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EPR STUDIES OF THERMALLY STERILIZED *VASELINUM ALBUM* *

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Abstract: Electron paramagnetic resonance (EPR) spectroscopy was used for examination of free radicals in thermally treated *vaselinum album* (VA). Thermal treatment in hot air as sterilization process was tested. Conditions of thermal sterilization were chosen according to the pharmaceutical norms. *Vaselinum album* was heated at the following conditions (T – temperature, t – time): T = 160°C and t = 120 min, T = 170°C and t = 60 min and T = 180°C and t = 30 min. The aim of this work was to determine concentration and free radical properties of thermally sterilized VA. EPR analysis for VA was done 15 min after sterilization. EPR measurements were done at room temperature. EPR spectra were recorded in the range of microwave power of 2.2-70 mW. g-Factor, amplitudes (A) and line width (ΔB_{pp}) of the spectra were determined. The shape of the EPR spectra was analyzed. Free radical concentration (N) in the heated samples was determined. EPR spectra were not obtained for the non heated VA. EPR spectra were detected for all thermally sterilized samples. The spectra revealed complex character, their asymmetry depends on microwave power. The lowest free radicals concentration was found for the VA sterilized at 180°C during 30 min. EPR spectroscopy is proposed as the method useful for optimization of sterilization process of drugs.

Keywords: free radicals, thermal sterilization, *vaselinum album*, EPR spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy was used for examination of free radicals in thermally treated *vaselinum album* (VA). Thermal treatment in hot air as sterilization process was tested. Sterilization is expected to exterminate microorganisms in drugs (1), but chemical structure and therapeutic interactions of drugs with tissues should not be modified. Conditions of thermal sterilization are described in the pharmaceutical norms (2, 3). It is expected that thermal sterilization breaks chemical bonds and produce free radicals in drugs depending on temperatures. A lot of our works deals with formation of free radicals in organic pharmaceutical substances at high temperature (4-11). The aim of this work was to determine concentration and free radical properties of thermally sterilized VA. In the literature, information about free radicals in VA was not found. The EPR measurements are proposed to optimize sterilization process. The optimal temperature and time of heating of the VA are those that produce the lowest amount of free radicals in the sample.

Free radicals should not exist in VA, because they may cause a lot of toxic effects in tissues (12). Free radicals may interact with dia- and paramagnetic molecules in organism. These reactions may cause large modifications of structures and destroy living functions.

EXPERIMENTAL

Samples

VA is a greasy, translucent, odorless, low melting, white color substance (13-15). It is obtained from the distillation of crude oil (13-15). VA is a mixture of paraffinic hydrocarbons (alkanes) from the border of the solid and liquid state of matter at room temperature, docosane and tricozane (13-15). VA is used as a sterile base for prescribed antibiotics ointment and other biologically active substances and ophthalmic ointments (13-15).

VA was sterilized according to the Polish Pharmacopoeia recommendationss (2) at the following temperatures: 160°C (during 120 min), 170°C (during 60 min), and 180°C (during 30 min), respectively. Sterilization process was performed in a dryer with hot air.

The samples of VA were measured in the thin-wall glass tubes. EPR signals were not obtained for

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empty tubes, they do not contain paramagnetic impurities.

VA was obtained from Sigma-Aldrich.

EPR measurements

The electron paramagnetic resonance (EPR) measurements were performed 15 minutes, 2, 8, 10, 13, 16, 22, 32 and 40 days after sterilization. The samples were examined at room temperature. The first-derivative spectra were recorded by the use of an X-band (9.3 GHz) EPR spectrometer of Radiopan (Poznań). Magnetic modulation was 100 kHz. Microwave frequency was directly measured by MCM 101 recorder of Eprad (Poznań).

