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THE EPR EXAMINATION OF FREE RADICALS FORMATION IN THERMALLY STERILIZED β-LACTAM ANTIBIOTICS

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EPR examination of free radicals formed in β-lactam antibiotics sterilized at different temperatures was performed. Asymmetrical EPR lines were measured for the studied heated drugs. Properties and free radical concentration in the heated antibiotics changed during storage after thermal treatment. Effect of microwave power on the spectra was analysed. Optimal conditions of thermal sterilization of ampicillin, crystal penicillin, and piperacillin were determined.

INTRODUCTION

Free radicals are formed in heated organic substances and in drugs during thermal sterilization [Pilawa, Pietrzak, Wachowska & Babeł, 2005; Pilawa, Więckowski & Lewandowski, 1995; [a)]Kościelniak, Pilawa & Wilczyński, 2008; [b)]Kościelniak, Pilawa & Wilczyński, 2008; Pilawa, Wilczyński, Ramos & Tomasik, 2008; Ramos, Pilawa & Kawka, 2009; Ramos, Pilawa, Wilczyński, Czyż & Adamczyk, 2009; Pilawa, Ramos, Wilczyński & Czyż, 2008].

The aim of this work was to determine influence of thermal sterilization on concentration and properties of free radicals in the analysed β-lactam antibiotics. Effect of temperature and time of sterilization on free radicals in these drugs was evaluated. The optimal conditions of thermal sterilization of the studied antibiotics were searched. Effect of the storage time on free radical concentration in the antibiotics sterilized at different temperatures was tested. The results will help to determine of storage conditions for the studied drugs.

EXPERIMENTAL DETAILS

The three β-lactam antibiotics: ampicillin, crystal penicillin, and piperacillin, were studied. The chemical structure of these drugs are presented in figure 1 [Zejc, 2004]. β-lactam antibiotics are a broad class of antibiotics that include penicillin, cephalosporins, monobactams, and carbapenems [Zejc, 2004]. They are the most widely-used group of antibiotics. β-lactam antibiotics are indicated for the prophylaxis and treatment of bacterial infections caused by susceptible organisms [Zejc, 2004]. β-lactam antibiotics are bactericidal, and they act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls [Zejc, 2004].

Sterilization was performed according to the Polish norms [Farmakopea Polska VIII, 2009; PN-EN 552, 1999; PN-EN 556, 2002] in hot air oven 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.

EPR analysis was done for powdered samples at room temperature 20 minutes after thermal sterilization. Changes of EPR spectra of the sterilized drugs with increasing the storage time in air up to 3 months were tested. Storage conditions were the same as in pharmaceutical practice. The samples were storage in air at room temperature. An X-band (9.3 GHz) EPR spectrometer with modulation of magnetic field of 100 kHz produced by RADIOPAN Firm (Poznań, Poland) was used. Microwave frequency was measured by MCM101 recorder of EPRAD firm (Poznań, Poland). EPR spectra were recorded in the range of microwave power from 2.2 mW to 70 mW. g-Factor, amplitudes (A), integral intensities (I), and linewidth (ΔB_{pp}) of the spectra were determined. The shapes parameters of the spectra were analysed. Free radical concentration (N) in the samples was determined by the use of ultramarine as the reference. The relative correlations between spin-lattice relaxation times T_1 of the individual samples were presented by the use of the effect of microwave

power on amplitudes of their EPR lines.

