



Effect of microwave power on EPR spectra of thermally sterilized *eucerinum anhydricum*

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Abstract. Free radicals formed during thermal sterilization of *eucerinum anhydricum* – the pharmaceutical base were examined by an X-band (9.3 GHz) spectrometer. *Eucerinum anhydricum* was sterilized at different physical conditions according to the Polish Pharmacopeia norms. The samples were heated at temperatures: 160°C (120 min), 170°C (60 min), and 180°C (30 min). The aim of this study is to compare free radical concentration and effect of microwave power on EPR spectra of *eucerinum anhydricum* base thermally sterilized at different temperatures and periods of time. The effect of time storage on the free radicals in the heated samples was tested. Free radical concentrations in the sample stored 15 min strongly decreased with the increasing of sterilization temperature, probably as the result of recombination. Storage caused strong decrease of free radical concentrations in the samples, probably as the result of interactions with oxygen. It was observed to be independent of sterilization conditions from 2 days of storage and longer. Because of the lowest free radical concentration, for *eucerinum anhydricum* thermal sterilization at 180°C for 30 min is recommended. The sterilized samples should be stored at inert atmosphere without oxygen molecules. Fast spin-lattice relaxation processes existed in sterilized *eucerinum anhydricum*. The character of changes of amplitudes and linewidths of EPR lines with increasing of microwave power was the same for different storage times. The parameters of thermal sterilization and storage time influenced free radical concentration in *eucerinum anhydricum*, but magnetic spin-lattice interactions were unchanged. The usefulness of EPR spectroscopy in optimization of thermal sterilization process of *eucerinum anhydricum* was confirmed.

Key words: EPR • microwave saturation • free radicals • *eucerinum anhydricum* • thermal sterilization

Introduction

Substances interacting pharmacologically and the pharmaceutical bases during drug production are sterilized [1, 2]. The sterilized products should be free from high levels of free radicals and their structure should be unchanged [1, 2]. In this work, the original and sterilized *eucerinum anhydricum* base was tested. The popular method of removing microorganisms from the base is thermal sterilization [1–5]. Different temperatures and relative times of heating are necessary [1–3]. The physical parameters of thermal sterilization are determined by norms [1, 2]. The properties of sterilized substances should be also stable during their storage after sterilization.

The aim of our studies was to compare free radical concentration and the effect of microwave power on electron paramagnetic resonance (EPR) spectra of *eucerinum anhydricum* thermally sterilized at different temperatures and times. The changes in free radical system of this pharmaceutical base after heating in hot air with increasing time of storage was observed. The conditions of thermal sterilization producing lower amounts of free radicals in *eucerinum anhydricum* were searched.

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The examination of the effect of microwave power on EPR spectra of the thermally sterilized pharmaceutical base was proposed to test stabilization of its structure. It was expected by us that the similar character of correlations between parameters of the EPR spectra of the original and sterilized *eucerinum anhydricum* indicates that heating of the samples did not considerably modify its chemical building.

Experimental

Samples

Eucerinum anhydricum – the standard pharmaceutical base used in pharmacy was tested in this work. *Eucerinum anhydricum* is the anhydrous base homogeneous, slightly translucent soft-mass sample [3, 4, 6]. Its consistency is very soft, and *eucerinum anhydricum* is ointment is used as standalone or in ointment base combination with other components [3, 4, 6]. The total water contents in *eucerinum anhydricum* is about 220–300 g of water per 100 g of base at a temperature of 20°C, which makes the base a valuable absorbent substrate [3, 4, 6].

Eucerinum anhydricum was sterilized according to the Polish Pharmacopeia IX norms [1]. Sterilization was performed in hot air oven with air circulation produced by Memmert firm (Germany). The following conditions of sterilization processes were used: (a) $T = 160^\circ\text{C}$ and $t = 120$ min, (b) $T = 170^\circ\text{C}$ and $t = 60$ min, and (c) $T = 180^\circ\text{C}$ and $t = 30$ min, where T is temperature and t is time of sterilization, respectively. The sterilized samples were stored at room temperature in air.

