EPR spectra, microwave saturation,
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EFFECT OF STORAGE TIME ON MICROWAVE SATURATION OF EPR SPECTRA OF THERMALLY STERILIZED STREPTOMYCIN AND ITS PRACTICAL APPLICATIONS

Electron paramagnetic resonance spectroscopy at X-band (9.3 GHz) is used as the tool of optimalization of storage of thermally sterilized aminoglycoside antibiotic – streptomycin. Thermal sterilization at different conditions was performed according to the norms. Changes in free radicals system of this drug during storage after sterilization were detected. Lineshape analysis and microwave saturation of EPR spectra were done. Free radical concentrations were determined. Microwave saturation EPR analysis is proposed as useful method to determine the best conditions of storage of sterilized drugs.

1. INTRODUCTION

Thermal sterilization damages microorganisms in drugs [1], but it often produces free radicals in the samples by the way of thermolysis [2-5]. Free radicals may interact on tissues and they are responsible for toxic effects in organism [6-7]. Free radical reactions changes chemical structure of thermally sterilized drugs during storage. Both formation of free radicals and their modification during storage are negative effects, so the tools for reduction of them are searched. In this work we proposed spectroscopic analysis to optimalization of storage of thermally sterilized popular antibiotic as streptomycin. Numerical analysis of lineshape of electron paramagnetic resonance spectra is applied. The practical application of the analysis of microwave saturation of EPR spectra is bring to light.

2. EXPERIMENTAL

2.1. SAMPLES

Free radicals in thermally sterilized streptomycin were examined. Streptomycin is the natural aminoglycoside antibiotic applied in medicine against tuberculosis [8-9]. This drug is mainly used as the sulfate of streptomycin. Chemical structure of streptomycin is shown in Fig. 1 [9].

Thermal sterilization of the studied drug was performed according to standard [10, 11] in hot air with air circulation at the following temperatures and times: 160 ^{o}C and 120 minutes, 170 ^{o}C and 60 minutes, and 180 ^{o}C and 30 minutes.

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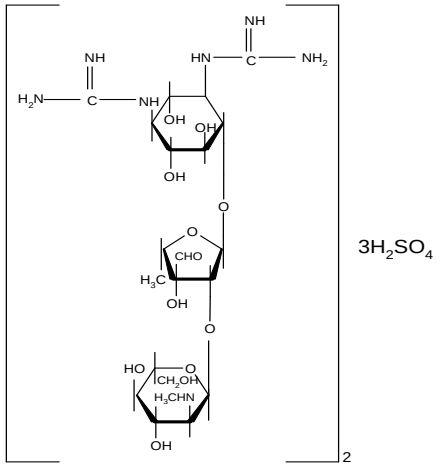

Fig. 1. Chemical structure of streptomycin [9].

2.2. EPR MEASUREMENTS

EPR spectra of powdered samples (Fig. 2) were measured at room temperature after 7 and 30 days after thermal sterilization. The spectra were recorded by Radiopan (Poznań) X-band (9.3 GHz) electron paramagnetic resonance spectrometer and Jagmar (Kraków) Rapid Scan Unit of Jagmar Firm (Kraków). Modulation of magnetic field was 100 kHz and the first-derivative EPR lines were obtained. Microwave frequency was obtained by MCM 101 recorder of EPRAD Firm (Poznań). Microwave powers in the range of 2.2-70 mW was used.

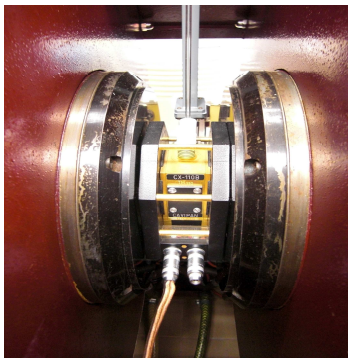

Fig. 2. The streptomycin sample in the resonance cavity of the EPR spectrometer.

