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EPR studies of free radicals in thermally sterilized famotidine

Paweł Ramos,
Barbara Pilawa,
Edyta Stroka

Abstract. Free radicals formation in thermally sterilized famotidine was studied by the use of an X-band (9.3 GHz) electron paramagnetic resonance (EPR) spectroscopy. The influence of temperature and time of sterilization on free radicals generated in famotidine was determined. The best sterilization conditions for the tested drug were found. Sterilization was done according to the pharmaceutical norms at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min). It was pointed out that the optimal temperature of thermal sterilization for famotidine was 170°C and time of heating 60 min in this conditions the lowest free radicals concentration was found. The highest free radicals concentration was measured in famotidine heated at 160°C during 120 min, the long time of sterilization is responsible for this effect. Free radicals concentration changes during storage of famotidine after thermal sterilization. Its value increased with storage time for the drug sterilized at both temperatures of 170°C and 180°C. Free radicals concentration decreases from 7 days after sterilization. Complex system of free radicals with the shape of EPR lines, dependent on microwave power exist in thermally sterilized famotidine. Slow spin-lattice relaxation processes, strong dipolar interactions characterize thermally sterilized famotidine. Microwave saturation indicates that EPR lines of famotidine are homogeneously broadened. The usefulness of EPR spectroscopy to examine of the sterilized drugs was evaluated.

Key words: free radicals • thermal sterilization • famotidine • electron paramagnetic resonance (EPR) spectroscopy

P. Ramos✉, B. Pilawa, E. Stroka
Department of Biophysics,
School of Pharmacy and Laboratory Medicine,
Medical University of Silesia in Katowice,
8 Jedności Str., 41-200 Sosnowiec, Poland,
Tel.: +48 32 364 1164, Fax: +48 32 364 1160,
E-mail: pawelramos@sum.edu.pl

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Introduction

Sterilization process as the main stage during drugs production may be exactly controlled [1, 4, 10]. During sterilization microorganisms are killed, and their final contents in the samples should be, as the probability, lower than 10^{-6} [10]. Drugs may be sterilized by radiation or thermally by heating. The norms used in pharmacy and medicine determine the conditions of the sterilization process [4, 10]. The type of sterilization should be choosen to the chemical properties of the materials. Thermal sterilization may be used only for these drugs which reveal stabile chemical structure at higher temperatures. According to the Polish Pharmacopoeia [4] drugs should be sterilized in air at temperatures 160, 170 or 180°C with relative heating times of 120, 60 and 30 min [4]. There are no norms for the free radicals contents in the sterilized drugs and materials. In this work we present an important problem of the formation of free radicals in the samples during their sterilization. The free radicals were found in thermally sterilized drugs earlier [7–9, 11]. Free radicals should not exist in drugs, because they may cause a number of toxic effects in tissues [2].

It is proposed by us that the conditions of sterilization should be found for the individual drugs with the test of a free radical contents in the samples. The con-

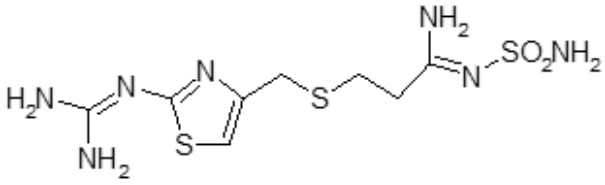

Fig. 1. Chemical structure of famotidine [14].

ditions with the absence or low contents of free radical should be used. In this work thermal sterilization of the exemplary drug – famotidine was studied. The aim of this examination was to determine properties and concentrations of free radicals in thermally sterilized famotidine. The influence of temperature and time of heating on free radical formation for this drug was evaluated. Changes of free radical concentrations during storage of famotidine after thermal sterilization were tested. Free radicals formed in thermally sterilized famotidine were not examined earlier.

Experimental

Samples

Free radicals in famotidine were studied by the EPR method. Famotidine is a histamine H_2-receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcer disease and gastroesophageal reflux disease [5, 6]. Chemical structure of famotidine is show in Fig. 1 [14].

Thermal sterilization was performed to kill microorganisms in the drug samples. Famotidine was sterilized according to the Polish Pharmacopoeia Norms [4] in the following conditions: T = 160°C and t = 120 min, T = 170°C and t = 60 min, T = 180°C and t = 30 min, where T is the temperature and t is the time of heating, respectively. The individual sample was sterilized by the use of one of the mentioned three methods. Sterilization process was performed in a dryer with hot air.