The first-derivative EPR spectra were measured with microwave power in the range 2.2-70 mW. g-Factor, amplitudes (A) and line width (ΔB_{pp}) of the EPR lines were analyzed (Fig. 1a). The parameters A_1/A_2 , B_1-B_2, B_1/B_2, and B_1-B_2 of asymmetry of line shape of EPR spectra were determined (Fig. 1b).

g-Factor was calculated from the resonance condition according to the formula (16):

$$g = h\nu/\mu_B B_r,$$

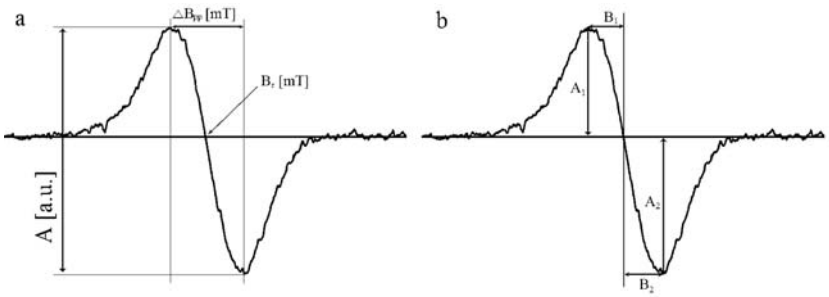

Figure 1. The analyzed parameters of the EPR spectra (a): amplitude (A), line width (ΔB_{pp}), B_r – resonance magnetic induction, and the asymmetry of line shape parameters (b): A_1, A_2, B_1, B_2

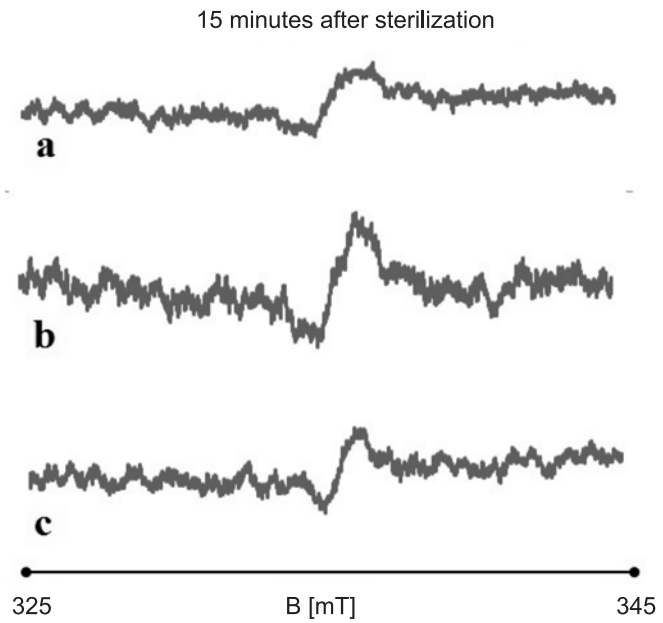

Figure 2. EPR spectra of *vaselinum album* sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min) (c). The measurement was done 15 min after sterilization with microwave power of 2.2 mW. B - induction of magnetic field

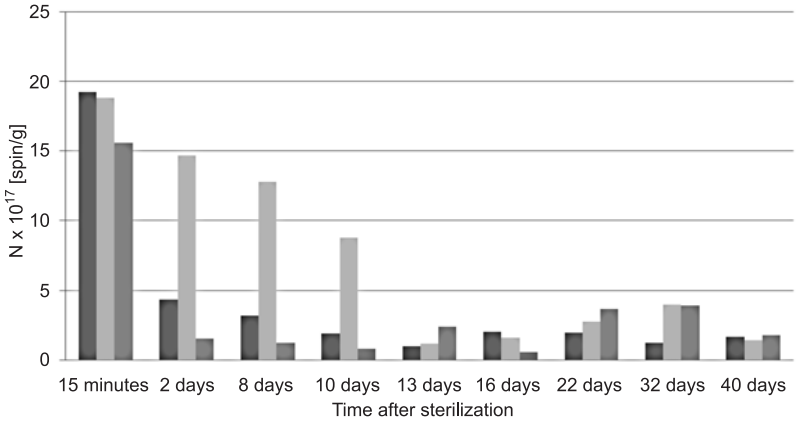

Figure 3. Change of free radical concentration (N) in the stored thermally sterilized *vaselinum album*

Table 1. Free radical concentration (N) in the sterilized *vaselinum album*, g factor and linewidth (ΔB_{pp}) of its EPR spectra. T – temperature of sterilization of *vaselinum album*, t – times of sterilization of *vaselinum album*.

Samples	Sterilization		$N \times 10^{17}$ [spin/g]	g [± 0.0002]	ΔB_{pp} [± 0.02 mT]
	T [^oC]	t [min]			
Vaselinum album	160	120	19.2	1.9948	2.16
	170	60	18.8	1.9953	1.69
	180	30	15.6	1.9949	2.64

where: h – Planck constant, ν – microwave frequency, μ_B – Bohr magneton, B_r – resonance magnetic induction.

