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EPR examination of free radicals thermally formed in *vaselinum flavum*

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Abstract. The popular pharmaceutical base used in pharmacy – *vaselinum flavum* – was studied by an X-band (9.3 GHz) EPR spectrometer in the range of microwave power of 2.2–70 mW. The samples were sterilized in hot air oven at temperatures: 160°C (120 min), 170°C (60 min), and 180°C (30 min). The aim of this work was to determine properties and free radical concentrations in *vaselinum flavum* thermally sterilized at different conditions. The changes in free radical system in *vaselinum flavum* during storage were analyzed. Free radicals were found in all the heated samples. The lowest free radical concentration was obtained for *vaselinum flavum* heated at 180°C for 30 min; so these parameters are proposed for the thermal sterilization of this pharmaceutical base. Interactions with oxygen decreased free radical concentration in *vaselinum flavum* during storage. Strong quenching of free radicals in *vaselinum flavum* was observed after 2 days for the samples sterilized at temperatures 160 and 180°C. Such an effect for *vaselinum flavum* heated at temperature 170°C was observed later, 13 days after sterilization. Fast spin-lattice relaxation processes exist in thermally sterilized *vaselinum flavum*. The EPR lines of heated *vaselinum flavum* were homogeneously broadened. EPR spectroscopy and its use for examining the thermal sterilization process in pharmacy was confirmed.

Key words: *vaselinum flavum* • thermal sterilization • free radicals • EPR spectroscopy

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Introduction

Different methods of sterilization are used in pharmacy during production of drugs [1–3]. Thermal sterilization is the economic method with simple apparatus [1]. The thermally stable pharmaceutical bases are often sterilized by heating in hot air [1, 2]. The aim of this work was to determine properties and free radical concentrations in *vaselinum flavum* – pharmaceutical base – thermally sterilized at three physical conditions differ in temperature and time of heating. These conditions are described in pharmacological norm [2, 3]. The parameters of the sterilization process with the lowest free radical formation in *vaselinum flavum* were searched. The pharmaceutical base used to prepare drugs should not contain high amounts of reactive free radicals, which produce a lot of destruction in organisms [4].

Experimental

Samples

Vaselinum flavum from AMARA firm (Szczecin, Poland) was tested by EPR method. *Vaselinum flavum* is used as a base for the prescription of

drugs and biologically active substances [1, 5, 6]. *Vaselinum flavum* is obtained from the distillation of crude oil [1, 5, 6]. *Vaselinum flavum* is a greasy, translucent, odorless, low melting, no dries, yellow colored substance [1, 5, 6].

Vaselinum flavum was thermally sterilized in hot air oven with air circulation produced by Memmert firm (Germany). The conditions of sterilization as proposed Pharmacopoeia IX [2] were applied. Three different temperatures of sterilization were chosen: 160, 170, and 180°C. The times of sterilization were fitted to the relative temperatures according to the norms, and they were 120, 60, and 30 min, respectively. After thermal sterilization, *vaselinum flavum* was stored at room temperature in air.

The samples as the sterilization products were tested by EPR method at room temperature. EPR spectra of the heated *vaselinum flavum* were measured at 15 min, and 2, 8, 10, 13, 16, 22, 32, and 40 days, after thermal sterilization. The samples were located in thin glass tubes with an external diameter of 3 mm. The empty tubes did not give EPR signals in the used measurement conditions. The mass of the samples was determined. Weight of the samples was: 0.027 g (160°C/120 min), 0.020 g (170°C/60 min), and 0.043 g (180°C/30 min).

An X-band (9.3 GHz) EPR spectrometer with a magnetic modulation of 100 kHz of Radiopan firm (Poznań, Poland) connected with the numerical acquisition system – the Rapid Scan Unit of Jagmar firm (Kraków, Poland) – were used in this study. The cylindrical resonance cavity type CX-01N with the quality factor of 10 000–12 000 was in the EPR measurements. The measurements and analysis of EPR spectra of *vaselinum flavum* were done by the use of the professional spectroscopic programs of Jagmar (Kraków, Poland) and LabView (National Instruments, U.S.A.). The EPR spectra were obtained in the range of microwave power 2.2–70 mW. The total microwave power produced by klystron of the spectrometer was 70 mW. Attenuation was changed in the range from 15 dB to 0 dB.