The samples were kept in contact with air, because in practice famotidine is kept in air environment. The powdered samples of famotidine were measured in thin-walled glass tubes. EPR signals were not obtained for the empty tubes, they do not contain paramagnetic impurities. Famotidine was obtained from Aldrich-Sigma.

EPR measurements

The electron paramagnetic resonance (EPR) measurements were performed 20 min, 7 days and 14 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 an MCM 101 recorder of Eprad (Poznań).

The first-derivative EPR spectra were measured with a microwave power in the range 2.2–70 mW. g-Factor, amplitudes (A) and linewidth (ΔB_{pp}) of the EPR lines were analyzed (Fig. 2a). The parameters A_1/A_2 and B_1/B_2 of asymmetry of lineshape of EPR spectra were determined (Fig. 2b).

g-Factor was calculated from the resonance condition according to the formula [13]

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

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 the examination of spin-lattice relaxation processes [13].

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

$$N = N_u[(W_u A_u)/I_u][I/(WA\,m)],$$

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

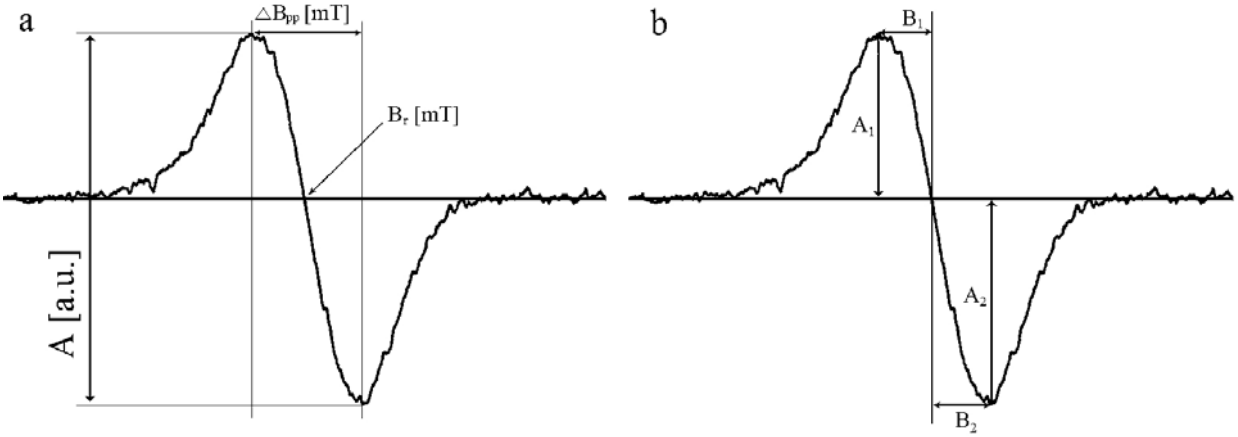

Fig. 2. The analyzed parameters of the EPR spectra (a) amplitude (A), linewidth (ΔB_{pp}), B_r – resonance magnetic induction, and the asymmetry of lineshape parameters (b) A_1, A_2, B_1, B_2. Data for famotidine sterilized at 160°C (120 min). Microwave power was 70 mW.

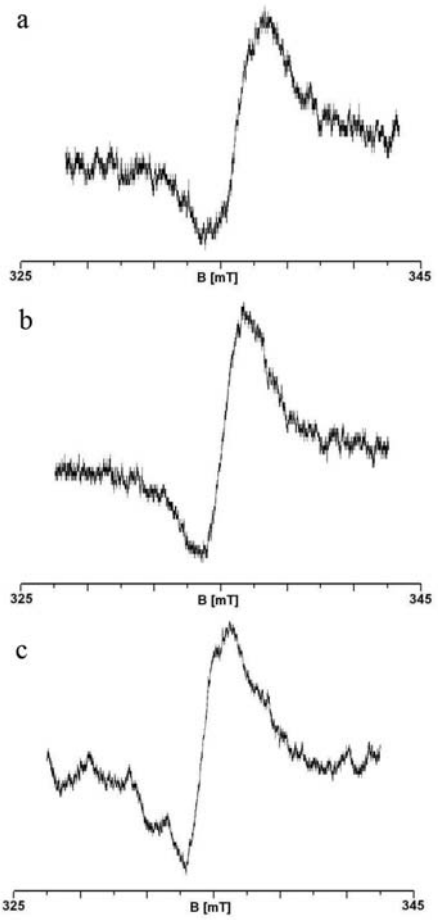

Fig. 3. EPR spectra of famotidine sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min) (c). The measurement was done 20 min after sterilization with a microwave power of 2.2 mW.

