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ANALYSIS

FREE RADICAL FORMATION IN SALICYLIC ACID AND HEATING PARAMETERS – APPLICATION OF EPR, UV-VIS, TGA AND COLORIMETRY EXAMINATION TO OPTIMIZE THERMAL STERILIZATION

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Abstract: Salicylic acid heated at different temperatures and times was examined by an X-band (9.3 GHz) EPR spectroscopy, UV-Vis spectrophotometry, TGA and colorimetry test to optimize its thermal sterilization process. Free radical formation (~10^{18} spin/g) during thermal sterilization of salicylic acid according to the pharmaceutical norms at temperature 120°C and time of 120 min were compared with those for heating at the new tested temperatures and times: 130°C and 60 min, and 140°C and 30 min. It was obtained that the relatively lower free radical concentrations characterized salicylic acid heated at temperatures (times): 120°C (120 min), and 130°C (60 min.), than at temperature (time) 140°C (30 min). So treatment at temperature 120°C during 120 min, and temperature 130°C during 60 min, were recommended as the optimal for thermal sterilization of salicylic acid. Salicylic acid should not be sterilized at temperature 140°C for 30 min, because of the highest free radical formation. Free radical systems of thermally treated salicylic acid revealed complex character. Fast spin-lattice relaxation processes existed in heated salicylic acid. Strong dipolar interactions characterized all the heated salicylic acid samples. EPR spectroscopy, UV-Vis spectrophotometry, thermogravimetry, and color measurement may be helpful besides microbiological analysis to optimize thermal sterilization conditions of salicylic acid.

Keywords: salicylic acid, thermal sterilization, free radicals, EPR spectroscopy, UV-Vis spectrophotometry, thermogravimetry, colorimetry test

Salicylic acid (SA) is an organic compound that belongs to the group of aromaticity alpha hydroxy acids and occurs as a white crystalline powder (1). Oral intake of SA causes gastric ulcers (2-4). For this reason, it is used only externally (2-4). SA is used as a disinfectant and fungicide (2-4). SA at higher concentrations is used as a keratolytic agent (2-4). In the pharmacy formulation salicylic acid is mainly used in the form of solutions and ointments (5-7). SA in the pharmaceutical industry is used as a substrate for the production of acetylsalicylic acid (1, 2).

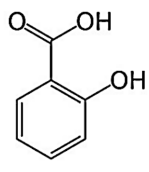

Figure 1. Chemical structure of salicylic acid.

The drugs should be sterile, independent on the way of administration (8-12). Salicylic acid is used in drugs and cosmetics applied on the skin (2-7) so that its sterility seems to be so important. Drugs given to skin diseases should not contain microbes. The sterilization process should be fitted to the type of drug, its stability in the sterilization conditions (8-12).

Polish (8), European (9) and International (10) Pharmacopoeia, WHO norms (11) and the others norms (5, 6, 13, 14) determine sterilization methods and conditions of thermal sterilization of salicylic acid, but they did not consider the formation of free radicals during heating. In our work, we proposed broadening the tests of sterilization products of salicylic acid from the free radical point of view. Sterile salicylic acid after removing of microorganisms by hot air in sterilizer should not contain high amounts of free radicals. Free radicals concentration and properties were tested by us earlier in other drugs,

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for example: chloramphenicol (15), bismuth subgallate (16), boric acid (17, 18), cefaclor (19), and ursodeoxycholic acid (20). We focused on thermal sterilization, because of economic reasons. The other method of sterilization such as radiative sterilization by gamma irradiation of drugs was connected with the higher costs, and free radicals were also produced in the samples (21-23).

The aim of this work was to characterize free radical properties and concentration formed in salicylic acid during heating at different temperatures and times and to optimize thermal sterilization parameters. The best potential temperatures and times for thermal sterilization of SA were searched by the use of EPR spectroscopy, UV-Vis spectrophotometry, thermogravimetry, and colorimetry methods. The performed studies of products of thermal sterilization of salicylic acid were innovatory. We were heading in the direction of electron paramagnetic resonance (EPR) spectroscopy practical application in the pharmacy during the process of production of drugs.

EXPERIMENTAL

Samples

Salicylic acid

Salicylic acid of chemical formula $C_7H_6O_3$ was examined. The molecular mass of tested substance is 138.12 g/mol (1). The density of SA is 1.440 g/cm^3 (20^{o}C) (1). Salicylic acid is soluble in ether, carbon tetrachloride, benzene, propanol, acetone, ethanol, methanol, oil of turpentine, toluene, but is weakly soluble in water (1). The tested substance was purchased from Sigma-Aldrich company.