The following EPR parameters were analyzed: g factors (±0.0002), amplitudes (A) (±0.01 a.u.), integral intensities (I) (±0.02 a.u.), and linewidths (ΔB_{pp}) (±0.02 mT). The changes of amplitudes and linewidths with microwave power were tested. g-Factors were calculated from the formula [7, 8]: $g = h\nu/\mu_B B_r$, where: h is Planck constant, ν is microwave frequency, μ_B is Bohr magneton, and B_r is induction of resonance magnetic field. Microwave frequency (ν) was measured by MCM101 recorder of EPRAD firm (Poznań, Poland). Integral intensity (I) was obtained by the double integration of the first-derivative EPR spectra.

Free radical concentrations (N) (±0.2 × 10^{17} spin/g) in thermally sterilized *vaselinum flavum* were determined as [7, 8]:

$$N = N_u[(W_u A_u)/I_u]\cdot[I/(WAm)]$$

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;

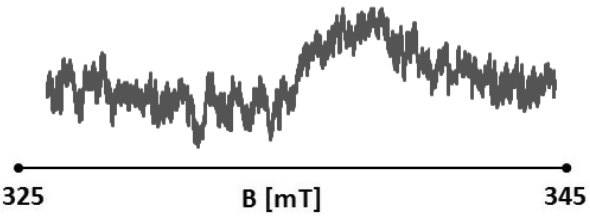

Fig. 1. The exemplary EPR spectrum of *vaselinum flavum* sterilized at temperature 160°C for 120 min. The measurement was done 20 min after sterilization with a microwave power of 2.2 mW.

A, A_u – the amplitudes of ruby signal for the tested samples and the ultramarine; I, I_u – the integral intensities for the tested samples and ultramarine, m – the mass of the sample.

As free radical concentration reference, ultramarine was used. The number of paramagnetic centers in ultramarine was determined chemically. The ruby crystal (Al_2O_3:Cr^{3+}) was the second reference. The EPR lines were detected for each pharmaceutical base samples and ultramarine.

Results and discussion

Free radicals (~10^{18} spin/g) were formed in *vaselinum flavum* during sterilization at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min). The EPR spectra were obtained for all the tested heated pharmaceutical base samples. The exemplary EPR spectrum of *vaselinum flavum* sterilized at temperature 160°C for 120 min is shown in Fig. 1. The original *vaselinum flavum* sample was free of paramagnetic centers and EPR lines were not observed.

Free radicals concentrations in heated *vaselinum flavum* depended on temperature and time of heating the samples in air. Free radical concentrations (N) changed during storage of the sterilized samples. Free radicals concentrations (N) in thermally sterilized *vaselinum flavum* at times from 15 min to 40 days after thermal treatment are presented in Fig. 2. g-Factors of EPR lines of *vaselinum flavum* heated at temperatures 160, 170, and 180°C, were: 1.9994, 1.9882, and 2.0012, respectively.

Similar free radical concentrations (N) were stated for *vaselinum flavum* sterilized at tempera-

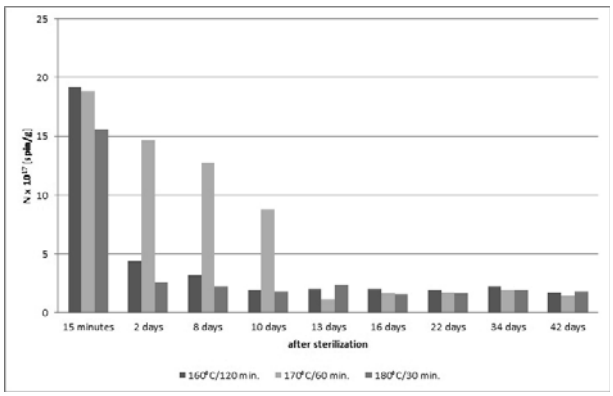

Fig. 2. The effect of storage time on free radical concentrations (N) in *vaselinum flavum* sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min).

tures 160°C and 170°C for observation 15 min after heating (Fig. 2). The considerably lower free radical concentration (N) characterized *vaselinum flavum* heated at temperature 180°C, measured 15 min after heating (Fig. 1). The lowest free radical concentrations (N) in the sample sterilized at 180°C may be due by the recombination of free radicals formed at thermolysis reactions. Additionally, reactions with oxygen may play the role. As the best conditions for *vaselinum flavum* thermal sterilization at temperature 180°C during 30 min is proposed, because of the lowest existence of free radicals in the sample.