2.3. ANALYSIS OF EPR SPECTRA

Analysis of EPR spectra was performed by the use of spectroscopic program of Jagmar Firm, LabView 8.5 program and program to analysis of microwave saturation. The following parameters of EPR spectra: g-factor, amplitude (A), integral intensity (I) and linewidth (ΔB_{pp}), were determined (Fig. 3). g-Factor was calculated from the resonance condition as [12-14]:

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

where:

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

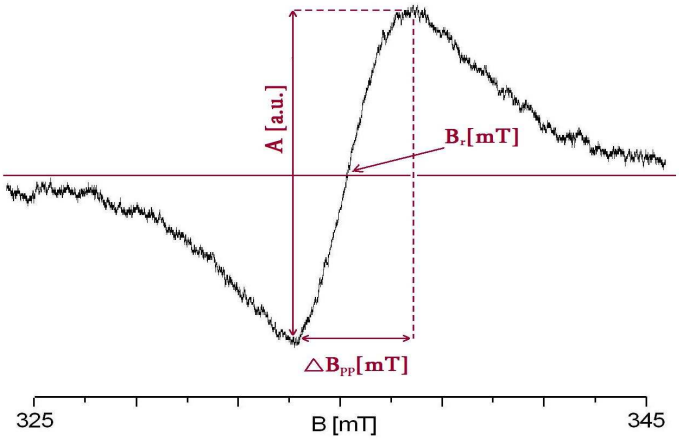

Fig. 3. The first derivative EPR spectrum of streptomycin thermally sterilized at 180 °C during 30 minutes. The spectrum was recorded 7 days after sterilization at room temperature with microwave power of 2.2 mW. A [a. u.] – amplitude, ΔB_{pp} [mT] – linewidth, and B_r – resonance magnetic induction.

The lineshape parameters A_1/A_2, $A_1–A_2$, B_1/B_2, and $B_1–B_2$, were calculated. The exemplary EPR spectrum of thermally sterilized streptomycin with A_1, A_2, B_1, and B_2 is presented in Fig. 4. We analysed influence of microwave power on the parameters and lineshape of EPR spectra.

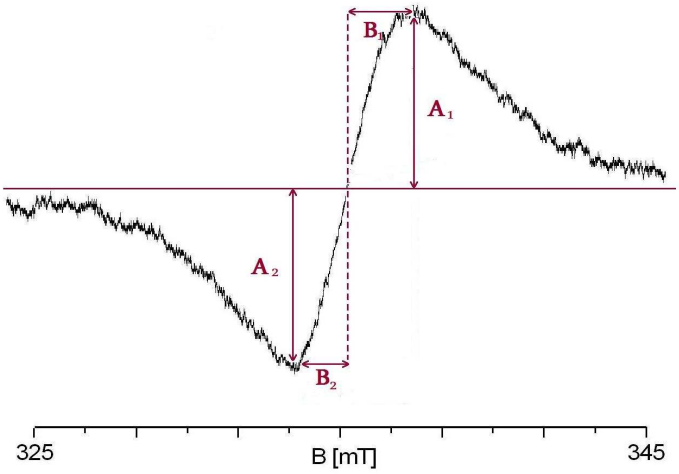

Fig. 4. The parameters of the lineshape (A1, A2, B1, and B2) of the EPR spectrum of streptomycin thermally sterilized at 180 oC during 30 minutes. The spectrum was recorded 7 days after sterilization at room temperature with microwave power of 2.2 mW.

Concentration (N) of free radicals and its dependence on storage time of the sample were determined. Ultramarine (Fig. 5) and a ruby crystal (Fig. 6) were used as the references. The value of concentration (N) was calculated as:

$$N = n_u \frac{I_p A_{ru} W_u}{I_u A_{rp} W_p m} \quad (2)$$

where:

n_u – number of paramagnetic centers in the reference (ultramarine) (n_u = 1.2x10^{19}spin),
I_p, I_u – integral intensities of EPR lines of the sample and ultramarine,
A_{rp}, A_{ru} – amplitude of EPR lines of a ruby crystal for the sample and ultramarine in the resonance cavity,
W_p, W_u – receiver gain of EPR signal for the sample and ultramarine,
m – mass of the sample.

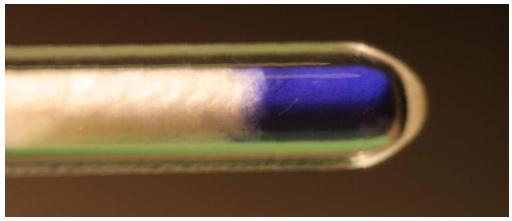

Fig. 5. Ultramarine used as the reference during the measurements of free radicals concentration.