The first-derivative EPR spectra were measured at room temperature at 15 min, and on days 2, 8, 10, 13, 16, 22, 32, and 40 after thermal sterilization. The EPR measurements were performed by using an X-band (9.3 GHz) EPR spectrometer with magnetic modulation of 100 kHz produced by Radiopan firm (Poznań, Poland). Numerical acquisition of the spectra was done by the Rapid Scan Unit of Jagmar firm (Kraków, Poland). The spectroscopic programs of Jagmar (Kraków, Poland) and LabView (National Instruments, U.S.A.) were applied during the measurements and analysis of the EPR spectra. The EPR spectra were recorded in the range of microwave power from 2.2 to 70 mW.

Free radical concentrations (N) in the tested samples were determined as the comparison with ultramarine – the reference. The second reference – the ruby crystal was also used. The values of free radical concentrations (N) were calculated from the tests with the low microwave power of 2.2 mW as [7, 8]:

$$N = N_u [(W_u A_u) / I_u] \cdot [I / (W A m)]$$

where: N_u – the number of paramagnetic centers in the reference – ultramarine; W , W_u – the receiver gains for the tested samples and the ultramarine; A , A_u – the amplitudes of ruby signal for the tested samples and the ultramarine; I , I_u – the integral

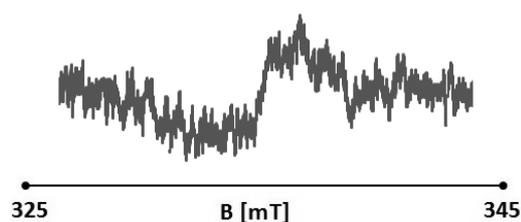


Fig. 1. EPR spectrum of *eucerinum anhydricum* sterilized at temperature 160°C for 120 min. The spectrum was recorded with microwave power of 2.2 mW. B – magnetic induction.

intensities for the tested samples and ultramarine, m – the mass of the sample.

The number of paramagnetic centers in ultramarine was determined chemically. The integral intensities (I) of the EPR lines were obtained from double integration of the first-derivative curves.

g -Factors, amplitudes (A), and linewidths (ΔB_{pp}) of the EPR spectra, were obtained. The influence of microwave power (2.2–70 mW) on amplitudes (A), and linewidths (ΔB_{pp}), was evaluated.

g -Factors were calculated from the formula [7, 8]:

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

where: h – Planck constant, ν – microwave frequency, μ_B – Bohr magneton, B_r – induction of resonance magnetic field. Microwave frequency (ν) was measured by MCM101 recorder of EPRAD firm (Poznań, Poland).

Results and discussion

EPR spectra were not obtained for the original *eucerinum anhydricum* – pharmaceutical base. EPR signals were observed for all the thermally sterilized *eucerinum anhydricum* samples. The exemplary EPR spectrum of the sample heated at 160°C is shown in Fig. 1.

The parameters of the EPR spectra of the heated *eucerinum anhydricum* depended on microwave power. The changes of amplitudes (A) of the EPR spectra of *eucerinum anhydricum* sterilized at 160, 170, and 180°C with increasing of microwave power are shown in Figs. 2a–c, respectively. The influence of microwave power on linewidths (ΔB_{pp}) of the EPR spectra of *eucerinum anhydricum* sterilized according to the norms are shown in Figs. 3a–c, respectively. The data on Figs. 2 and 3 reflect correlations for the *eucerinum anhydricum*, pharmaceutical base, tested 15 min and 2 days after sterilization.

Amplitudes (A) of the EPR spectra of thermally sterilized *eucerinum anhydricum* increases with increasing of microwave power in the used range from 2.2 to 70 mW for the samples heated in all the tested temperatures (160, 170, and 180°C) (Figs. 2a–c). The effect of microwave saturation of the EPR lines was not observed. The correlations between amplitudes (A) and microwave power (M/M_0) presented in Figs. 2a–c are characteristic for fast spin-lattice relaxation processes in the samples [7, 8]. Fast spin-lattice relaxation processes existed

in *eucerinum anhydricum* 15 min and 2 days after thermal sterilization. The EPR lines of *eucerinum anhydricum* were not microwave saturated for our observation performed up to 40 days after thermal sterilization. It indicates that spin-lattice magnetic interactions are similar in the base sterilized in different temperatures and they did not change during storage of the samples.

The increase of linewidths (ΔB_{pp}) of *eucerinum anhydricum* sterilized at temperature 160°C with increasing of microwave power (M/M_o) was observed (Fig. 3a). We obtained the broadening of EPR lines with increase of microwave power for the samples examined 15 min after thermal sterilization as well as for the longer storage samples. The rise of linewidths of EPR lines for higher microwave power (Fig. 3a) is characteristic of homogeneously broadened lines [7, 8]. The increase of linewidths with microwave power was not clearly visible for *eucerinum anhydricum* sterilized at temperatures 170 and 180°C (Figs. 3b,c).