Thermal sterilization – heating parameters

Salicylic acid was thermally sterilized. The sterilization process was performed in a hot air oven with air circulation, which was the professional apparatus in pharmacy produced by Memmert company (Germany). Recommended norms for thermal sterilization of salicylic acid are temperature 120^{o}C and heating time 120 min (5, 8-10, 13). Additionally, the two other conditions were tested. Salicylic acid was heated at the following temperatures and times: (a) 120^{o}C and 120 min (the proposed by norms), and (b) 130^{o}C and 60 min, and (c) 140^{o}C and 30 min. The two additional conditions (b, and c) were tested to optimize the thermal sterilization process of salicylic acid. It was checked if the better thermal parameters may be found for salicylic acid sterilization. Temperatures and times resulted in the lowest free radical formation in salicylic acid were considered as the optimal parameters of thermal sterilization. The advanced EPR spectroscopic analysis was done for free radicals in heated salicylic acid. The chemical structure of salicylic acid should remain stable after thermal sterilization. The UV-Vis spectra and color measurement (CIE L*a*b* color system) of the non-heated and heated salicylic acid gives us the information in this area.

UV-Vis analysis

UV-Vis spectrophotometer Thermo Genesys 10S produced by Thermo Scientific (Waltham, MA, USA) was used. Methanol solution of salicylic acid in a concentration of 1 mg/100 mL was prepared. The absorption spectra in the wavelength range of λ: 220-400 nm for the non-heated and heated salicylic acid in methanol solution were obtained. The individual measurement was zeroed on methanol. Methanol was purchased from Sigma-Aldrich company.

Thermogravimetric analysis (TGA)

The thermal stability of salicylic acid was determined by thermogravimetric analysis. Thermogravimeter TG 209 F3 Tarsus produced by Netzsch (Germany) was used. The thermogravimetric (TG) and derivative thermogravimetric (DTG) thermograms were recorded for 10 mg of salicylic acid at a heating rate of 10 K/min. in the temperature range of 35-900^{o}C under N_2 atmosphere. The total nitrogen flow rate was 50 mL/min. Al_2O_3 crucible type use for measured.

Color measurements

Color measurement in the CIE L*a*b* color system was used as the research techniques. NH 310 colorimeter produced by 3nh (China) was used. Analyses of color values were done 10 min after the heating process. The results were compared with the unheated salicylic acid. Measurements collected three times for each sample. The received values were averaged.

The parameters lightness (L*), redness (a*), and yellowness (b*), were used to study changes in the color. L* refers to the lightness of the samples and ranges from black (0) to white (100). A negative value of a* indicates green, while a* positive one indicates red color. Positive b* indicates yellow and negative b* indicates blue color (24, 25).

EPR measurements

Preparation of the samples to the EPR spectra detection

The solid-state salicylic acid samples were put into thin-walled glass tubes, especially to EPR

measurements. For the quantitative analysis, salicylic acid samples were weighted. Mass of the individual sample was obtained as the difference between the mass of the tube containing sample and mass of the empty tube. The analytical weight of the CPA Series Sartorius company (Gottingen, Germany) was used.

The glass tubes had high paramagnetic purity. EPR signals were not observed for them at the experimental conditions. The external diameter of the glass tubes was 3 mm.

Parameters and conditions for recorded EPR spectra

EPR spectra for salicylic acid samples were measured at room temperature 15 min after thermal sterilization by the use of electron paramagnetic resonance spectrometer of Radiopan company (Poznań, Poland). The microwaves were from the X-band with a frequency of 9.3 GHz. The microwave frequency was measured by the MCM101 recorder produced by the EPRAD company (Poznań, Poland). The magnetic modulation was 100 kHz. Microwave power was in the range from 2.2 mW to the total microwave power produced by klystron of 70 mW. The attenuations corresponding to these microwave powers were 15 dB – 0 dB. Numerical acquisition of the EPR spectra was performed by the Rapid Scan Unit produced by Jagmar (Kraków, Poland). The EPR spectra were measured as the first-derivative lines.

EPR spectra were measured and analyzed by the use of professional spectroscopic programs of Jagmar company (Kraków, Poland) and LabView of National Instruments company (Austin, TX, USA).

Analyzed parameters of the EPR spectra

The following 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] of the EPR spectra. g-Factors were calculated according to the formula (26, 27): $g = h\nu/\mu_B B_r$, where: h – Planck constant, ν – microwave frequency, μ_B – Bohr magneton, B_r – induction of resonance magnetic field. Integral intensity (I) as the area under the absorption line was obtained by double integration of the first-derivative EPR spectra.

The following shape parameters as the asymmetry parameters: A_1-A_2, and B_1-B_2, were analyzed. Figure 2 shows amplitude (A), linewidth (ΔB_{pp}), and the parameters: A_1, A_2, B_1, and B_2. The influence of microwave power on these parameters was determined.