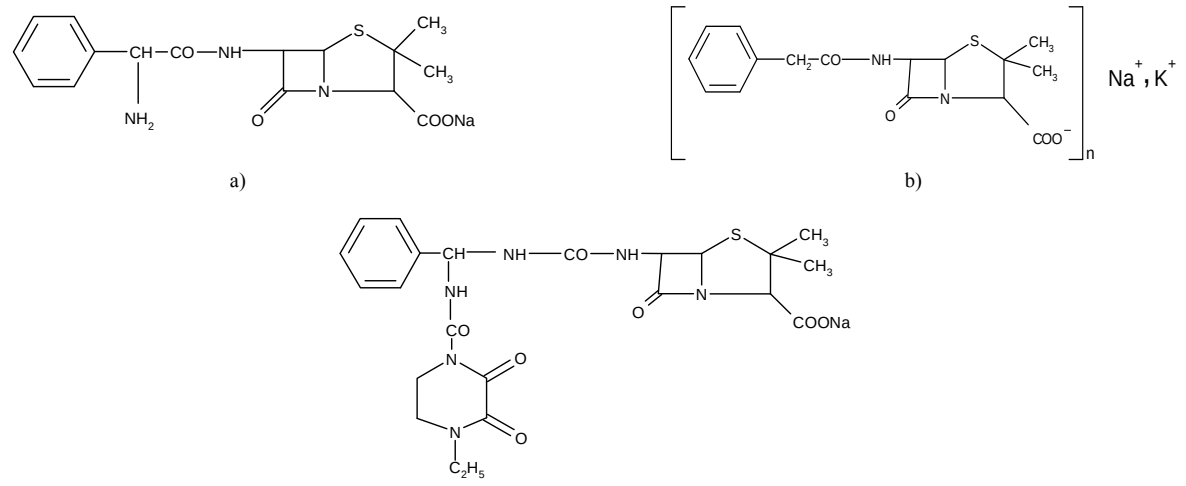

Fig. 1. Chemical structure of: a) ampicillin, b) crystal penicillin, and c) piperacillin [Zejc, 2004].

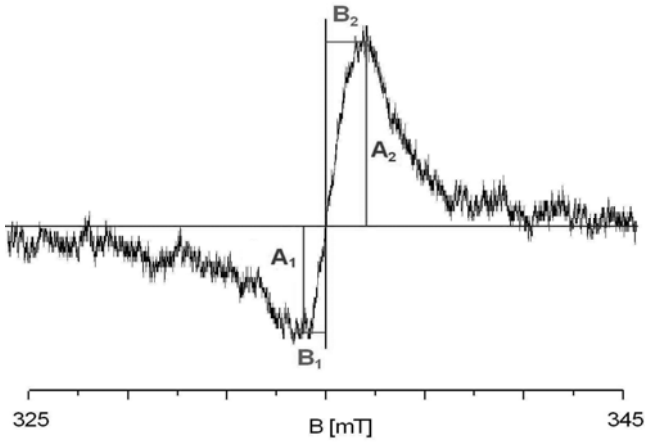

Fig. 2. EPR spectrum of ampicillin sterilized at 160 °C during 120 minutes. The measurement with microwave power of 2.2 mW was done at room temperature 20 minutes after sterilization.

RESULTS AND DISCUSSION

EPR spectra were not obtained for the original non-heated drugs. Thermal sterilization at temperatures 160 °C (120 minutes), 170 °C (60 minutes), and 180 °C (30 minutes) forms free radicals in β-lactam antibiotics. The free radical concentrations in the samples 20 minutes after sterilization were in the range $(0.2\text{-}8.0)\text{x}10^{18}$ spin/g. The highest concentrations of free radicals in ampicillin and crystal penicillin was observed for samples sterilized at 180 °C during 30 minutes. The highest formation of free radicals in piperacillin appeared at 170 °C during heating by 60 minutes. The amounts of free radicals changes during storage time, but the changes were lower compared to the radiosterilized samples [Wilczyński, 2008]. Interactions with oxygen during storage of the sterilized drugs are responsible for changes in free radicals in the samples.

Shapes of all the spectra of β-lactam antibiotics changes with increasing of microwave power. It probably indicates that more than one group of free radicals exist in the samples. Unpaired electrons in the studied drugs are mainly localized on oxygen atoms. Localization of unpaired electrons is concluded taking to

account the chemical structure of the tested drugs, which is presented in figure 1. Probably additionally free radicals with unpaired electrons localized at carbon atoms exist in the samples. Influence of microwave power on amplitudes and selected asymmetry parameter of EPR spectra of the examined antibiotics is presented in figures 3-6. The spin-lattice relaxation time in the studied drugs depends on sterilization temperature. Relative slower spin-lattice relaxation processes were found in ampicillin and penicillin sterilized at temperature 180 °C during 30 minutes. EPR lines of these two samples saturated at the relatively lower microwave powers (figs. 3-4). EPR lines of the others samples: ampicillin and penicillin sterilized at temperatures 160 °C (120 minutes) and 170 °C (60 minutes) (figs. 3-4), and piperacillin sterilized at the three temperatures (160 °C, 170 °C, and 180 °C) (fig. 5), were not saturated at the used range of microwave power. The relatively faster spin-lattice relaxation processes exist in these sterilized drugs. Differences between spin-lattice relaxation times in the samples result from modifications of their chemical structures during thermal sterilization at different conditions. The asymmetry of the spectra of all the tested samples changes with microwave power. The exemplary changes of the asymmetry parameter $|A_1-A_2|$ for the drugs sterilized at 180 °C during 30 minutes are visible in figure 6. Changes of asymmetry of EPR spectra with microwave power indicate that the spectra are complex and the individual components changes differently with microwave power. Complex character of paramagnetic centers system in the examined drugs was expected, because different chemical bonds may be ruptured by heating of the samples.

Free radicals of different types in the thermally sterilized ampicillin, penicillin and piperacillin may be responsible for negative biochemical reactions in tissues. Especially this effect is expected in paramagnetic melanin biopolymers which prolongate interactions of drugs in organism.