Continuous microwave saturation of EPR lines was applied to examination of spin-lattice relaxation processes (16-18).

Free radical concentration (N) in the samples was determined as follows:

$$N = N_u[(W_uA_u)/I_u][I/(WAm)],$$

where: N_u - the number of paramagnetic centers in ultramarine, W, W_u - the receiver gains for sample and ultramarine, A, A_u - the amplitudes of ruby signal for the sample and ultramarine, I, I_u - the integral intensities for the sample and ultramarine, m - the mass of the sample.

Ultramarine with the strong stable EPR line was the reference for free radicals concentration in the samples. A ruby crystal was permanently placed in the resonance cavity, and it was used as the secondary reference during measurements of the concentration.

RESULTS AND DISCUSSION

EPR spectra were not measured for the original, non-heated samples and were obtained for all the thermally sterilized VA. The original samples are free of paramagnetic impurities. Free radicals were found in VA sterilized at 160^{o}C (120 min), 170^{o}C (60 min) and 180^{o}C (30 min). The EPR spectra of the sterilized VA are presented in Figure 2a-c. Free radicals are formed in VA as the result of thermolysis. Thermal energy breaks chemical bonds in the tested VA. It is expected that thermal transformation changes the therapeutic interactions of VA. The interactions of free radicals of VA and tissues may be the source of toxic effects. The EPR spectra changed with temperature of sterilization. The parameters of the EPR spectra of the samples sterilized at different temperatures and times are shown in Figure 2a-c.

Concentrations (N) of free radicals, g-factors, and line widths (ΔB_{pp}) of EPR spectra of VA sterilized at different conditions are presented in Table 1. The tested EPR spectra were broad lines with line widths in the range 1.69-2.64 mT. Dipolar interactions are probably responsible for such line broadening. The apparent g-factors 1.9948-1.9953 indicate that unpaired electrons in the heated VA are located on carbon atoms. The high free radical concentration (~10^{17} spin/g) characterized all the sam-