Free radical concentration – formula and determination

Free radical concentration (N) [$\pm 0.2\times10^{18}$ spin/g] in the heated salicylic acid samples was determined according to the formula (26-28): $N = N_u[(W_u A_u)/I_u]\times[I/(WAm)]$, where: N_u – number of paramagnetic centers in ultramarine (reference); W,

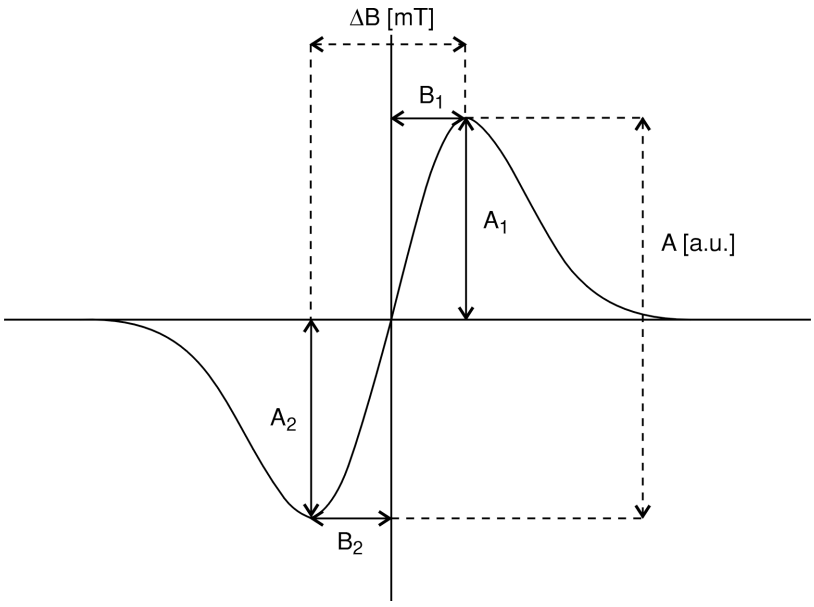

Figure 2. The analyzed parameters of the EPR spectra: amplitude (A), linewidth (ΔB_{pp}), and shape parameters: A_1, A_2, B_1, and B_2.

W_u – receiver gains for the tested drug samples and ultramarine; A, A_u – amplitudes of ruby signal for the tested samples and the ultramarine; I, I_u – integral intensities for the tested salicylic acid samples and ultramarine, m – mass of the salicylic acid sample.

The used reference – ultramarine was paramagnetic stabile and it revealed a strong EPR signal.

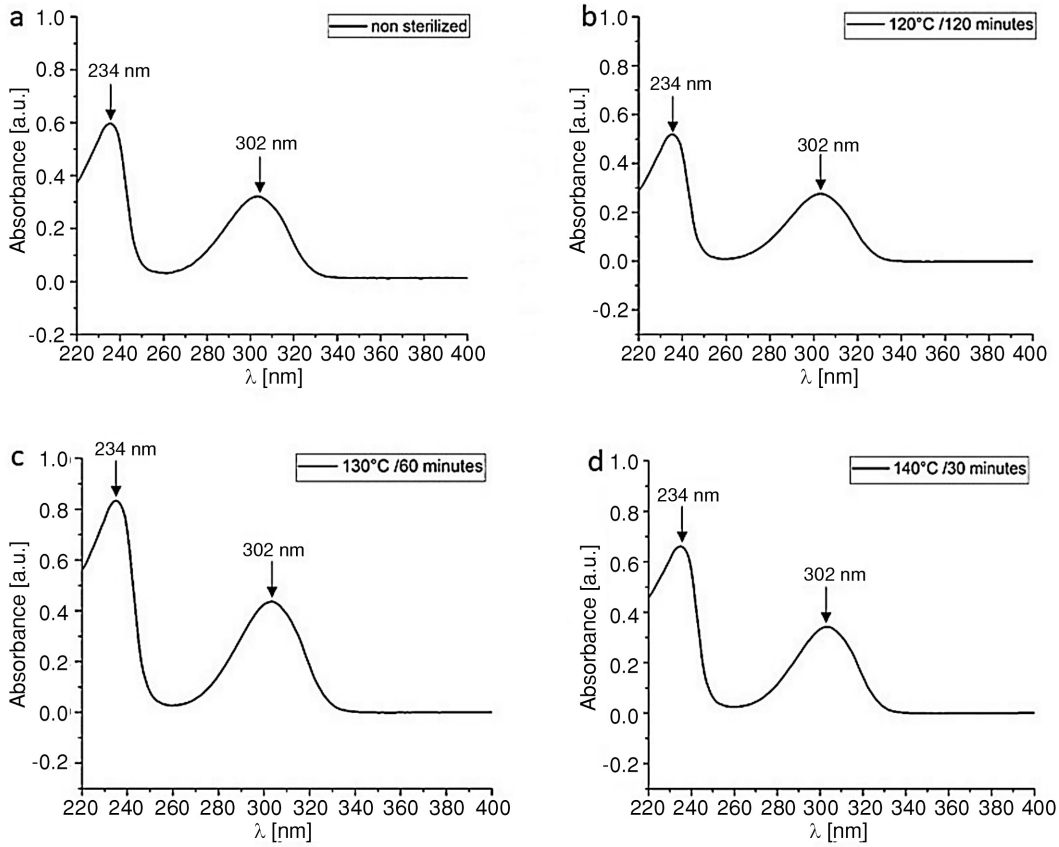

Figure 3. UV-Vis spectra of salicylic acid non-heated (a), and heated at temperatures and times: (b) 120°C, 120 min, (c) 130°C, 60 min, and (d) 140°C, 30 min.