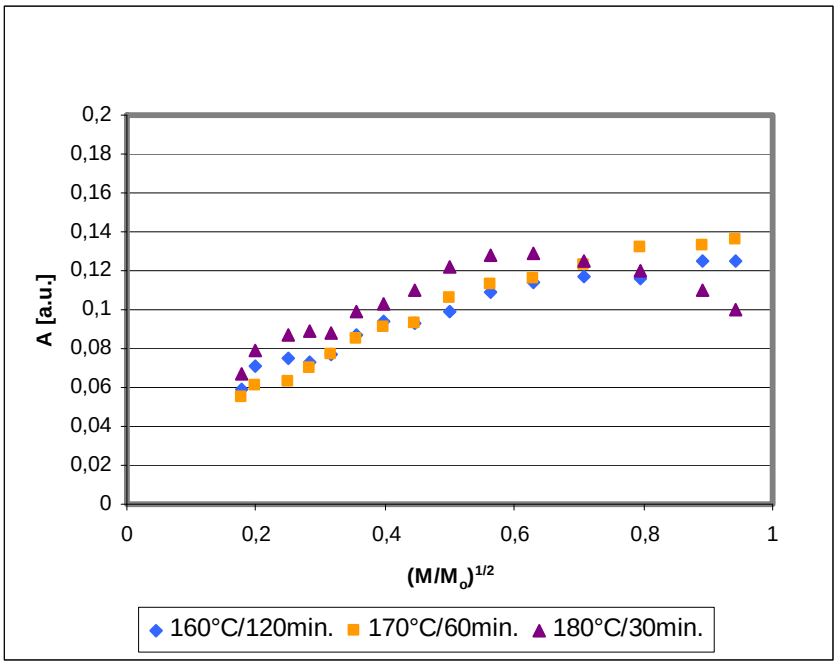

Fig. 3. Influence of microwave power (M) on amplitude (A) of EPR lines of ampicillin sterilized at 160 °C (120 minutes), 170 °C (60 minutes), and 180 °C (30 minutes). M – microwave power used during the measurement, M_o – the maximal microwave power produced by klystron (70 mW). EPR spectra were recorded in the day of sterilization.

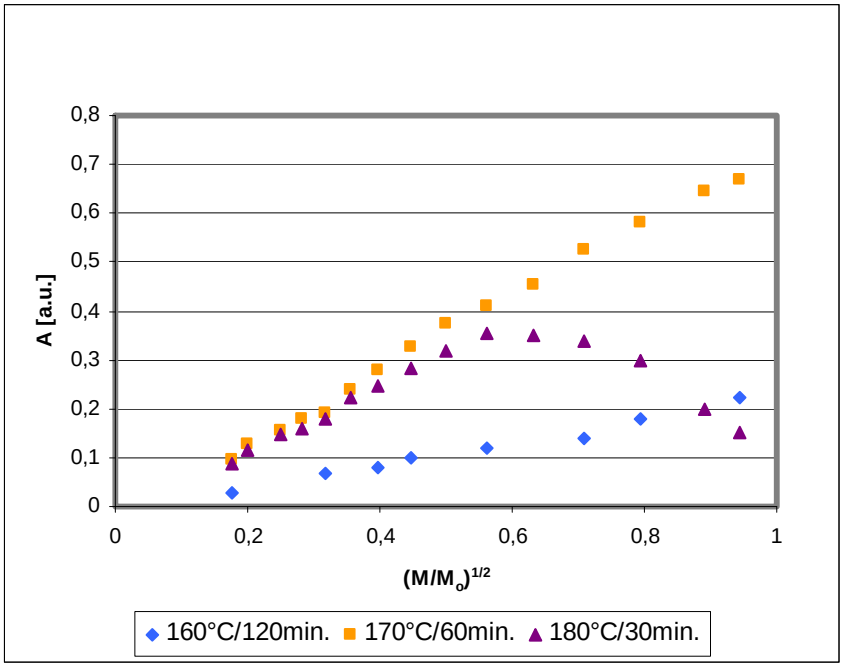

Fig. 4. Influence of microwave power (M) on amplitude (A) of EPR lines of crystal penicillin sterilized at 160 °C (120 minutes), 170 °C (60 minutes), and 180 °C (30 minutes). M – microwave power used during the measurement, M_o – the maximal microwave power produced by klystron (70 mW). EPR spectra were recorded in the day of sterilization.