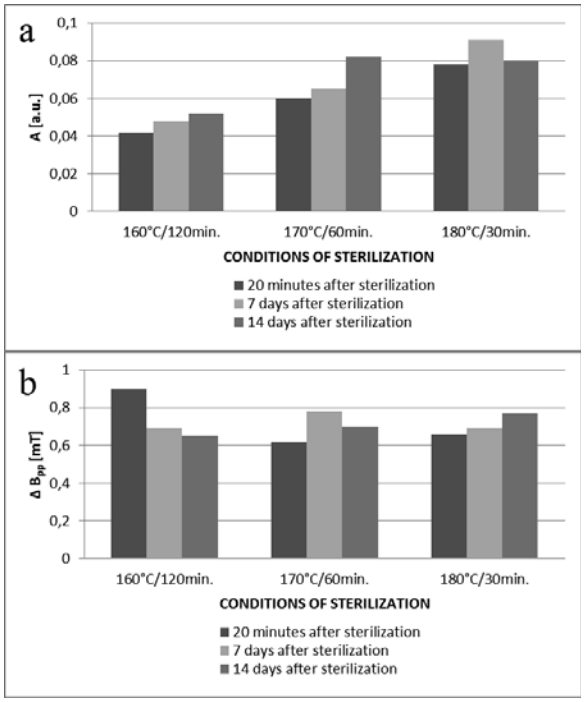

Fig. 4. Amplitudes (A) (a) and linewidths (ΔB_{pp}) (b) of EPR lines of famotidine sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min). The spectra were measured 20 min, 7 days, and 14 days after sterilization with a microwave power of 2.2 mW at room temperature.

Results and discussion

Free radicals were found in all the tested thermally sterilized famotidine samples, independently of the physical condition of this process. EPR spectra were measured for famotidine sterilized at 160°C (120 min) (a), 170°C (60 min) (b), and 180°C (30 min). The EPR spectra of the sterilized drug samples measured with a low microwave power of 2.2 mW in the absence of microwave saturation effect at room temperature are shown in Fig. 3. Free radicals appeared as the result of thermolysis of the samples, when the chemical structures of the analyzed drug was destroyed. This effect of formation of free radicals is not desirable, because the interactions of famotidine with cells and tissues may be responsible for the major toxic effects in the organism.

Contrary to paramagnetic sterilized samples, the non heated samples were diamagnetic and the EPR signals were not obtained. The paramagnetic defects with unpaired electrons do not exist in the tested original famotidine.

The parameters of the EPR spectra of the sterilized samples depended on the temperature and time of heating, and changed during storage of famotidine after sterilization. The amplitudes (A) and linewidths (ΔB_{pp}) of EPR spectra of famotidine sterilized in different conditions for the measurements during 20 min, 7 days, and 14 days after sterilization are compared in Figs. 4a–4b.

Free radicals concentrations (N) in famotidine were different for samples sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min), and changed during storage of the drug after heating. The concentrations of free radicals in heated famotidine samples are presented in Fig. 5. The high values of free radicals concentrations ($\sim 10^{17}$ spin/g) were obtained for all the tested thermally sterilized famotidine samples. At a time of 20 min after sterilization, the lowest free radicals concentration was obtained for famotidine sterilized at 170°C during 60 min (Fig. 5). For this drug sterilized at 160°C and 180°C, the concentrations were higher (Fig. 5). Taking into account these mentioned above correlations, we proposed a temperature of 170°C and a time of 60 min for sterilization process of famotidine. We proposed such conditions to this process in practice, because of the lowest value of free radical concentration in the drug heated under these conditions.