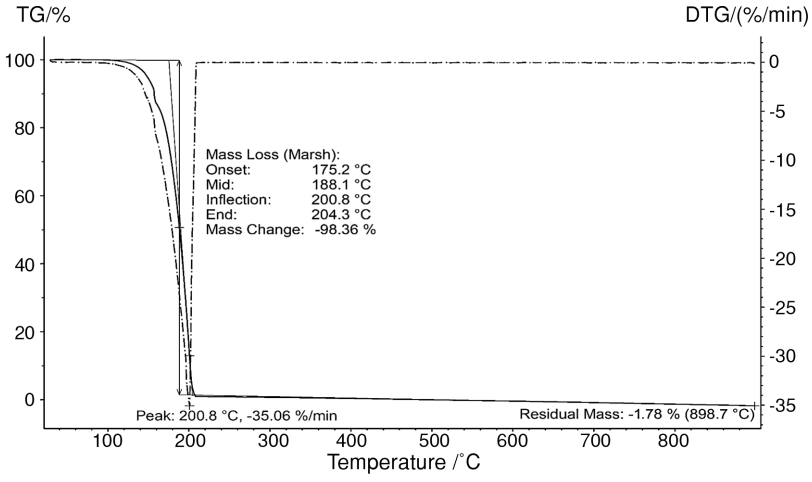

Figure 4. Decomposition of salicylic acid, illustrated by the TG, and DTG curves.

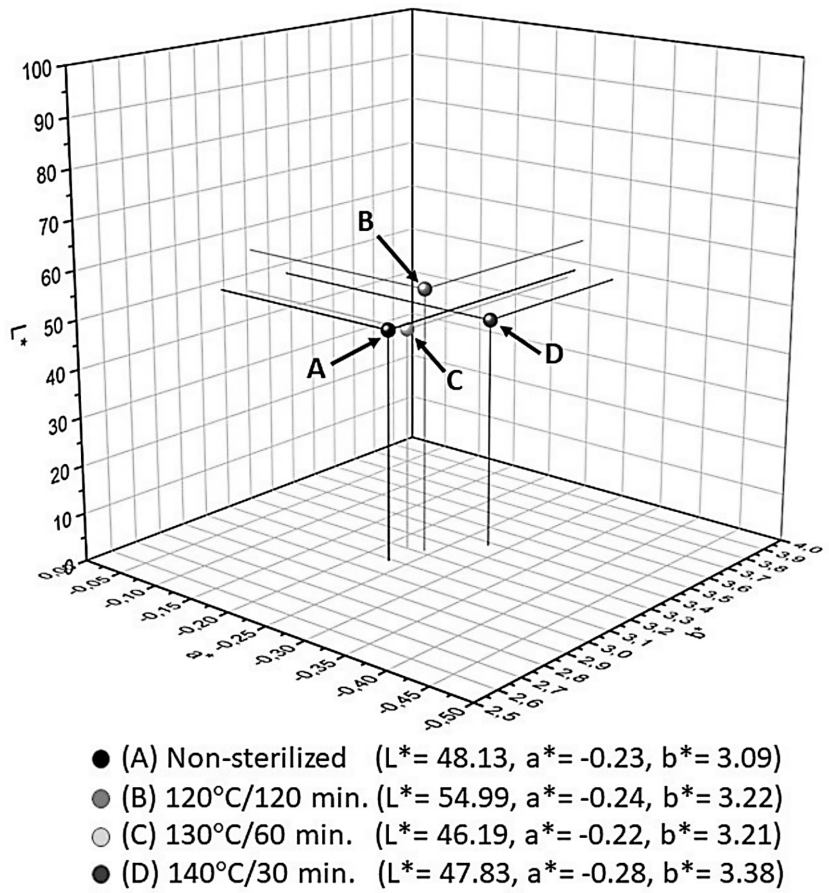

Figure 5. Analysis of color in the 3D (CIE L*a*b*) space for non-sterilized and sterilized salicylic acid.

The second reference, a ruby crystal (Al_2O_3:Cr^{3+}) was permanently placed in the resonance cavity below the samples, and its EPR signals were measured when both ultramarine or salicylic acid were located in the cavity.

RESULT AND DISCUSSION

UV-Vis examination indicated that the used temperatures and times of heating may be acceptable from the point of view of the stability of the chemical structure of salicylic acid. Chemically SA is a 2-hydroxybenzoic acid, contained one hydroxyl group and one carboxyl group in the ortho position (29, 30). Salicylic acid has a free phenyl group, which means that the UV spectrum shows the bathochromic shift of the benzenoid band to a wavelength of 302 nm in methanol (30). Similar UV spectra for salicylic acid in methyl alcohol were recorded at work (31). UV-Vis absorption lines for the original non-treated by higher temperatures salicylic acid and salicylic acid heated at temperatures 120^{o}C for 120 min, 130^{o}C for 60 min, and 140^{o}C for 30 min, were shown in Figure 3 a-d. UV-Vis spectra were similar for the non-heated and heated salicylic acid.