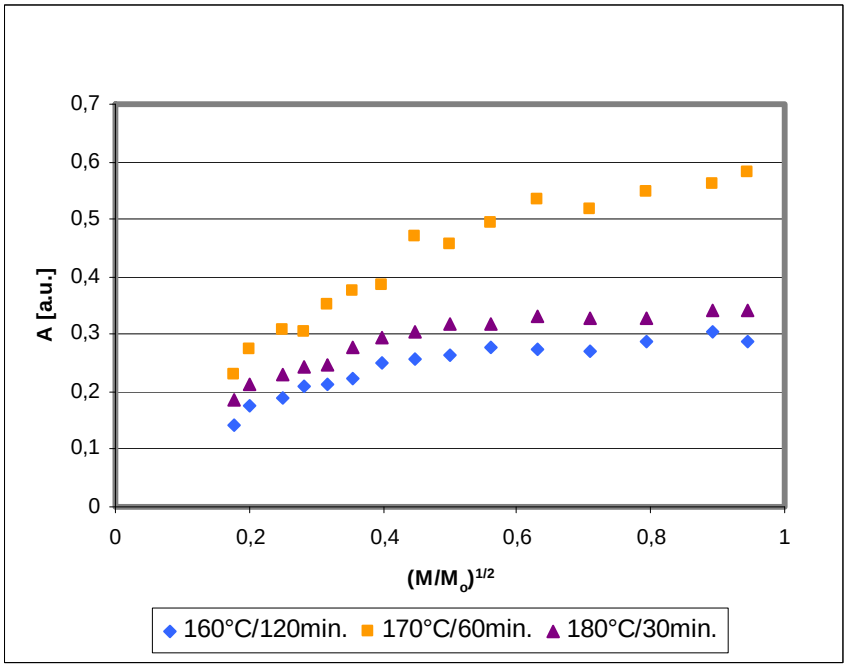

Fig. 5. Influence of microwave power (M) on amplitude (A) of EPR lines of crystal piperacillin sterilized at 160 °C (120 minutes), 170 °C (60 minutes), and 180 °C (30 minutes). M – microwave power used during the measurement, M_o – the maximal microwave power produced by klystron (70 mW). EPR spectra were recorded in the day of sterilization.

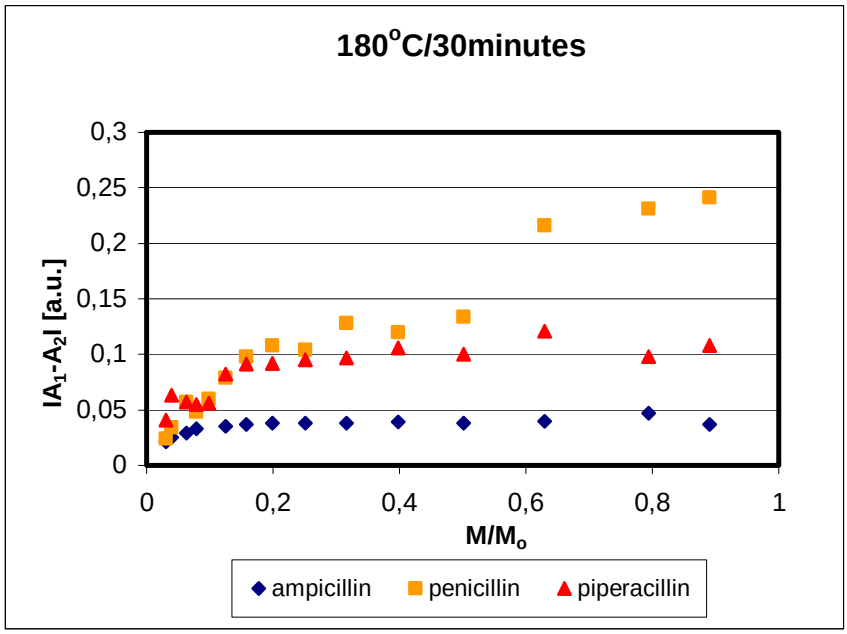

Fig. 6. Influence of microwave power (M) on asymmetry parameter $|A1-A2|$ of EPR lines of crystal piperacillin, ampicillin and piperacillin sterilized at 180 °C (30 minutes). M – microwave power used during the measurement, M_o – the maximal microwave power produced by klystron (70 mW). EPR spectra were recorded in the day of sterilization. A_1 and A_2 are presented in figure 2.

CONCLUSIONS

EPR studies indicates that stable paramagnetism (~10^{17}-10^{18} spin/g) characterizes thermally sterilized β-lactam antibiotics: ampicillin, crystal penicillin, and piperacillin. Free radicals exist in these drugs 3 months after sterilization. Complex paramagnetic centers system exist in the samples. Dipolar interactions and microwave saturation of EPR lines of the tested drugs depend on temperature of sterilization process. Optimal temperature of sterilization for ampicillin and crystal penicillin is 180 °C. Piperacillin should be sterilized at 170 °C.

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