Free radical concentrations (N) in *eucerinum anhydricum* – pharmaceutical base – depended on

sterilization conditions and on the storage time. Free radical concentrations (N) in the *eucerinum anhydricum* thermally sterilized at temperatures 160, 170, and 180°C at different storage times are presented in Fig. 4.

Thermolysis produced free radicals in the tested base in all physical conditions. A short time after thermal sterilization (15 min), strong decrease of free radical concentration (N) in *eucerinum anhydricum* with increase of heating temperature was observed (Fig. 4). Probably, the thermally formed free radicals recombine in the heated samples, and this process is more active at higher temperatures. Additionally, free radicals reactions with oxygen during sterilization, performed in circulation of air in the samples environment, may be responsible for the lowering of their levels in the products. Free radical concentrations (N) in the heated *eucerinum anhydricum* decreased strongly during storage of the samples independent of sterilization conditions from 2 days of storage and longer (Fig. 4). The samples were stored in air, so it was expected that reactions

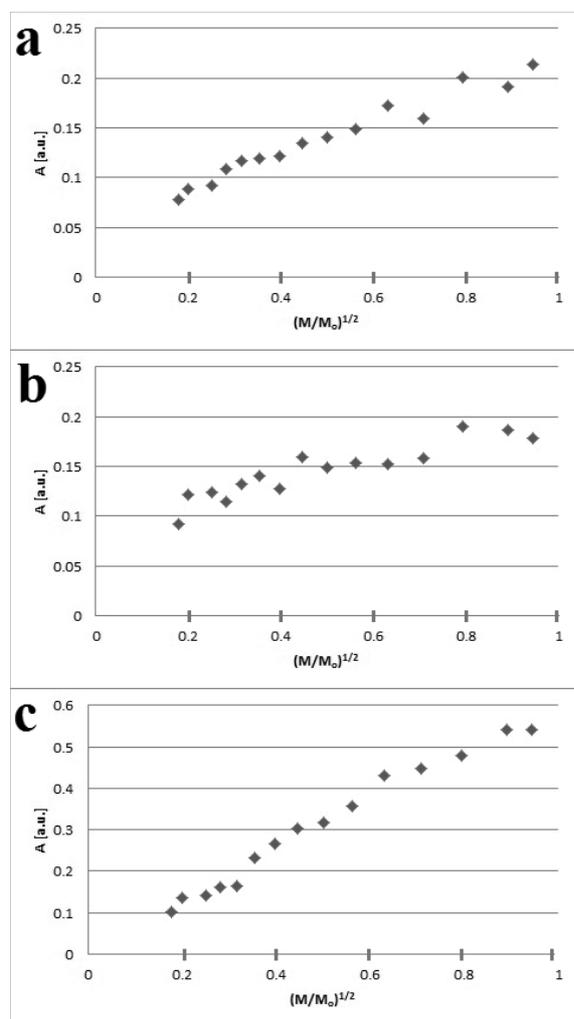


Fig. 2. The influence of microwave power (M/M_o) on amplitude (A) of the EPR line of *eucerinum anhydricum* sterilized at temperature 160°C for 120 min (a), 170°C for 60 min (b), and 180°C for 20 min (c). M is microwave power used during the measurement of the EPR spectra, M_o is the total microwave power produced by klystron (70 mW). The errors of amplitudes are (± 0.01 a. u.).

