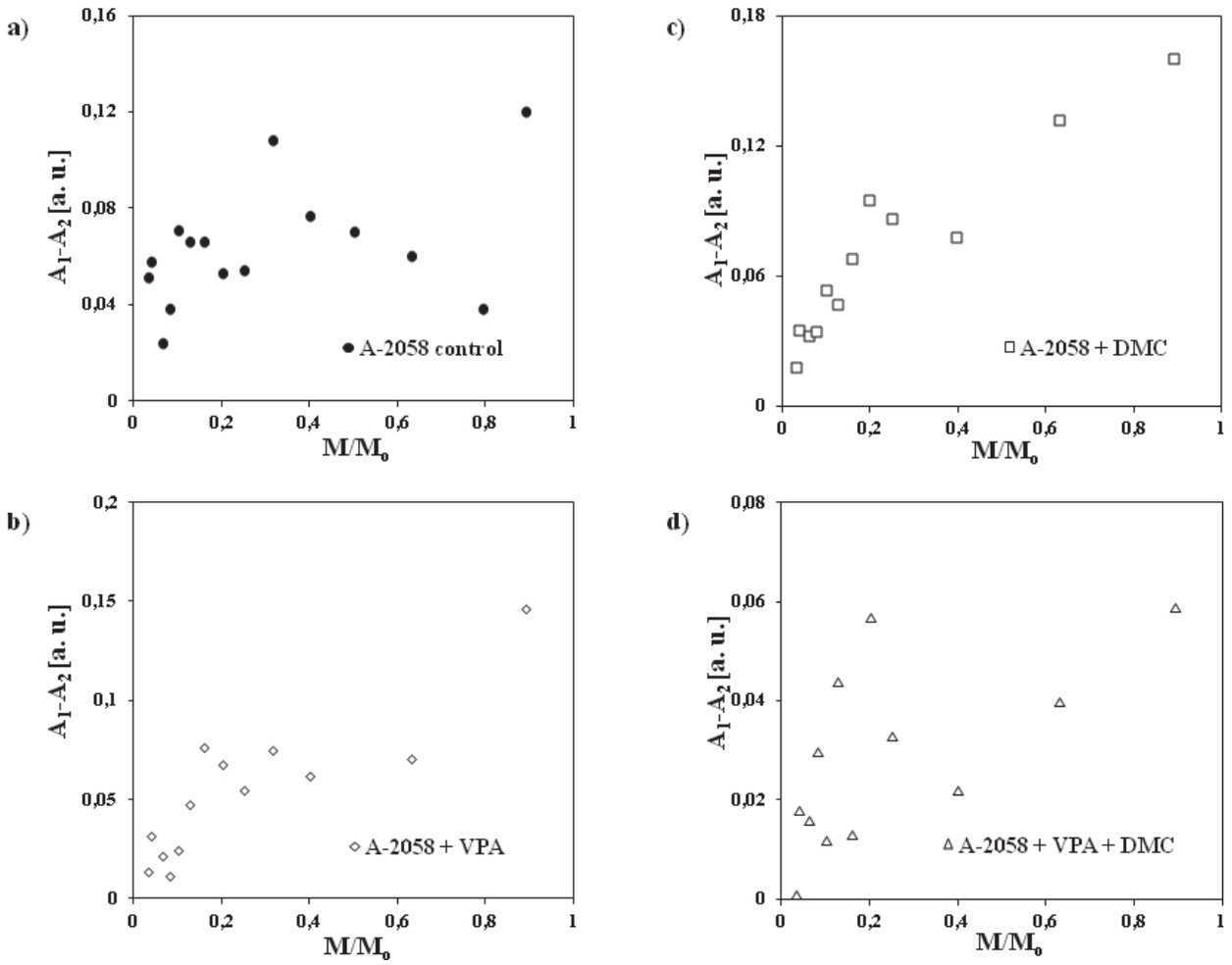

Figure 10. The influence of microwave power (M/M_o) on the lineshape parameter A_1-A_2, for the control A-2058 cells (●) (a) and A-2058 cells cultured with VPA (◊) (b), DMC (□) (c), and both VPA and DMC (Δ) (d). *M* is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

We proposed these shape parameters, A_1/A_2, A_1-A_2, B_1/B_2, and B_1-B_2, for checking the multi-component type of free radical in cells. They supported in the analysis of complex free radicals in the other paramagnetic samples, for example, for drugs [64, 65]. The EPR spectra of the cells were superposition of several lines resulted from the individual groups of free radicals. The microwave power differently influenced these EPR components, dependent on the type of free radicals. Amplitudes (A), linewidths (ΔB_{pp}), and integral intensities (*I*) of each component lines changed differently with microwave power. The component EPR lines saturated at different microwave powers. All these facts resulted in the summary effects of nonregular changes of shape parameters with microwave power used during the measurements of the EPR spectra of A-2058 cells. The existence of several groups of free radicals in A-2058 cells was expected. The o-semiquinone free radicals, biradicals, and free radicals formed, for example, by UV irradiation of the cells, may exist in the A-2058 cells. The studies of the complex free radicals system in tumor cells with application of the spectral shape analysis in the broad range of microwave power will be continued. The numerical analysis of the components will be performed.