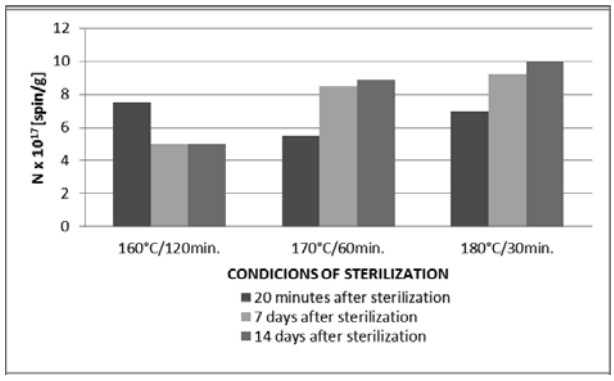

Fig. 5. Free radicals concentrations (N) in famotidine sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min). The data for the sterilized samples 20 min, 7 days, and 14 days after the process.

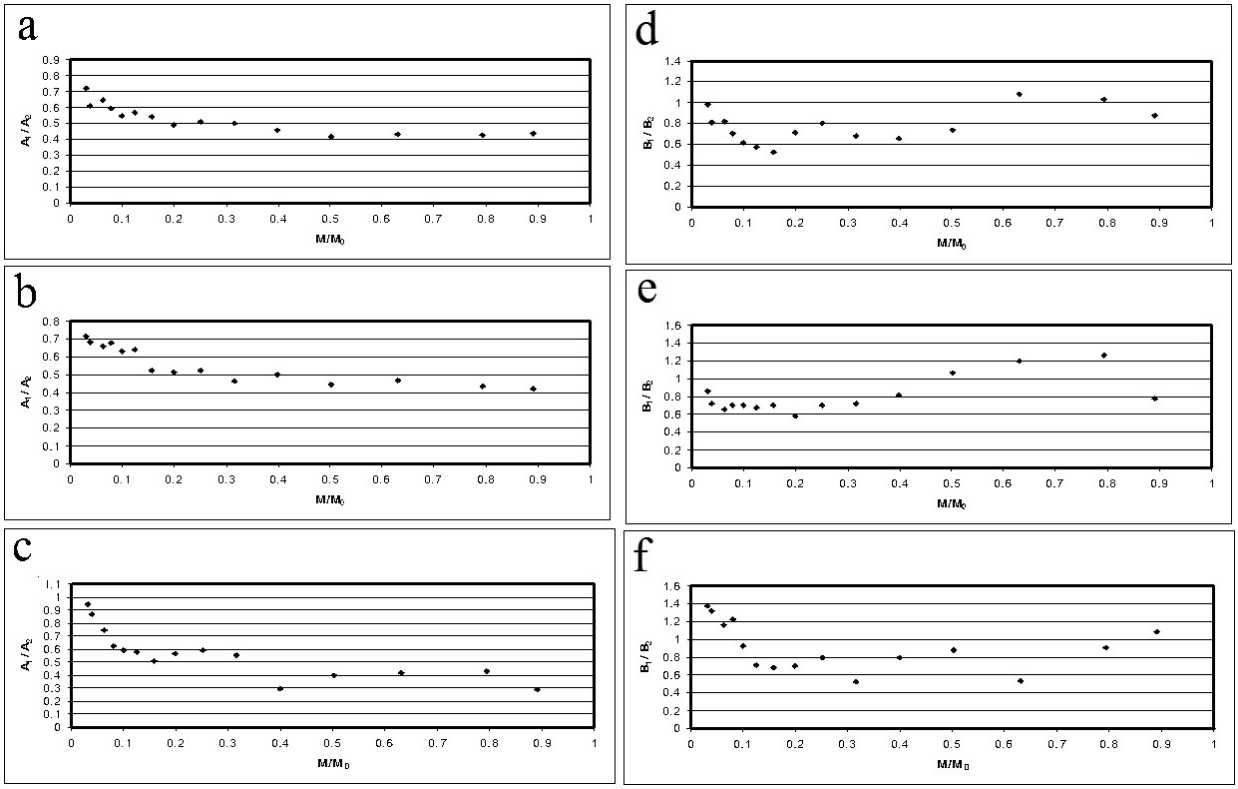

Fig. 6. Influence of microwave power (M) on the asymmetry parameters A_1/A_2 and B_1/B_2 of EPR spectra of famotidine sterilized at 160°C (120 min) (a, d), 170°C (60 min) (b, e), and 180°C (30 min) (c, f). The measurement was done 20 min after sterilization. M is a microwave power used during the measurement of EPR spectrum. M_0 is the total microwave power produced by klystron (70 mW).