Thermogravimetric analysis curves of salicylic acid were shown in Figure 4. There is only one single mass-loss step representing an almost complete decomposition (residual mass of only 1.78%). The corresponding 1st derivative of the TG curve, DTG, provides the decomposition rate. The DTG peak temperature (200.8^{o}C) is usually used as a characteristic value to specify the appropriate step. The salicylic acid onset the mass lost at 175.2^{o}C, and end the mass lost at 204.3^{o}C. The mass change of the decomposition process of salicylic acid was 98.36%. Similar results were obtained at work (32). Lindquist E. and Yang Y. showed the degradation of salicylic acid in water solution at 150^{o}C for 30 min is 5% (33).

The location of colors in CIE L*a*b* space is illustrated in Figure 6. Axes L*, a* and b* define the 3D color space CIE. So, if coordinates L*, a* and b*

are known, then the color is described and also located in space. Inside this system, any color can be denoted through coordinates L*, a* and b* (25).

Measurement in the CIE L*a*b* color system has shown that the temperatures and heating times may be acceptable from the point of view of the salicylic acid color change. The CIE L*a*b* parameters were similar for the non-heated and heated salicylic acid. Most similarly CIE L*a*b* color parameters for non-heated salicylic acid have salicylic acid heated at 130°C for 60 min (new proposed conditions) (Fig. 5). The most different CIE L*a*b* color parameters for non-heated salicylic acid have salicylic acid heated at 140°C for 30 min (Fig. 5).

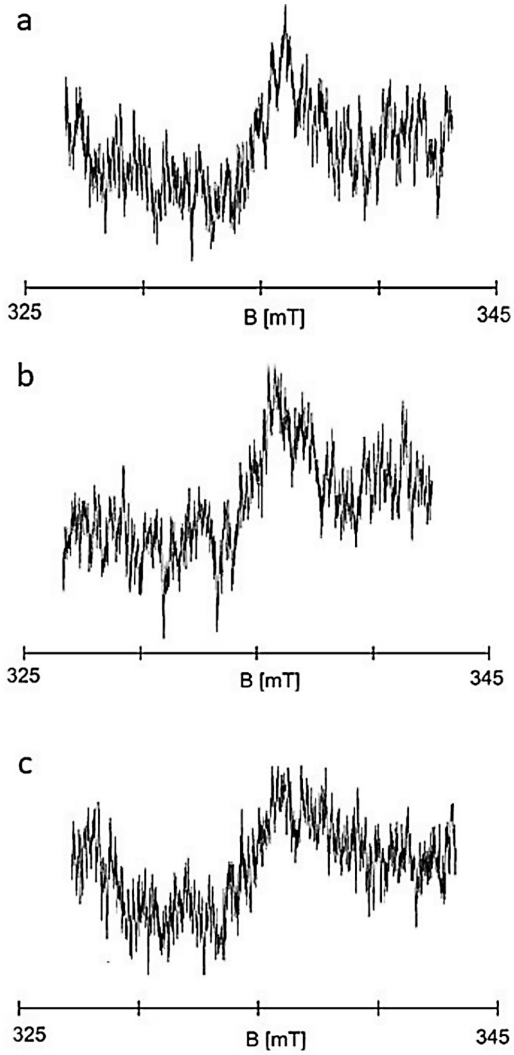

Figure 6. EPR spectra of salicylic acid heated at temperatures and times: (a) 120°C, 120 min, (b) 130°C, 60 min, and (c) 140°C, 30 min. **B** – magnetic induction. The spectra were measured with a microwave power of 2.2 mW.

Table 1. Parameters of the EPR spectra of salicylic acid heated at different temperatures: amplitudes (A), integral intensities (I), and linewidths (ΔB_{pp}). T, t - temperature and time of heating, respectively. Data for the EPR spectra measured with a microwave power of 2.2 mW (attenuation of 15 dB) were presented.

T [°C]	t [minutes]	A [± 0.01 a. u.]	I [± 0.02 a. u.]	ΔB_{pp} [± 0.02 mT]
120°C	120	0.24	1.13	0.98
130°C	60	0.18	0.81	0.84
140°C	30	0.20	2.32	1.31