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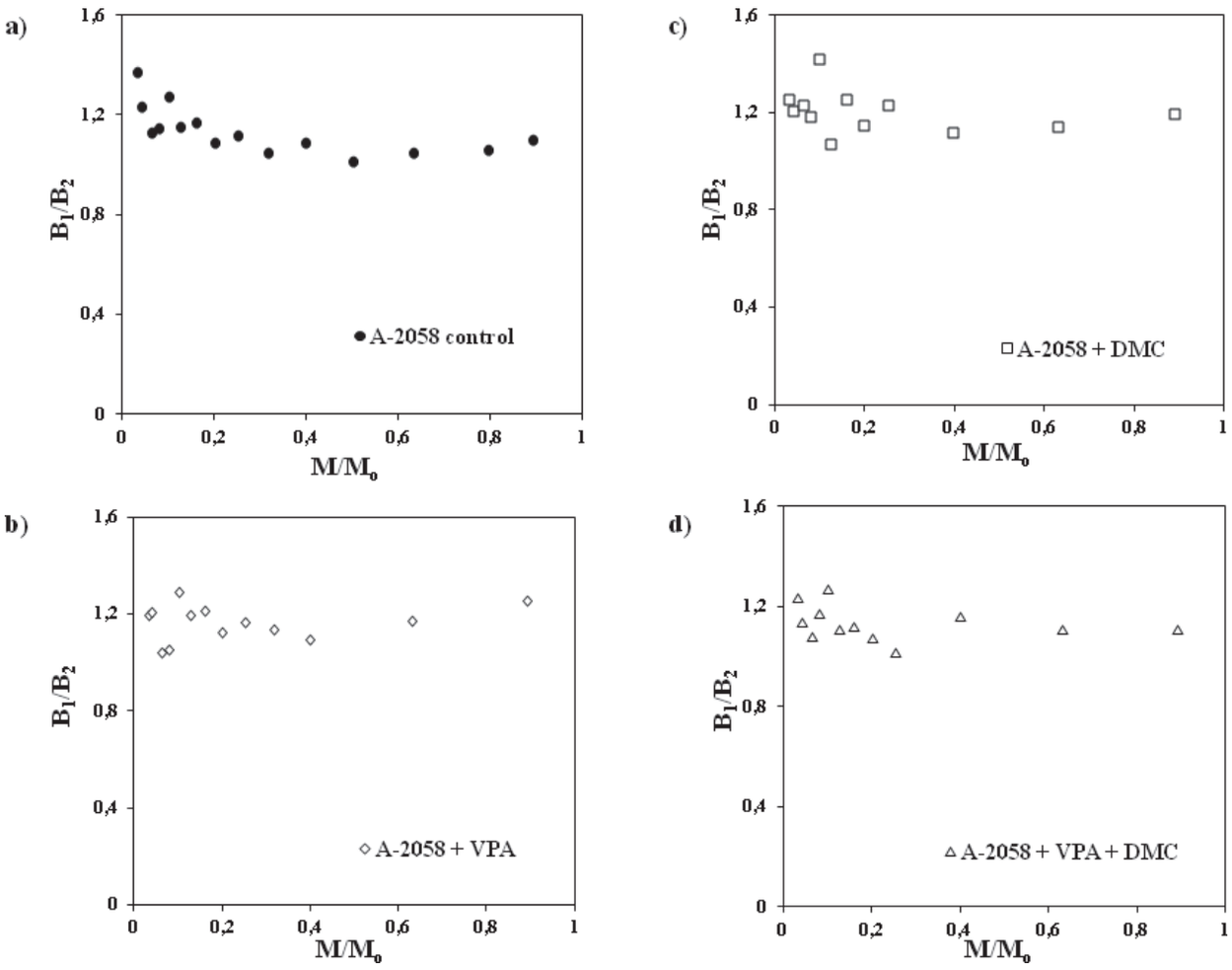