Fig. 6. A ruby crystal – the secondary reference during the measurements of free radicals concentration.

3. RESULTS AND DISCUSSION

Free radicals exist in all the sterilized samples. EPR spectra were obtained for streptomycin sterilized at the used conditions of thermal sterilization. Parameters of these spectra changes with storage time. Concentrations of free radicals in streptomycin sterilized at 160 ^{o}C (120 minutes) were 1.25 x 10^{18} spin/g and 1.75 x 10^{18} spin/g for 7 and 30 days after this process. These concentrations in the antibiotic sterilized at 170 ^{o}C (60 minutes) were 1.95 x 10^{18} spin/g and 1.60 x 10^{18} spin/g, respectively. Free radicals concentrations in streptomycin sterilized at 180 ^{o}C (30 minutes) changed from 2.0 x 10^{18} spin/g to 1.95 x 10^{18} spin/g from 7 to 30 days after sterilization. Oxygen interactions are responsible for the evolution of free radicals amount in the tested system.

Influences of microwave power on amplitudes (A) and linewidths (ΔB_{pp}) of EPR spectra of thermally sterilized streptomycin for 7 and 30 days after sterilization are compared in Figs. 7 and 8, respectively. Amplitudes of EPR lines increase with increasing of microwave power and they begin saturate at the used range of microwave power (Fig. 7). These correlations indicate that slow spin-lattice relaxation processes exist in all the tested samples. This conclusion was confirmed by the numerical analysis of the first derivative to the experimental curves. Linewidths of the spectra increases with increasing of microwave power (Fig. 8). Changes of amplitudes and linewidths of the EPR spectra of streptomycin (Fig. 7-8) pointed out that free radicals are homogeneously distributed in the samples.

Spectroscopic analysis shown that thermal sterilization of streptomycin at the used conditions is optimal process for this drug, because the temperature effect similarly on the whole sample, and free radicals are formed in all volume of them.

Lineshape of EPR spectra of sterilized streptomycin strongly changed with microwave power. Multicomponent free radicals system is responsible for this effect. The exemplary changes of lineshape parameters with increasing of microwave power are presented in Figs. 9-10.

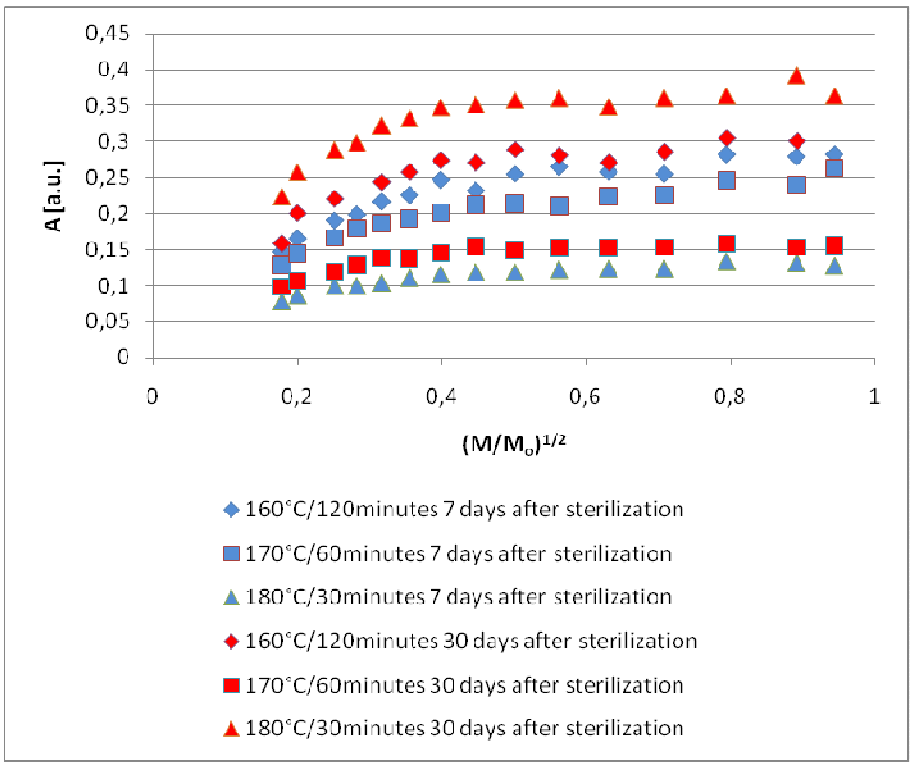

Fig. 7. Effect of microwave power (M) on amplitude (A) of EPR lines of thermally sterilized streptomycin. M_o – total microwave power produced by klystron (70 mW), M – microwave power used during the measurement.