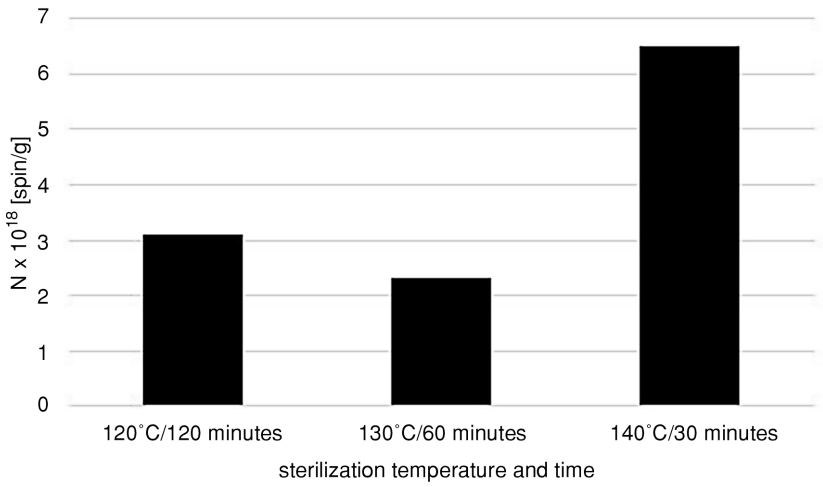

Figure 7. The effect of temperature and time of thermal treatment on free radical concentrations (N) in salicylic acid. Temperatures and times of heating were: (a) 120°C and 120 min, (b) 130°C and 60 min, and (c) 140°C and 30 min.

EPR spectra were not obtained for the non-heated salicylic acid, because of the absence of unpaired electrons in its structure (1). The heating of salicylic acid caused free radical formation in the samples independent of temperature and time. EPR spectra with g-factors near 2 characteristic for free radicals appeared after the thermal treatment of salicylic acid. EPR spectra of salicylic acid heated at temperatures (times): 120°C (120 min), 130°C (60 min), and 140°C (30 min), were shown in Figure 6 a-c. Parameters of the EPR spectra: amplitudes (A), integral intensities (I), and linewidths (ΔB_{pp}), were compared in Table 1. The lower amplitude (A), integral intensity (I), and linewidth (ΔB_{pp}), revealed EPR spectrum of salicylic acid heated at temperature 130°C for 60 min (Table 1). The highest integral intensity (I) was obtained for salicylic acid heated at temperature 140°C for 30 min. All the EPR lines were broad with linewidths (ΔB_{pp}) in the range 0.84-1.31 mT (Table 1). Strong dipolar interactions between free radicals were responsible for line broadening. The higher dipolar interactions were observed from the highest line broadening for salicylic acid thermally treated at temperature 140°C for 30 min. The relatively weakest dipolar interactions existed in salicylic acid heated at temperature 130°C during 60 min, because of the relatively lowest linewidths (ΔB_{pp}). Broad EPR lines were also observed by us for the other heated drugs, for example for chloramphenicol (15) and boric acid (17).

Concentrations of free radicals (N) in the salicylic acid samples depended on temperature and time of heating. Free radical concentrations (N) in salicylic acid thermally treated at temperatures 120°C for 120 min, 130°C for 60 min, and 140°C for 30 min, were compared in the diagram in Figure 7. Free radical concentrations (N) in thermally treated salicylic acid were ~10^{18} spin/g. The relatively lower free radical concentrations (N) characterized salicylic acid thermally sterilized according to the pharmaceutical norms at temperature 120°C for 120 min (3.1×10^{18} spin/g), and according to proposed by us new conditions at temperature 130°C for 60 min (2.3×10^{18} spin/g). These above two conditions may be used for thermal sterilization of salicylic acid. The highest free radical concentration (N) obtained for salicylic acid thermally treated at temperature 140°C for 30 min. The UV-Vis spectra and EPR examination let us recommend the new parameters of thermal sterilization of salicylic acid as a temperature of 130°C and time of 60 min. This temperature and time generated the least free radicals in thermally sterilized salicylic acid. The chemical structure was not destroyed at these conditions (Fig. 3) and free radical concentration (N) was the lowest (Fig. 7). The negative result was obtained by us for temperature 140°C and heating time 30 min for thermal sterilization of salicylic acid.

EPR spectra of salicylic acid depended on microwave power used during the measurement. The changes of amplitudes (A) of the EPR spectra of salicylic acid heated at temperatures 120°C for 120 min, 130°C for 60 min, and 140°C for 30 min, were presented in Figure 8 a-c, respectively. Amplitudes (A) increased with the increase of microwave power, and the effect of microwave saturation of the EPR lines was not observed. Such correlation was characteristic for fast spin-lattice relaxation process-

es in the samples (26-28). The similar fastness of spin-lattice relaxation processes in salicylic acid heated at different conditions pointed out that the chemical structure was saved. The changes of linewidths (ΔB_{pp}) of the EPR spectra of salicylic acid heated at temperatures 120°C for 120 min, 130°C for 60 min, and 140°C for 30 min, were presented in Figure 8 d-f, respectively. The linewidths (ΔB_{pp}) of the EPR spectra increased with increasing of microwave power for all the heated salicylic acid samples. It was characteristic for homogeneously broadened EPR lines (26-28).