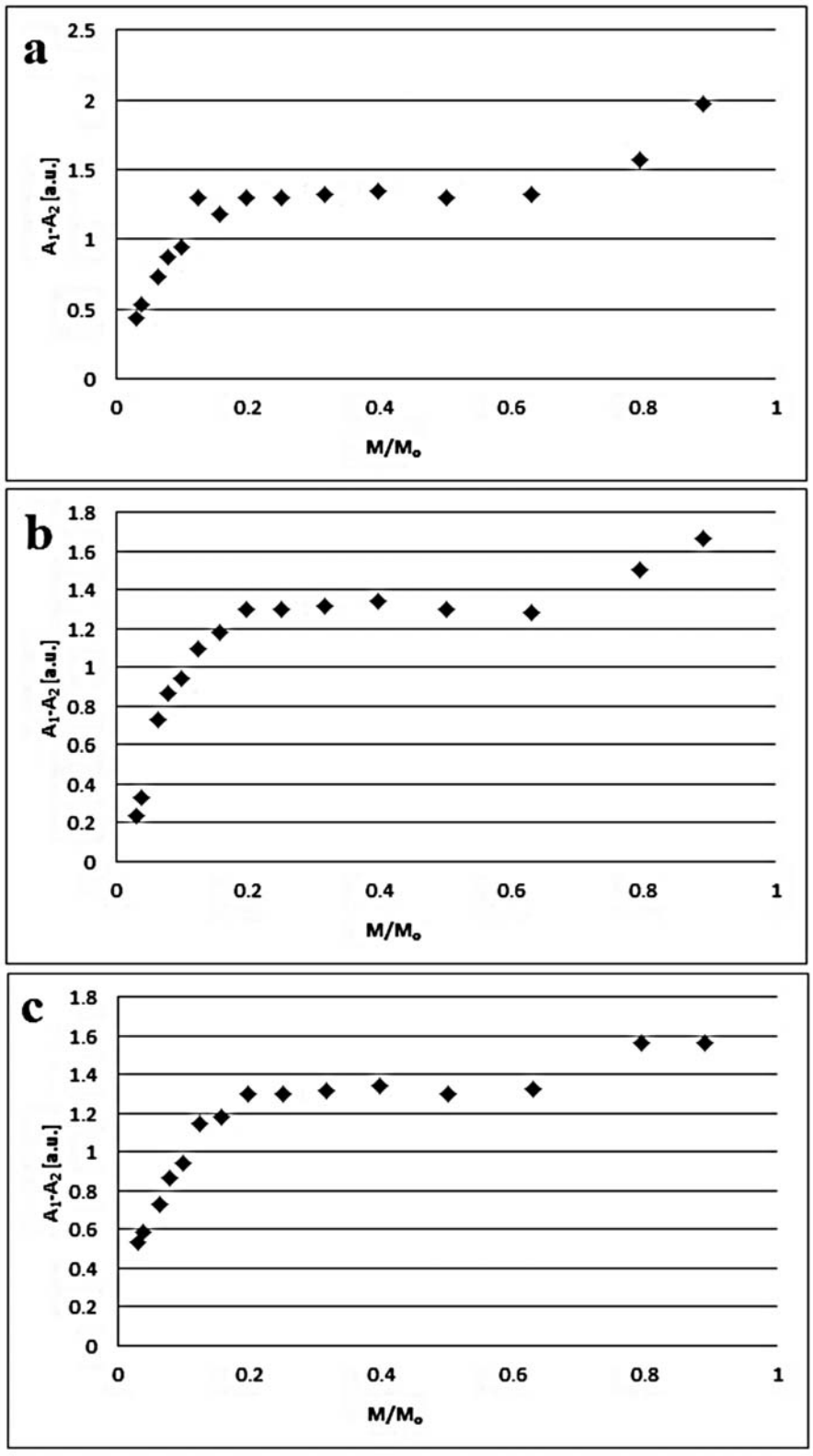

Figure 4. Influence of microwave power (M) on the asymmetry parameter (A_1-A_2) of EPR spectra of *vaselinum album* sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min) (c). The measurement was done 15 min after sterilization. M is microwave power used during the measure of the EPR spectrum. M_o is the total microwave power produced by klystron (70 mW)