Figure 11. The influence of microwave power (M/M_o) on the lineshape parameter B_1/B_2, for the control A-2058 cells (●) (a) and A-2058 cells cultured with VPA (◊) (b), DMC (□) (c), and both VPA and DMC (Δ) (d). *M* is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

Besides the shape analysis proposed in this work, the important qualitative results for free radicals in the human *melanoma malignum* A-2058 cells were obtained by us earlier [58]. It was pointed out that treatment by VPA, DMC, and both VPA and DMC decreased free radical concentration in A-2058 cells [58]. This effect was the strongest for VPA used together with DMC, so these substances were proposed as the antitumor drugs [58]. The used in the present work spectral parameter - integral intensity (*I*) - was more precise than the amplitude (*A*) [58] for examination of spin-lattice relaxation processes in A-2058 human melanoma cells.

3.2. EPR spectra of free radicals in melanin isolated from human *melanoma malignum* A-375 cells

Free radicals were also found in melanin biopolymer isolated from the control A-375 cells and the A-375 cells cultured with VPA, DMC, and both VPA and DMC. For all the melanin samples, EPR spectra were measured. The exemplary EPR spectra of melanin isolated from

A-375 cells cultured with VPA and DMC, recorded with microwave power attenuation of 7 dB, were shown in **Figure 13**. The other EPR spectra of melanin originated from A-375 cells were shown in [60].