The shape of the EPR spectra of salicylic acid was modified by the increase of microwave power. The influence of microwave power on the shape parameters: A_1-A_2, and B_1-B_2, for the EPR spectra of salicylic acid thermally treated at temperatures 120°C during 120 min, 130°C during 60 min, and 140°C during 30 min, were shown in Figure 9 a-c, and e-f respectively. All the tested parameters depended on microwave power and the character of the changes was visible in Figure 9. The changes in spectral shape with microwave power indicated the complex character of the free radical system in the heated salicylic acid. Free radicals with different localization of unpaired electrons existed in the samples. Taking into account its chemical structure (1), it may be said that mainly free radicals with unpaired electrons on carbon (C) and oxygen (O) atoms appeared in salicylic acid during thermal treatment. The complex structure of the free radical system was also observed by us for chloramphenicol (15), cholic acids (20), ampicillin (34), neomycin (35). This effect was probably the result of the thermal rupturing of different chemical bonds in the samples. Reactions with oxygen may be also taken into account.

The results obtained in this work pay attention to the important meaning of examination of thermal sterilization products by major experimental tech-

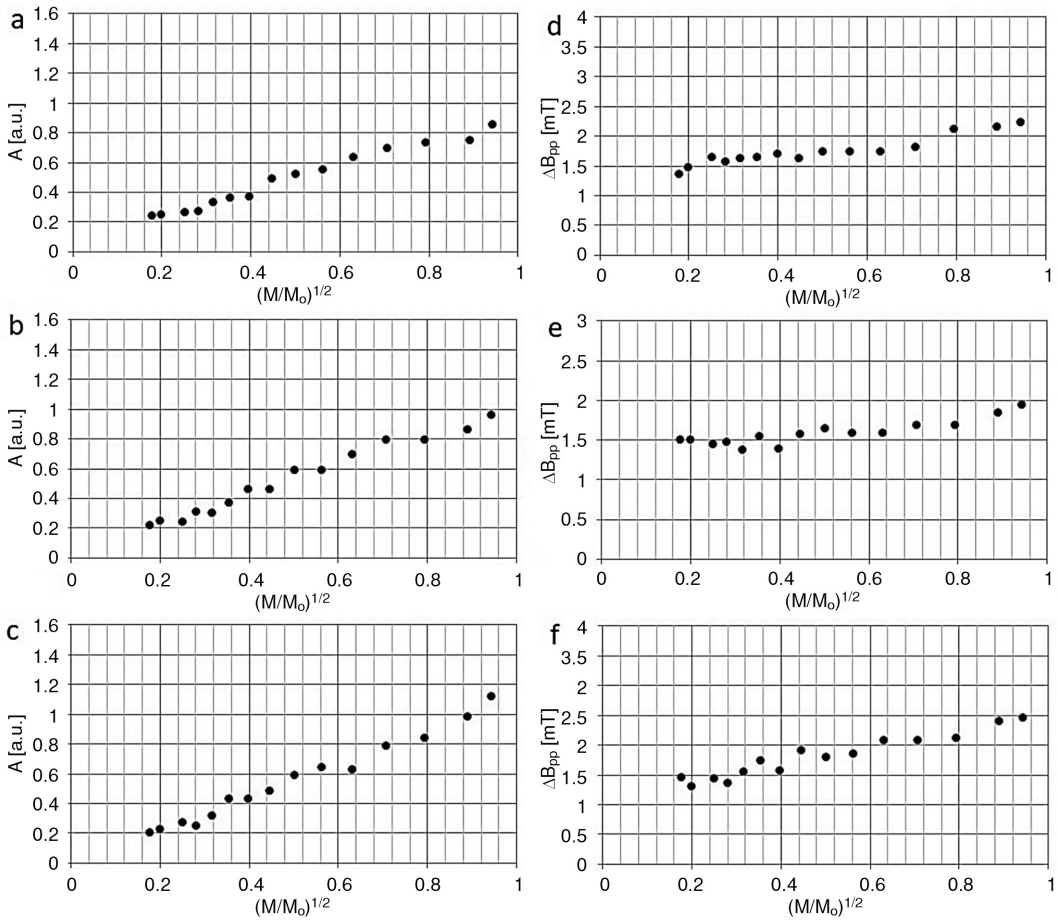

Figure 8. The influence of microwave power (M/M_o) on amplitudes (A) (a-c) and linewidths (ΔB_{pp}) (d-f) of the EPR line of salicylic acid heated at temperatures and times: (a, d) 120°C and 120 min, (b, e) 130°C and 60 min, and (c, f) 140°C and 30 min. M_o, M – the maximal microwave power (70 mW), and microwave power used during the measurement of the EPR spectra, respectively.