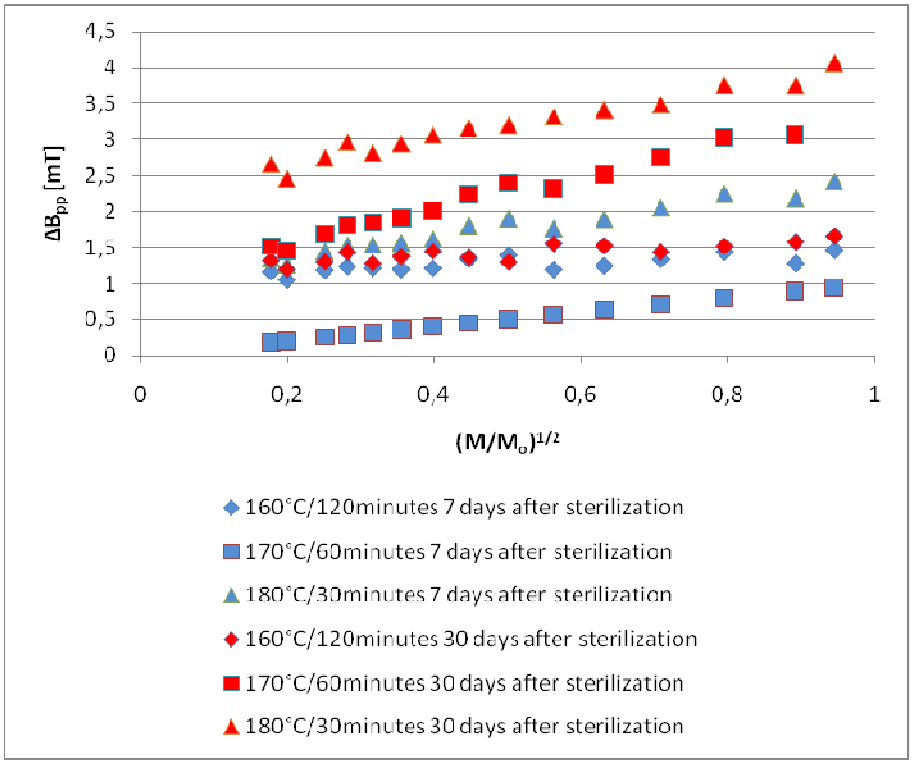

Fig. 8. Effect of microwave power (M) on linewidths (ΔB_{pp}) of EPR lines of thermally sterilized streptomycin. M_o – total microwave power produced by klystron (70 mW), M – microwave power used during the measurement.