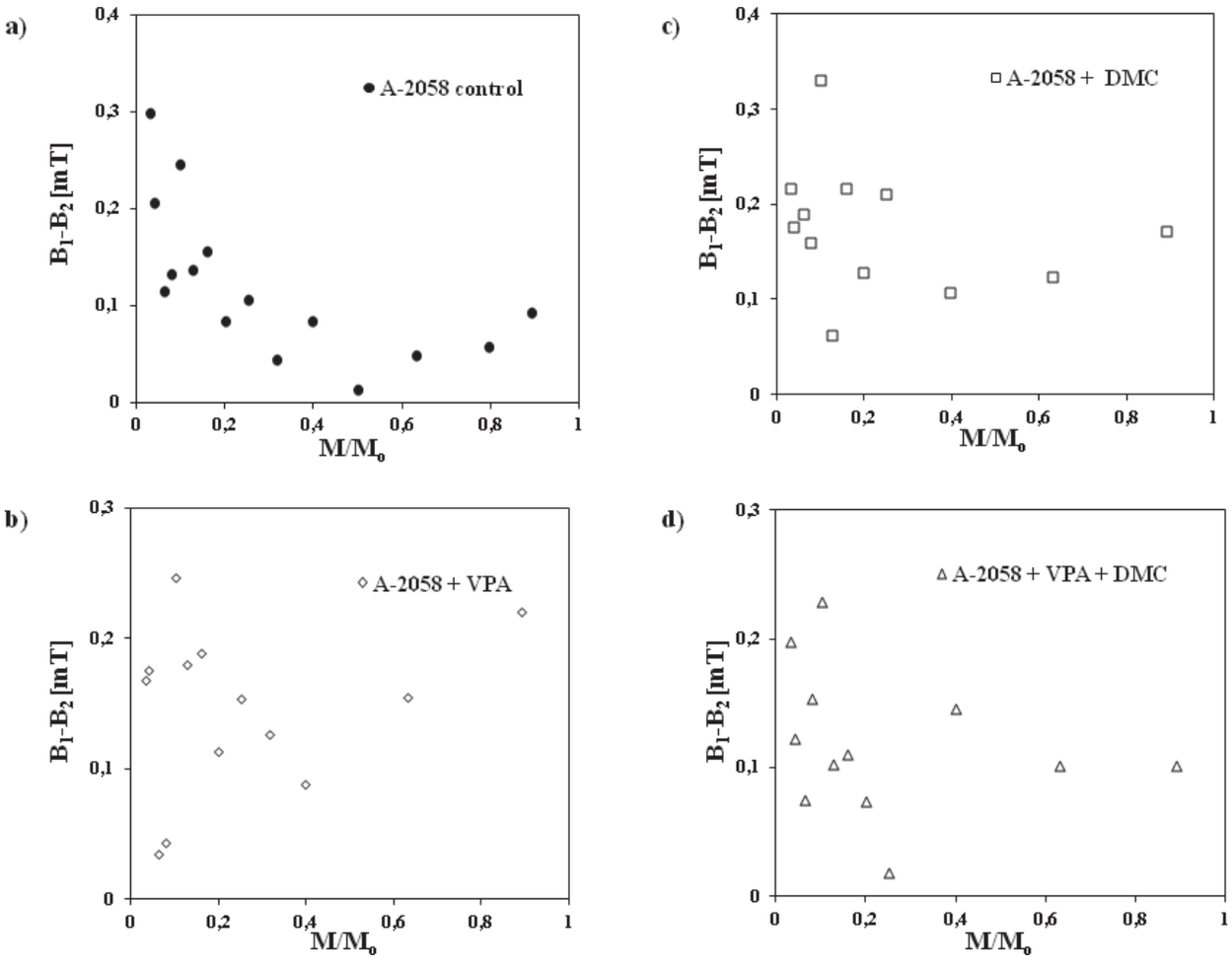

Figure 12. The influence of microwave power (M/M_o) on the lineshape parameter B_1–B_2, for the control A-2058 cells (●) (a) and A-2058 cells cultured with VPA (◊) (b), DMC (□) (c), and both VPA and DMC (Δ) (d). *M* is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

The parameters of the EPR spectra of the melanin obtained from A-375 cells changed with microwave power. In **Figure 14**, the influence of microwave power on integral intensities (*I*) of the melanin obtained from A-375 cells cultured with VPA, DMC, and both VPA and DMC was compared. The changes of the integral intensities (*I*) of the melanin isolated from the control A-375 cells and the other A-375 cell culture with VPA, with increasing of microwave power, were published in our earlier paper [59].

The integral intensities (*I*) of the EPR lines of melanin isolated from A-375 cells treated with VPA increased with increasing of microwave power (M/M_o) reached the maximum and started to saturate (**Figure 14**). The EPR lines of melanin isolated from the control A-375 cells saturated at the low microwave power [59]. Comparing the results for EPR lines of melanin from

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the control A-375 cells [59] and from the A-375 cells cultured with VPA (**Figure 14**), it may be concluded that the faster spin-lattice relaxation processes existed in melanin from the A-375 cells treated by VPA. Such effect was not observed for the melanin isolated from A-375 cells cultured with DMC. EPR lines of melanin from A-375 cells treated with DMC (**Figure 14**) saturated at similar microwave power than the lines of melanin from the control A-375 cells [59]. The EPR lines of melanin obtained from A-375 cells treated by both VPA and DMC (**Figure 14**) saturated at the lower microwave power than the EPR lines of the melanin isolated from control cells [59]. The slower spin-lattice relaxation processes existed in melanin from A-375 cells cultured with both VPA and DMC than the EPR lines of the melanin from the control cells.