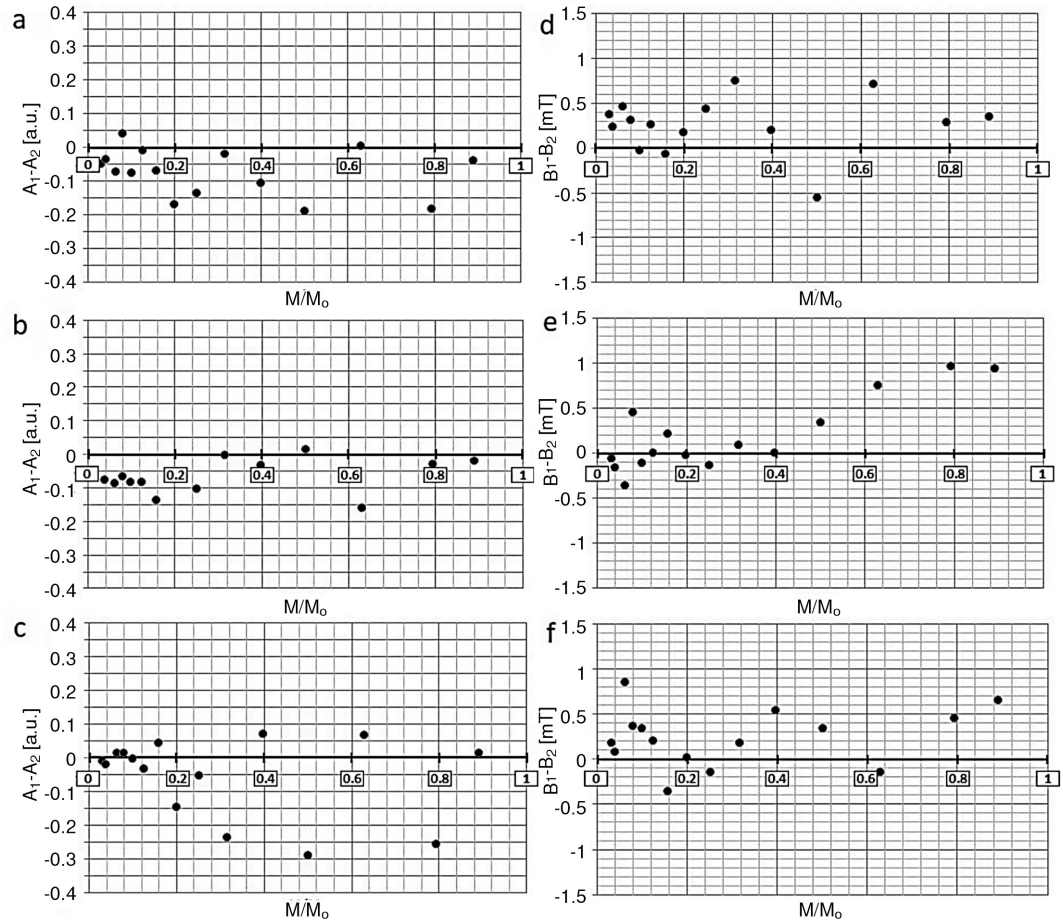

Figure 9. The influence of microwave power (M/M_o) on the shape parameters A_1-A_2 (a-c) and B_1-B_2 (d-f) of the EPR line of salicylic acid heated at temperatures and times: (a, d) 120°C and 120 min, (b, e) 130°C and 60 min, and (c, f) 140°C and 30 min. M_o, M – the maximal microwave power (70 mW), and microwave power used during the measurement of the EPR spectra, respectively.

niques and methods. UV-Vis, TGA, colorimetry, and EPR methods were useful, together with microbiological analysis, in optimization of the thermal sterilization process for salicylic acid. The high quality of the sterile product of thermal sterilization according to norms (5, 6, 8-11, 13) was confirmed. The new parameters of thermal sterilization, resulted in the low formation of free radicals in salicylic acid, was proposed. The other parameters, because of high free radical production, were rejected.

CONCLUSIONS

The UV-Vis, TGA, colorimetry and EPR examination of salicylic acid pointed out that:

1) Free radicals (~10^{18} spin/g) were formed during heating of salicylic acid at temperatures (times): 120°C (120 min), 130°C (60 min), and 140°C (30 min).
2) Thermal sterilization of salicylic acid may be performed at temperatures (times): 120°C (120 min) (norms) and 130°C (60 min) (the new proposed conditions), because of the lowest free radical formation. Sterilization process carried on the new proposed conditions (130°C, 60 min) produced the lowest free radical concentration in heated salicylic acid. UV-Vis, TGA and colorimetry examinations confirmed thermal stability of salicylic acid at the tested sterilization conditions.
3) Temperature 140°C and heating time 30 min were not recommended for thermal sterilization of salicylic acid, because of the highest free radical formation.
4) The influence of microwave power on EPR spectra of heated salicylic acid indicated fast spin-lattice relaxation processes in the samples.
5) Strong dipolar interactions between unpaired electrons of free radicals existed in thermally

treated salicylic acid, because of their broad EPR lines. The lowest dipolar interactions characterized salicylic acid heated at temperature 130°C for 60 min. The strongest dipolar interactions were obtained for salicylic acid heated at temperature 140°C for 30 min.

6) EPR spectroscopy, UV-Vis spectrophotometry, thermogravimetry, and color measurements may be used together with microbiological analysis during optimizing thermal sterilization conditions of salicylic acid.

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Conflict of interests

The authors declare no conflict of interest.

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