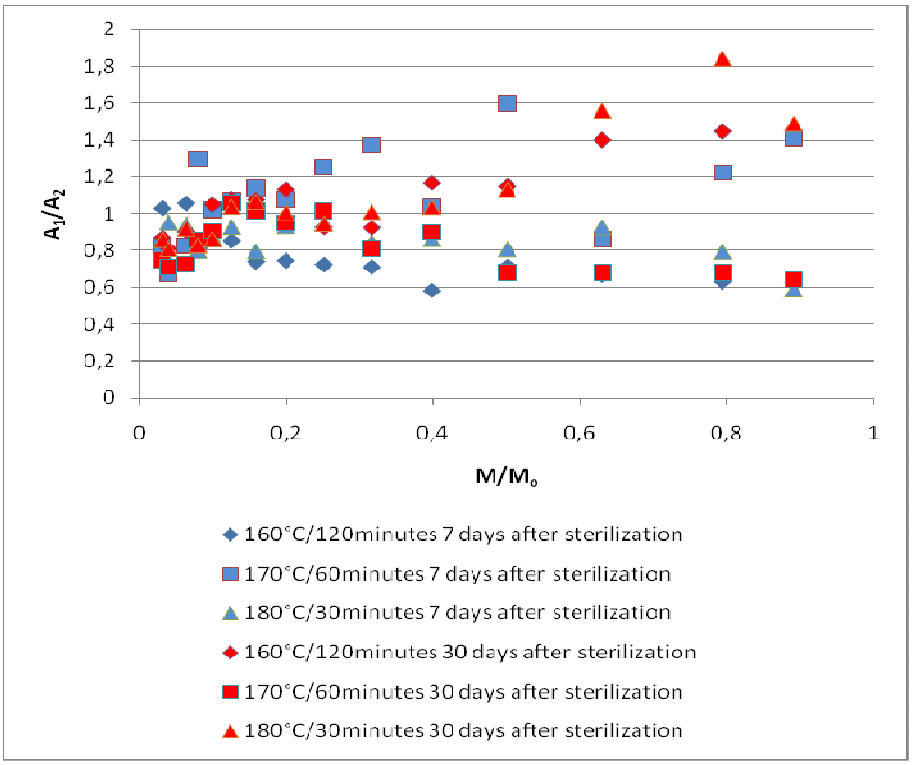

Fig. 9. Effect of microwave power (M) on parameter A_1/A_2 of EPR lines of streptomycin thermally sterilized at 180 ^oC during 30 minutes for 7 and 30 days after sterilization.. M_o – total microwave power produced by klystron (70 mW), M – microwave power used during the measurement.

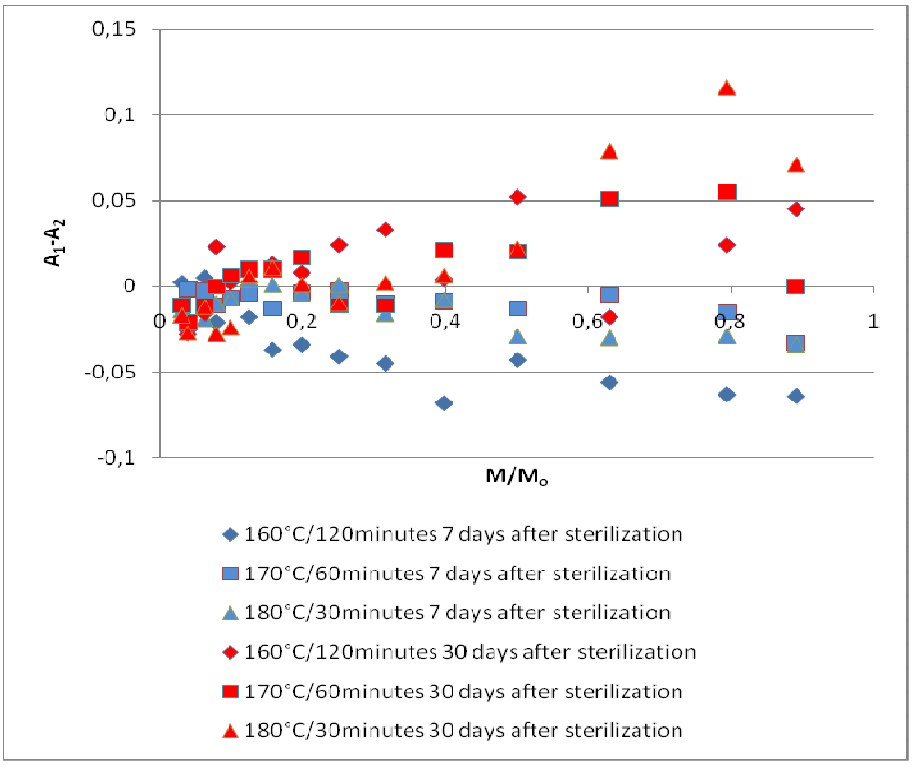

Fig. 10. Effect of microwave power (M) on parameter A_1-A_2 of EPR lines of streptomycin thermally sterilized at 180 ^oC during 30 minutes for 7 and 30 days after sterilization.. M_o – total microwave power produced by klystron (70 mW), M – microwave power used during the measurement.

4. CONCLUSIONS

Spectroscopic analysis shown that thermal sterilization of streptomycin at the used conditions is optimal process for this drug, because the temperature effect similarly on the whole sample, and free radicals are formed in all volume of them.

Continuous microwave saturation analysis of EPR spectra is the useful method for examination of storage conditions of thermally sterilized drugs. The absence of changes of microwave saturation of EPR spectra of the storage drug indicates that its chemical structure is not damaged.

ACKNOWLEDGEMENTS

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