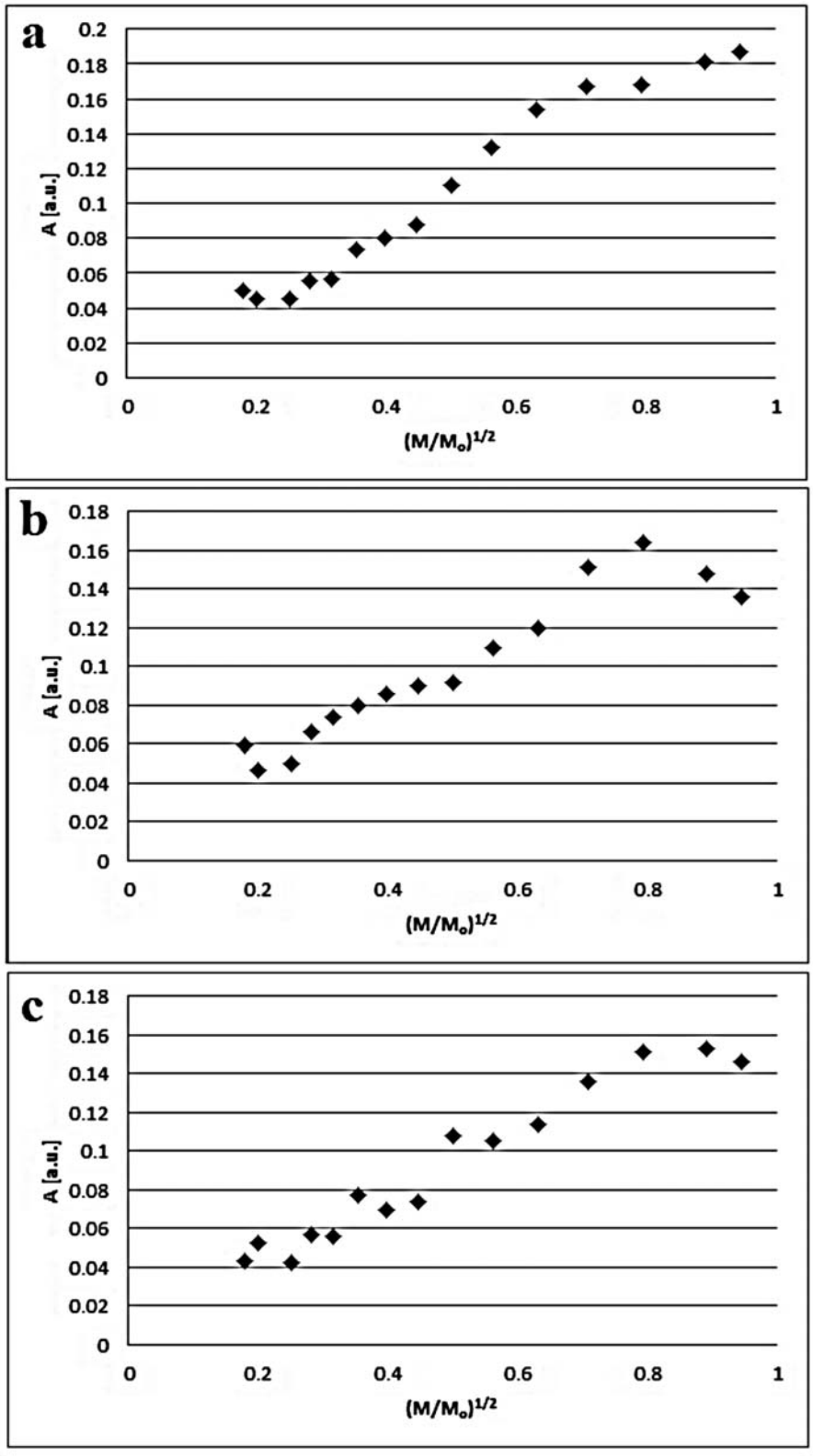

Figure 5. Influence of microwave power (M) on amplitude (A) of EPR spectra of *vaselinum album* sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min) (c). The measurement was done 15 min after sterilization. M is microwave power used during the measure of the EPR spectrum. M_o is the total microwave power produced by klystron (70 mW)