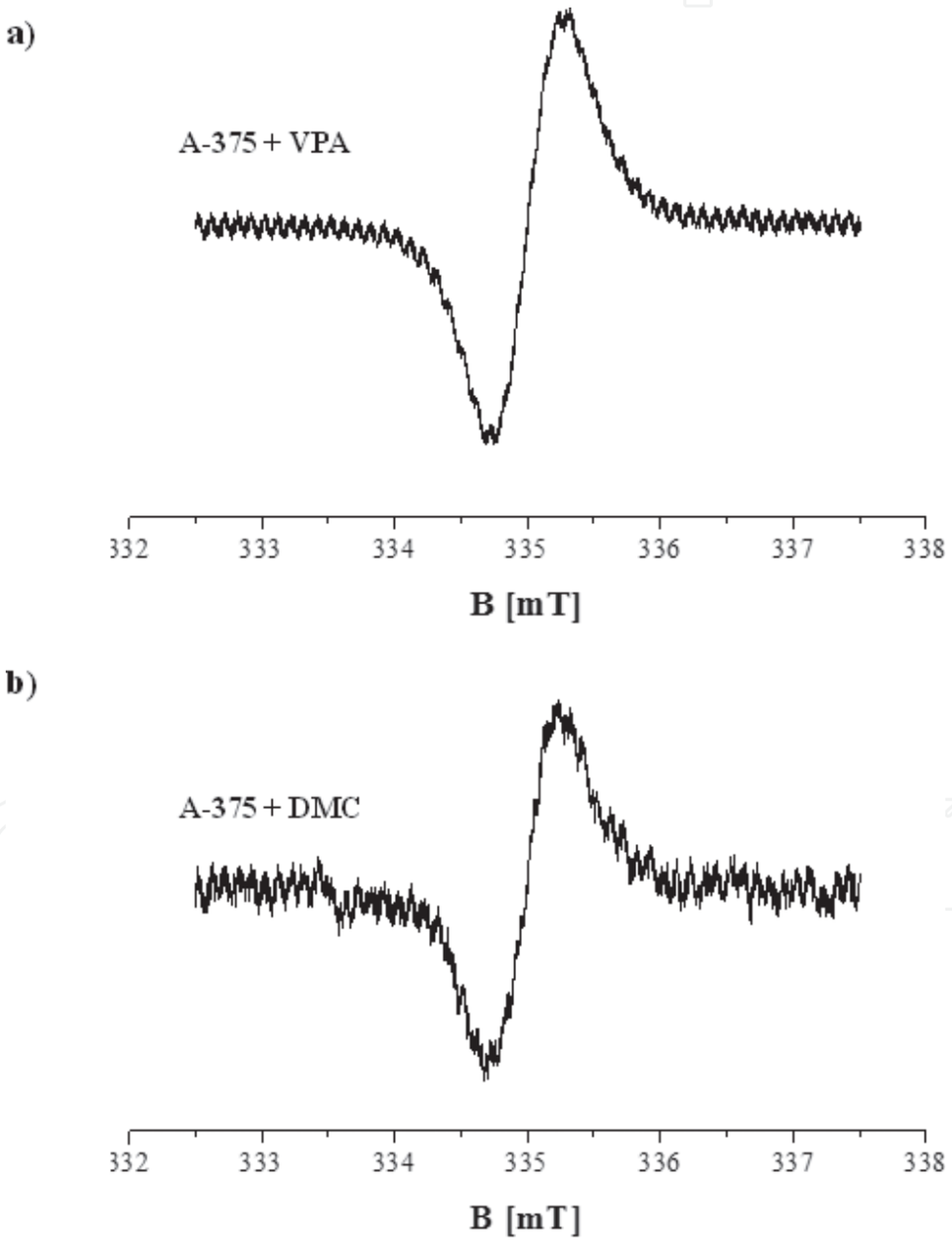

Figure 13. The EPR spectra of melanin isolated from the human *melanoma malignum* A-375 cells cultured with VPA (a) and DMC (b). The EPR spectra were measured with the attenuation of microwave power of 7 dB. *B*, Magnetic induction.