Free radical concentrations (N) strongly decreased during *vaselinum flavum* storage after thermal sterilization (Fig. 2). This effect was observed for *vaselinum flavum* heated at temperatures 160°C and 180°C after 2 days from sterilization, and for the sample heated at temperature 170°C after 13 days from sterilization. The earlier mentioned lower free radical concentrations (N) after storage of heated *vaselinum flavum*, may result from interactions of free radicals of the pharmaceutical base with oxygen. The influence of temperature and time of heating on free radical concentrations in thermally sterilized drugs, and their varying concentrations during storage were observed by us earlier spectroscopically for the other samples [9–16].

The influence of microwave power (M/M_o) on amplitudes (A) of EPR lines of *vaselinum flavum* sterilized at temperatures 160, 170, and 180°C for the storage times after heating 15 min, 2 days, and 40 days, are shown in Figs. 3–5, respectively. In Figs. 3–6, M is the microwave power used during the measurement of the EPR spectrum, and M_o is the total microwave power produced by klystron (70 mW).

Amplitudes (A) of EPR lines of the heated *vaselinum flavum* increased with increasing of microwave power (M/M_o) in the used range up to 70 mW. The correlations in Figs. 3–5 indicated that fast spin-lattice relaxation processes existed in thermally sterilized *vaselinum flavum* [7, 8] independent on temperature and time of heating. Fast spin-lattice relaxation processes existed in the tested samples independent on the storage time.

The influence of microwave power (M/M_o) on linewidths (ΔB_{pp}) of EPR spectra of *vaselinum flavum* sterilized at temperatures 160, 170, and 180°C for the storage times after heating for 15 min, 2 days, and 40 days are similar. The exemplary correlation between linewidths (ΔB_{pp}) and microwave power

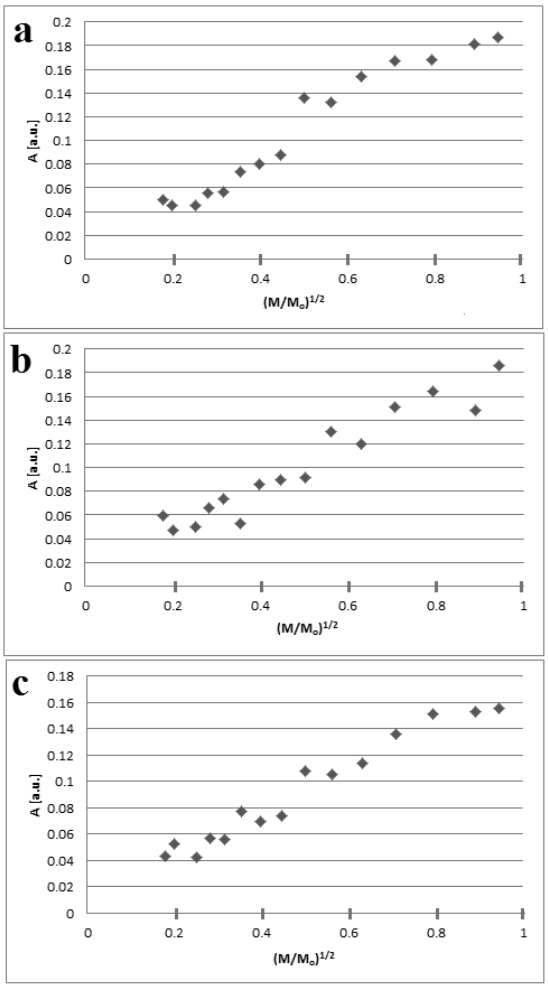

Fig. 3. The influence of microwave power (M/M_o) on amplitude (A) of the EPR line of *vaselinum flavum* sterilized at: (a) temperature 160°C for 120 min, (b) temperature 170°C for 60 min, and (c) temperature 180°C for 30 min. Data for the storage time after sterilization of 15 min.