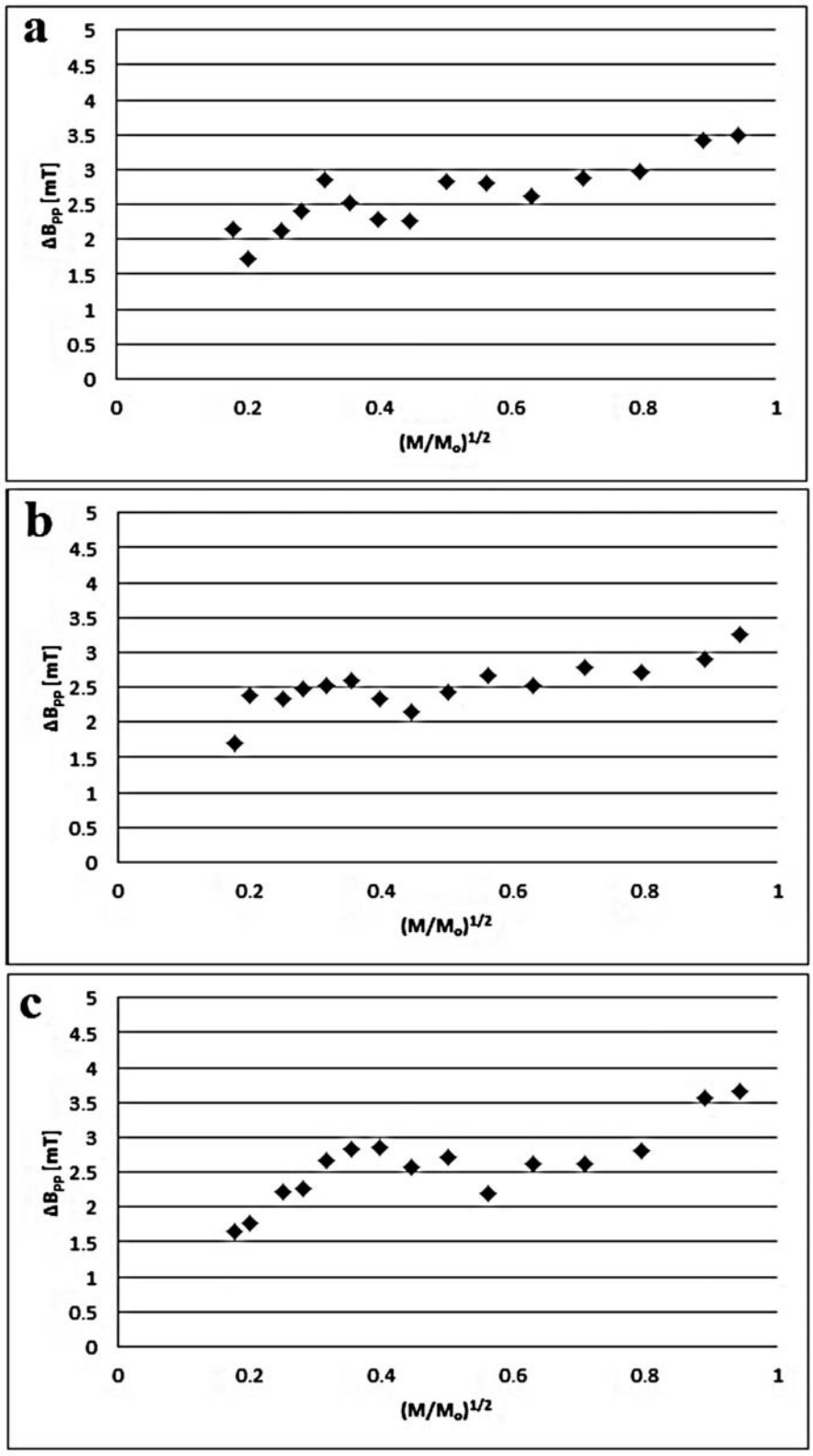

Figure 6. Influence of microwave power (M) on line width (ΔB_{pp}) of EPR spectra of *vaselinum album* sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min) (c). The measurement was done 15 min after sterilization. M is microwave power used during the measure of the EPR spectrum. M_o is the total microwave power produced by klystron (70 mW)

ples. The lowest free radicals concentration was obtained for VA sterilized at 180°C during 30 min. Such conditions of sterilization are proposed as the optimal for VA.

Free radicals concentrations change during storage of the examined VA samples and probably interactions with oxygen molecules may be responsible for this effect (Fig. 3).

Several types of free radicals are formed in VA during sterilization. Free radicals system in thermally sterilized VA was complex. Line shape of EPR spectra changed with the increasing of microwave power. The changes of the asymmetry parameters (A_1-A_2 of the EPR spectra of VA sterilized at 160°C (120 min), 170°C (60 min) and 180°C (30 min) are shown in Figures 4a-c, respectively. The correlations presented in Figure 4 resulted from the different changes of the individual EPR component lines with increasing of microwave power.

The changes of amplitudes and line widths of the EPR spectra of VA with an increase of microwave power are shown in Figures 5a-c and 6a-c, respectively. The amplitudes reached the maximum at low microwave powers, so it can be concluded that slow spin lattice relaxation processes exist in the analyzed samples. EPR lines of all the sterilized VA samples increase with increasing of microwave power and they reach the maximal values (Fig. 5). For VA sterilized at 170 and 180°C at the higher microwave powers amplitudes started to decrease (Fig. 5b, c). The slowest spin-lattice relaxation processes exist in VA sterilized at the highest temperature 170°C and 180°C. The slow spin-lattice relaxation processes were also found in thermally sterilized betamethasone (4), crystal penicillin (5), verapamil (6), famotidine (7), cefaclor (8), drotaverine (9) and streptomycin (10). The EPR spectra of VA are homogeneously broadened. The increase of line widths (Fig. 6) with the increasing microwave power was observed.

The obtained results indicate usefulness of electron paramagnetic resonance (EPR) studies to optimization of sterilization process. The EPR analysis of drugs should be done during the production of drugs. The Polish Pharmacopoeia recommendations (2) should be respected and the lowest amount of free radicals should be formed in VA.

CONCLUSIONS

EPR studies of thermally sterilized VA pointed out that:

1. Free radicals (10^{17} spin/g) are formed during thermal sterilization of VA at 160, 170 and 180°C.
2. The best thermal sterilization conditions of VA with the lowest concentration of free radicals are temperature 180°C and time of heating 30 min.
3. Complex system of free radicals exists in thermally sterilized VA.
4. Slow spin-lattice relaxation processes and strong dipolar interactions exist in thermally sterilized VA.
5. Continuous microwave saturation of EPR lines of VA indicated that the spectra are homogeneously broadened.

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