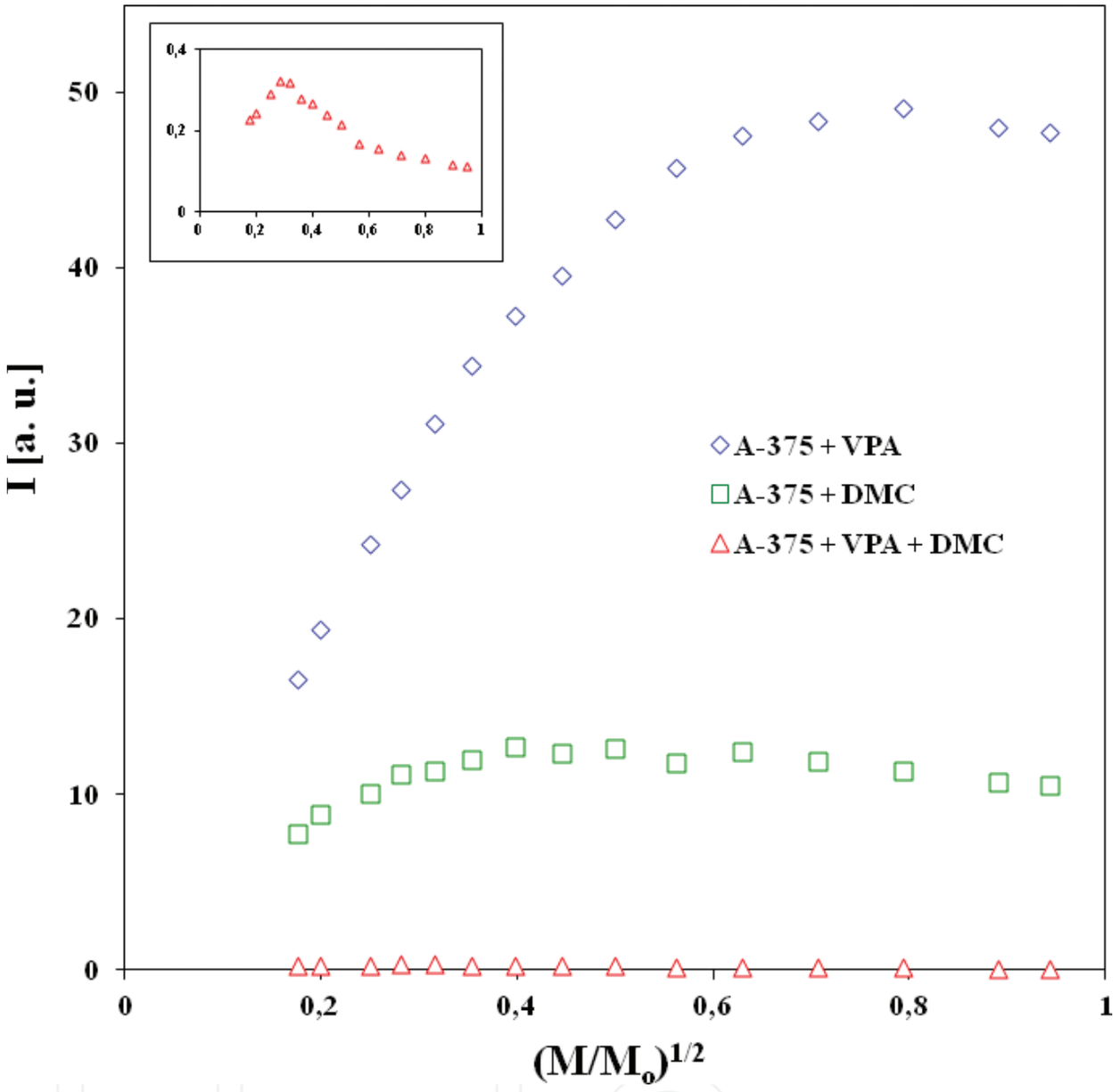

Figure 14. The influence of microwave power (M/M_o) on integral intensities (I) of the EPR spectra of melanin isolated from the human *melanoma malignum* A-375 cells cultured with VPA (◊), DMC (□), and both VPA and DMC (Δ). M is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

o-Semiquinone free radicals mainly existed in the melanin samples from A-375 cells. The quantitative results were published in the earlier paper [59, 60]. Considerable decrease of free radical concentration in melanin after treatment A-375 cells by both VPA and DMC was observed [60]. Free radical concentration in melanin isolated from A-375 cells cultured with DMC was lower than in melanin from the cells cultured with VPA [60]. The changes of amplitudes (A) and linewidths (ΔB_{pp}) with microwave power indicated homogeneous broadening of the EPR lines of melanin isolated from A-375 cells [60].

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3.3. EPR spectra of free radicals in melanin isolated from human *melanoma malignum* G-361 cells

EPR lines of o-semiquinone free radicals were also measured for melanin isolated from G-361 human melanoma cells. The EPR spectra of melanin isolated from the control G-361 cells, and the G-361 cells treated with VPA, DMC, and both VPA and DMC, measured with microwave power attenuation of 7 dB, were shown in **Figure 15**. The other spectra of these melanin samples were presented in paper [60]. The high level of the noise was visible in these spectra (**Figure 15**), so the lower contents of free radicals were found in melanin from G-361 cells than from A-375 cells (**Figure 13**).