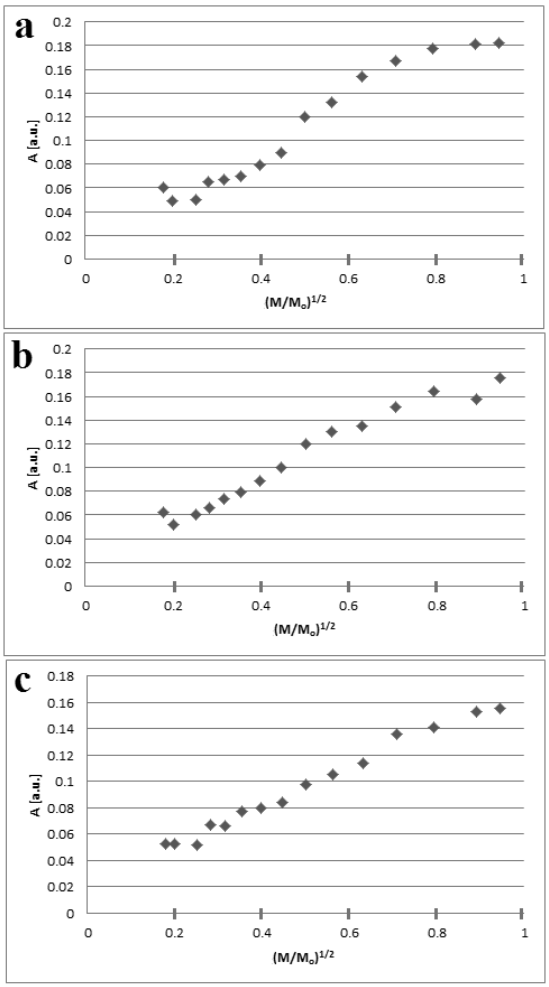

Fig. 4. The influence of microwave power (M/M_o) on amplitude (A) of the EPR line of *vaselinum flavum* sterilized at: (a) temperature 160°C for 120 min, (b) temperature 170°C for 60 min, and (c) temperature 180°C for 30 min. Data for the storage time after sterilization of 2 days.

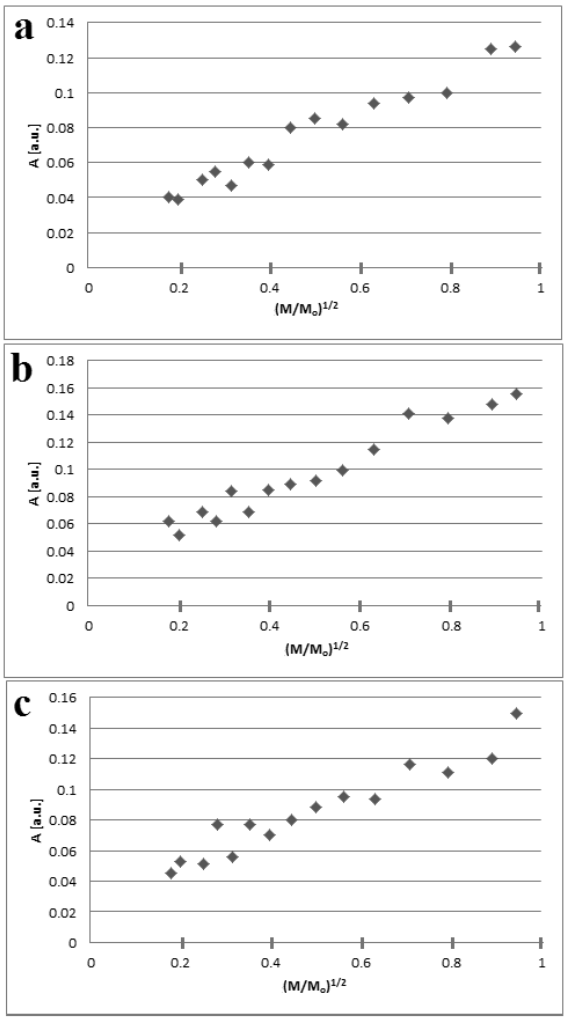

Fig. 5. The influence of microwave power (M/M_o) on amplitude (A) of the EPR line of *vaselinum flavum* sterilized at: (a) temperature 160°C for 120 min, (b) temperature 170°C for 60 min, and (c) temperature 180°C for 30 min. Data for the storage time 40 days after sterilization.