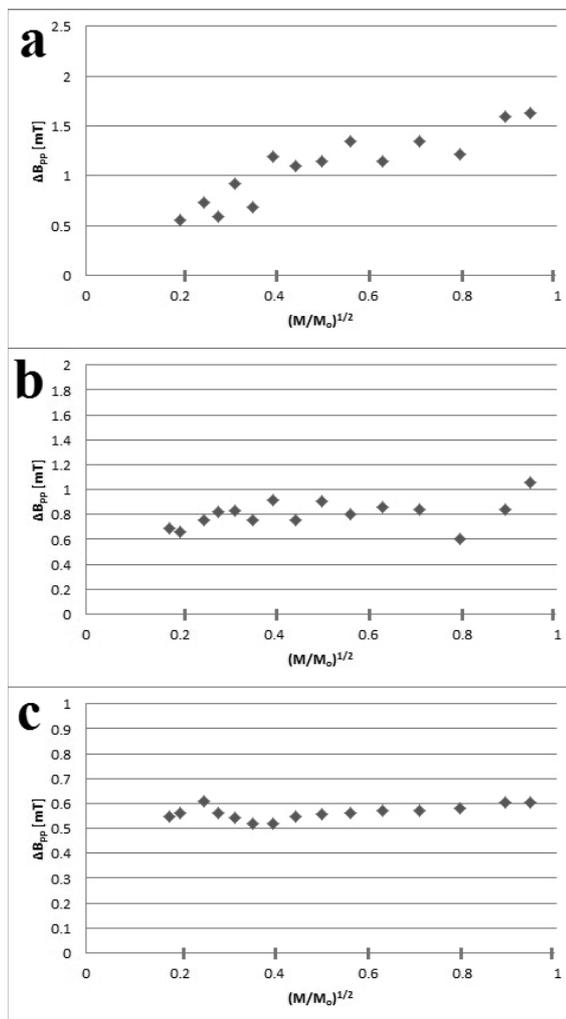


Fig. 3. The influence of microwave power (M/M_o) on linewidth (ΔB_{pp}) of the EPR line of *eucerinum anhydricum* sterilized at temperature 160°C for 120 min (a), 170°C for 60 min (b), and 180°C for 20 min (c). M is microwave power used during the measurement of the EPR spectra, M_o is the total microwave power produced by klystron (70 mW). The errors of linewidths are (± 0.02 mT).

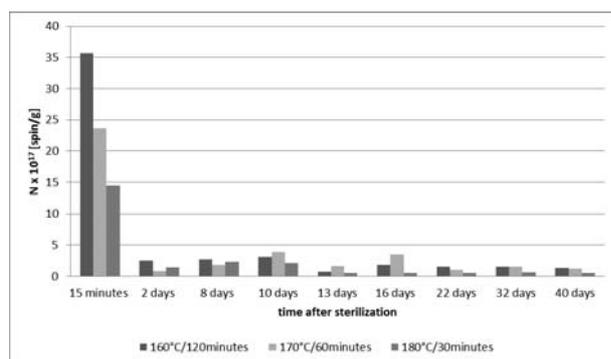


Fig. 4. The effect of storage time on free radical concentrations (N) in *eucerinum anhydricum* sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min).

with oxygen molecules may be responsible for such effect. The results contained in Fig. 4 indicated that the parameters of optimal conditions of thermal sterilization of *eucerinum anhydricum* were temperature of 180°C and time of 30 min, because of the lowest free radical concentration. The strong changes of free radical concentrations (N) in the sterilized *eucerinum anhydricum* during storage in air (Fig. 4) pointed out that the heated samples should be stored at inert atmosphere, which is free of oxygen molecules. The effect of temperature and time of thermal sterilization, and storage time on free radical concentrations in drugs was observed by us earlier [9–15].

EPR examination of heated *eucerinum anhydricum* performed in this study and our earlier EPR studies of pharmaceutical samples thermally sterilized according to the norms [1] confirmed usefulness of EPR spectroscopy in pharmacy. The basic EPR analysis and microwave effect on spectra may be applied to test optimal conditions of thermal sterilization process.

Conclusions

EPR studies of thermally sterilized *eucerinum anhydricum* – pharmaceutical base – pointed out that:

- 1) Free radicals are formed during sterilization of *eucerinum anhydricum* at temperatures 160, 170, and 180°C and free radical concentration decreases with increase of heating temperature and storage time. Recombination of free radicals and their interactions with oxygen molecules may be responsible for these effects.
- 2) Optimal parameters of thermal sterilization of *eucerinum anhydricum* are temperature of 180°C and time of 30 min, because of the lowest free radical concentration.
- 3) The effect of microwave power on amplitudes of EPR spectra are characteristic of fast spin-lattice relaxation processes in the samples. EPR lines of thermally sterilized *eucerinum anhydricum* are not saturated in the used range (2.2–70 mW) of microwave power.
- 4) The changes of linewidths with increase of microwave power indicate that EPR line of heated

eucerinum anhydricum heated at temperature 160°C are homogeneously broadened.

- 5) Examination of *eucerinum anhydricum* confirm usefulness of EPR measurements with different microwave powers to optimize thermal sterilization process and storage of the pharmaceutical base.

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