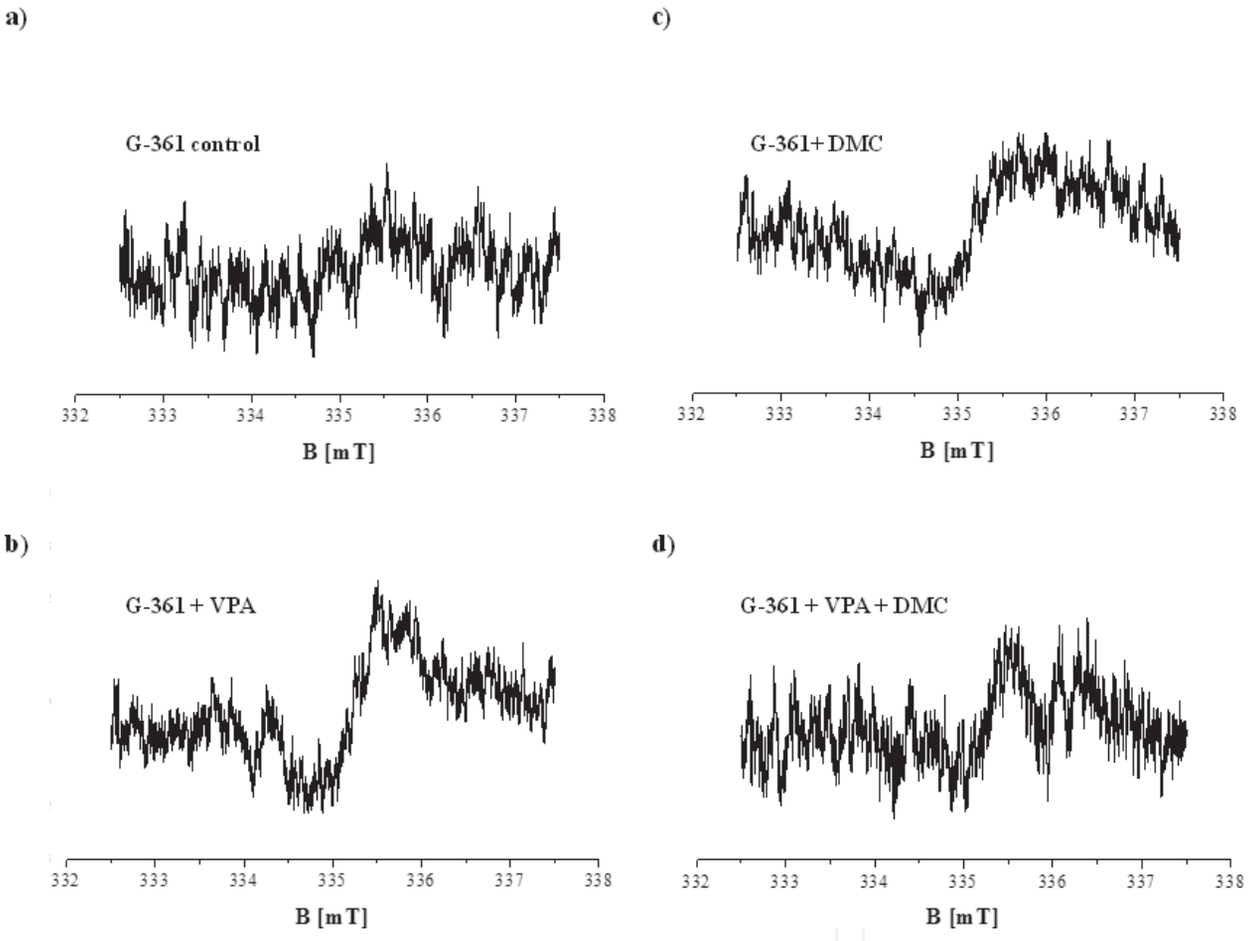

Figure 15. The EPR spectra of melanin isolated from the human *melanoma malignum* control G-361 cells (a), and the G-361 cells cultured with VPA (b), DMC (c), and both VPA and DMC (d). The EPR spectra were measured with the attenuation of microwave power of 7 dB. *B*, Magnetic induction.

The influence of the antitumor substances, VPA, DMC, and both VPA and DMC, on spin-lattice interactions in melanin obtained from G-361 human melanoma cells was not stated. The changes of integral intensities (*I*) of the melanin from G-361 cells for the control cells, and cells cultured with VPA, DMC, and both VPA and DMC, with increasing of microwave power

(M/M_o), were compared in **Figure 16**. The similar correlations between integral intensity (I) and microwave power for all the melanin samples were visible (**Figure 16**). The antitumor drugs did not change magnetic interactions in melanin structures of G-361 cells.