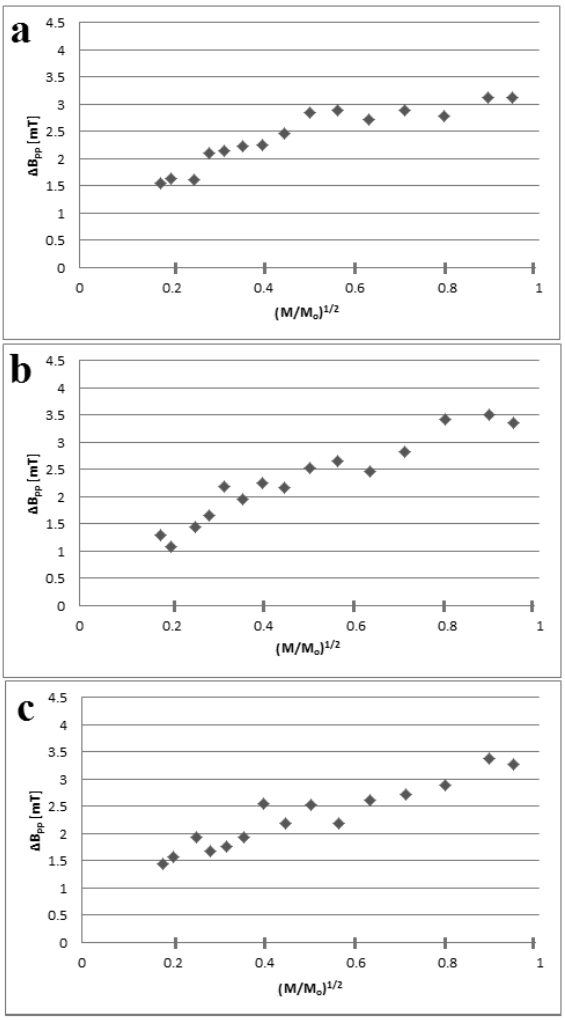

Fig. 6. The influence of microwave power (M/M_o) on linewidth (ΔB_{pp}) of the EPR line of *vaselinum flavum* sterilized at: (a) temperature 160°C for 120 min, (b) temperature 170°C for 60 min, and (c) temperature 180°C for 30 min. Data for the storage time 40 days after sterilization.

for the sample after 40 days from sterilization is shown in Fig. 6. The values of linewidths (ΔB_{pp}) increased with increase in microwave power for all the examined samples. It can be concluded that the EPR spectra of thermally sterilized *vaselinum flavum* were homogeneously broadened [7, 8]. Homogeneous broadening was observed by us for a lot of drugs earlier [9–16].

The EPR studies of *vaselinum flavum* indicated that not only microbiological analysis, but also electron paramagnetic resonance tests of free radical contents in pharmacological substances are necessary. We propose to form a database of EPR parameters for individual drugs and pharmaceutical bases to find the optimal conditions of their sterilization with the lowest free radical concentrations in the samples.

Conclusions

EPR examination of *vaselinum flavum* thermally sterilized at 160°C (120 min), 170°C (60 min), and 180°C (30 min), indicated that:

1) Free radicals are formed during thermal sterilization of *vaselinum flavum* and the lowest free radical concentration characterized the pharmaceutical base heated at 180°C, which is proposed as the optimal temperature of thermal sterilization for the tested sample.
2) Free radical concentration changed during *vaselinum flavum* storage after thermal sterilization, it decreased as the result of free radical recombination and interactions with oxygen. This effect was strong for the pharmaceutical base heated at temperatures 160 and 180°C, 2 days after sterilization, and for the base heated at temperature 170°C, 13 days after sterilization.
3) Fast spin-lattice relaxation processes exist in thermally sterilized *vaselinum flavum* independent of temperature and time of heating, and the storage time. The EPR lines did not saturate in microwave power up to 70 mW.
4) The EPR spectra of thermally sterilized *vaselinum flavum* were homogeneously broadened. The linewidths of EPR spectra increased with increase in microwave power for all the tested samples.

5) EPR spectroscopy and its use in pharmacy (of sterilized substances) was confirmed.

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