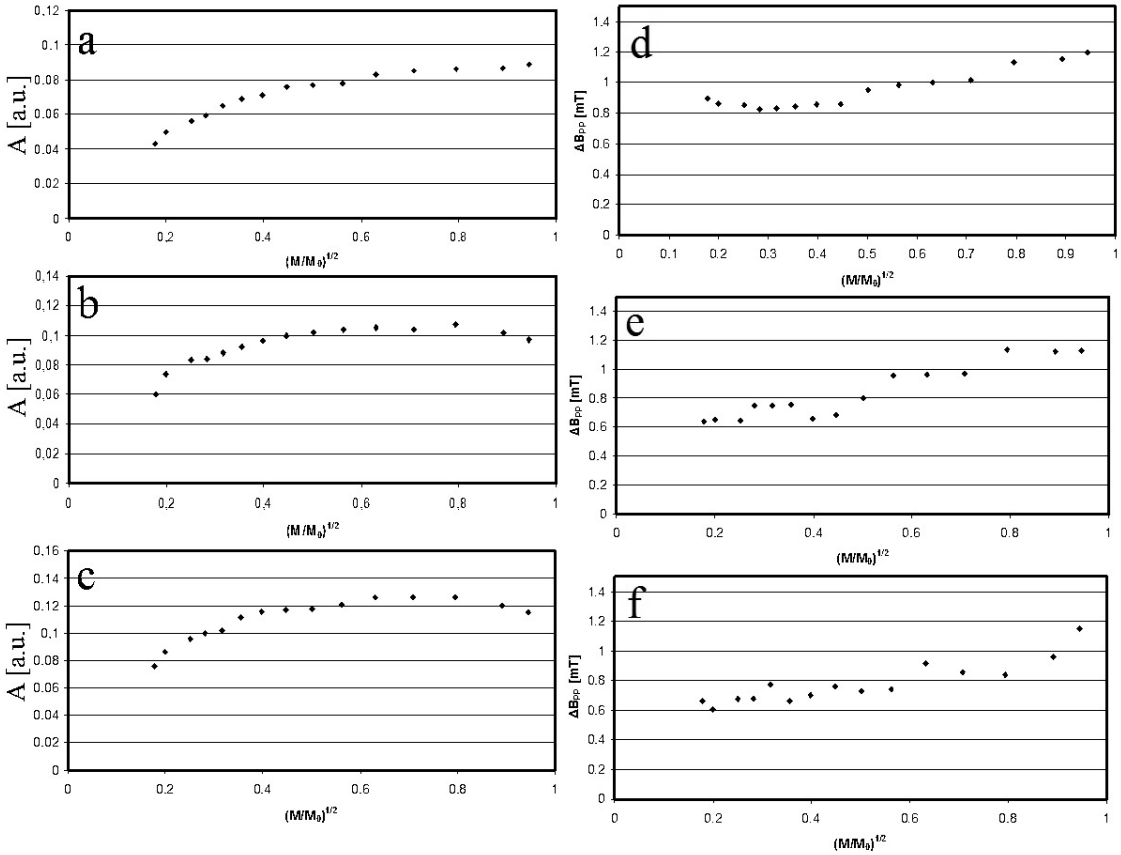

Fig. 7. Influence of microwave power (M) on amplitude (A) and on linewidth (ΔB_{pp}) of EPR spectra of famotidine sterilized at 160°C (120 min) (a, d), 170°C (60 min) (b, e), and 180°C (30 min) (c, f). The measurement was done 20 min after sterilization. M is a microwave power used during the measurement of the EPR spectrum. M_0 is the total microwave power produced by klystron (70 mW).