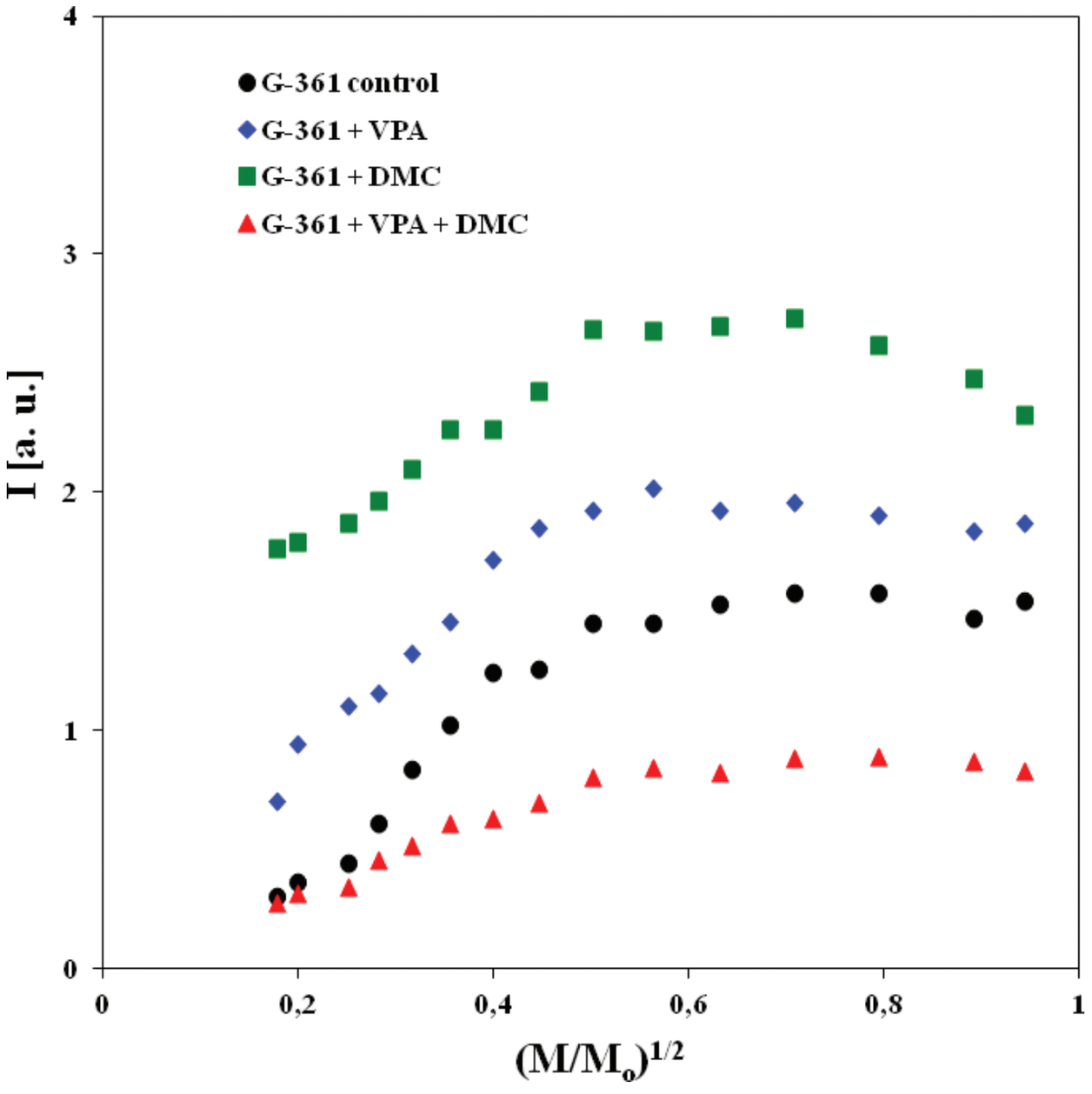

Figure 16. The influence of microwave power (M/M_o) on integral intensities (I) of the EPR spectra of melanin isolated from the human *melanoma malignum* control G-361 cells (●) and the G-361 cells cultured with VPA (◆), DMC (■), and both VPA and DMC (▲). M is the microwave power used during the measurement of the EPR spectrum, and M_o is the maximal microwave power produced by the klystron (70 mW).

The quantitative results of EPR examination of melanin originated from G-361 cells were described in paper [60]. It was obtained that after treating of G-361 cells with both VPA and DMC free radical concentration in melanin strongly decreased [60]. Free radical concentration

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in melanin isolated from G-361 cells cultured with DMC was higher than in melanin from the cells cultured with VPA [60]. The changes of amplitudes (A) and linewidths (ΔB_{pp}) with microwave power indicated homogeneous broadening of the EPR lines of melanin isolated from G-361 cells [60]. Our present spin-lattice relaxation studies by the use of integral intensities (I) dependence on microwave power confirmed the results obtained for melanin from G-361 cells from the amplitude (A) changes with microwave power [60].

4. Conclusions

The existence of o-semiquinone free radicals in melanin from the human *melanoma malignum* cells was confirmed. Free radicals of melanin were mainly responsible for the EPR lines of the tested tumor cells. The free radical concentrations depended on the type of tumor cells. The antitumor drugs changed the free radical concentrations. The changes depended on the drug amounts. The parameters and lineshape of the EPR spectra of melanin changed with increasing of the measuring microwave power. All the EPR lines of the tested melanins were very broad. The most of the spin-lattice relaxation processes in melanin samples characterized the long relaxation times, and their EPR lines saturated at the low microwave powers. The analysis of the lineshape of the EPR spectra measured in the wide range of microwave power was useful to obtain information about complex free radical system in the melanin biopolymers. The spectral EPR results may be applied in therapy of tumors contained melanin. The free radical concentrations in the tumors and the effect of the antitumor substances on their values may be obtained. The effective antitumor drugs as those which cause the decrease of free radical concentrations in the melanotic tumor cells may be spectroscopically found.

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