Free radical concentrations (N) depended on the time of storage of the thermally sterilized drug samples (Fig. 5). The concentration decreased with storage time for famotidine sterilized at 160°C during 120 min. The increase of free radical concentrations in famotidine sterilized at both 170°C during 60 min and 180°C during 30 min were observed after storage of the heated samples. The changes of the free radical concentrations in the sterilized famotidine during storage after thermal sterilization process may be caused by the reaction with oxygen. But the reactions of the samples with oxygen should be tested in the next our work. We think that these reactions are possible in parts of the samples exposed to oxygen. Our hypothesis about oxygen effect on free radicals in the analyzed drug samples should be checked.

Free radical system in thermally sterilized famotidine was complex. The characteristic of multi-component free radical system changes lineshape with increasing microwave power. The changes of the asymmetry parameters A_1/A_2 and B_1/B_2 of the EPR spectra of famotidine sterilized at 160°C (120 min), 170°C (60 min), and 180°C (30 min), are shown in Figs. 6a–6f, respectively. The correlations presented in Figure 6 resulted from the different changes of the individual EPR component lines with increasing microwave power. The shape of the resultant EPR spectra changed with microwave power. It can be seen that different chemical bonds were broken in famotidine during thermal sterilization and different types of free radicals were formed. g-Factors for the tested samples were about 2.00, so the unpaired electrons in the detected free radicals were mainly located on carbon (C). The low spin-orbit coupling was responsible for these g-values [13].

It was observed via drawing the correlations between amplitudes (A) of EPR lines of the heated famotidine and microwave power (M/M_0) that the slow spin-lattice relaxation exist in the drug samples. The influences of microwave power (M/M_0) on amplitudes (A) of the famotidine sterilized at temperatures of 160°C during 120 min, 170°C during 60 min, and 180°C during 30 min, are compared in Figs. 7a–7c, respectively. EPR lines of all the sterilized famotidine samples increase with increasing microwave power and they reach maximal values (Fig. 7). For famotidine sterilized at 170°C and 180°C at higher microwave powers amplitudes started to decrease (Figs. 7b–7c). Such an effect was not observed for famotidine sterilized at temperature 160°C (Fig. 7a). This indicates that the slowest spin-lattice relaxation processes exist in famotidine sterilized at higher temperatures (170°C and 180°C). The slow spin-lattice relaxation processes were also found in thermally sterilized prednisolone [7], diclofenac [8] and tramadole [9].

The influence of microwave power (M/M_0) on linewidths (ΔB_{pp}) of EPR spectra of famotidine sterilized at temperatures 160°C (120 min), 170°C (60 min), and 180°C (30 min) is shown in Figs. 7d and 7f. Linewidths (ΔB_{pp}) of EPR lines of the tested sterilized drug samples, independently of the temperature of heating, increased with increasing microwave power (M/M_0). This correlations (Figs. 7d–7f) are characteristic of homogeneously broadened EPR spectra [3, 12, 13]. Homogeneous broadening of EPR spectra was also observed earlier for prednisolone [7], diclofenac [8] and tramadole [9]. The high values of linewidths are characteristic of strong dipolar interactions in the samples. Such interactions appear for the low distances between free radicals.

The performed spectroscopic studies of thermally sterilized famotidine indicated that the electron paramagnetic resonance (EPR) method is useful to determine the optimal conditions of sterilization of drugs and to characterize their free radical properties and concentrations. We propose EPR spectroscopy as an additional method to microbiological tests of the sterilized drug samples. The thermally sterilized drugs should not contain microorganisms and should not contain a high amount of free radicals. EPR spectroscopy may be used to determine the amount and type of free radicals formed in drugs sterilized according to the Polish Pharmacopoeia Norms [4]. The spectral analysis brings to light the best conditions of sterilization for the individual drugs.

Conclusions

The performed EPR examination of thermally sterilized famotidine pointed out that:

1. Free radicals $4.9–9.8 \times 10^{17}$ spin/g are formed during thermal sterilization of famotidine at temperatures 160, 170, and 180°C.
2. The optimal thermal sterilization conditions of famotidine are the temperature 170°C and time of heating 60 min, because of the lowest free radicals concentrations in this drug sample.
3. During storage of thermally sterilized famotidine, free radical concentrations in the samples changes as a result of interactions with oxygen.
4. Complex system of free radicals exist in thermally sterilized famotidine.
5. Slow spin-lattice relaxation processes and strong dipolar interactions exist in thermally sterilized famotidine.
6. EPR lines of famotidine are homogeneously broadened.

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