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The Comparative Study of The Effects of Extremely Low Frequency Electromagnetic Fields and Infrasound on Water Molecule Dissociation and Generation of Reactive Oxygen Species

Final Project Technical Report

on the work performed from 02.01.2008 to 01.31.2009

Life Sciences International Postgraduate Educational Center

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14. ABSTRACT: Conclusions:
1.Temperature dependency of SEC in DW is higher than in PS. If the temperature dependency of DW SEC could be considered as a result of the increase of water molecules dissociation, in case of PS, SEC depends on ion hydration and velocity.
2.Heat fusion and melting rate of DW and PS depends on their initial temperature. This dependency is higher in case of PS than in DW.
3.The temperature-dependent decrease of CO_2 solubility in DW is more pronounced in range of temperature at 0-10oC , while in case of PS at 10-20oC.
4.The temperature dependency of H_2O_2 formation in DW was not pronounced while in case of PS H_2O_2 formation took place in range of 4-10oC temperature. It is worth to note that at 4 oC, when PS has maximum density, the H_2O_2 content in PS was less than at 0 oC.
5.At LBGR during the first 30 min the rate of water SEC decay was gradually increased and then stabilized, while after transferring the samples at NBGR the water SEC started to decrease.
6.The ELF EMF has frequency-dependent depressing effect on SEC of DW and this effect was more pronounced at 4 and 8 Hz frequencies. Such similarity of the effects of 4 and 8Hz on water SEC seems extremely interesting and could be a subject for more detailed investigation.
7.At LBGR we observed the time-dependent decrease of PS SEC, like as in case of DW SEC. However, in case of PS more pronounced effect was observed only at the frequency of 9 Hz, while in DW these frequency windows appeared at 4 and 8 Hz.
8.The frequency-dependent effect of IS on SEC of DW at LBGR shows the pronounced elevation effect at 1 and 4 Hz, while in case of PS 9Hz IS has maximum depressing effect on it.
9.The 1-10 Hz IS has elevation effect on SEC of PS at LBGR. This effect was less pronounced at 4Hz.
10.The effect of EMF treatment on melting process of DW at LBGR was significantly depressed (or disappeared) at 2-100 Hz. LBGR has depressing effect on EMF-induced heat fusion changes, as well as delays the 50 and 100 Hz EMF-induced DW melting process.
11.At NBGR EMF exposure leads to the decrease of H_2O_2 content in DW at 1, 7, 20 and 50 Hz and to the increase at 4 and 50 Hz. At LBGR we had reversed picture: the H_2O_2 content at 1, 7, and 20 Hz was closer to the control level and 50 Hz EMF had the maximal elevation effect on H_2O_2 formation in DW
12.The effect of EMF on H_2O_2 level in PS is time dependent: 1 Hz EMF exposure of PS during 10 minutes at LBGR depresses the H_2O_2 as compared with the control one, while 20-minute-exposure at the same EMF frequency has elevation effect on it.
13.As in case of DW, EMF has opposite effect on H_2O_2 content in PS at LBGR, as compared with NBGR, except 1 and 10 Hz frequency at which the H_2O_2 level is equal to the control one.
14.The above presented data show that 3Hz IS has elevation effect on H_2O_2 formation at NBGR while at LBGR it has strong depressing effect on it.
15.There are frequency windows (4 and 20 Hz) of ELF EMF and IS effects on water SEC. The EMF and IS effect on water SEC is decreased as a result of background radiation. The depressing effect of the latter was delayed at 4 and 8Hz EMF.
16.16.1 The IS pretreated DW samples have high heat fusion at 5 and 10 Hz ,while the PS has low heat fusion at 1, 3 and 10 Hz frequencies as

compared with non-treated DW and PS, correspondingly.
16.2 The EMF-pretreated DW samples have low heat fusion at 2 and 4 Hz, while the PS samples, like as in case of IS-exposure, at 1, 3 and 10 Hz than control samples.
17.17.1 Hz IS has stimulation while 8 Hz depressing effect on H2O2 formation in DW, while in case of PS IS has depressing effect on H2O2 formation at all frequencies. However in case of 5, 6 and 10 Hz this depression was less pronounced.
17.2 EMF has depressing effect on H2O2 formation in DW at less than 50 Hz frequency, except 4Hz, while at higher frequencies (60-100 Hz) it has elevation effect on H2O2 formation , but in PS, the activation effect was observed only in case of 10 Hz EMF. The rest of observed frequencies showed the decrease of H2O2 content. The most pronounced depressing effect was observed at 8 Hz.

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LIST OF CONTENTS

1. Brief description of the work plan: objective, expected results, technical approach

Objective

The present project had a basic research character, which was devoted to the study of the mechanisms of the effect of non-ionizing (extremely low frequency electromagnetic field and infrasound) radiation and gas composition of the medium on water dissociation and hydrogen peroxide (H_2O_2) formation in water and water solutions.

Expected results

Our little knowledge upon the effect of extremely low frequency electromagnetic field (ELF EMF) and infrasound (IS) on physicochemical properties of water and water solutions (which is the dominant component of cell) is the main barrier for precise estimation of beneficial and harmful effects of these factors on cell and organism (from the point of public health and biotechnology). On the basis of the obtained data it would be possible to estimate the messenger role of cell bathing medium in realization of the biological effect of ELF EMF and IS on cells and organisms and to determine their beneficial and harmful effects on the latter.

The obtained data on high sensitivity of physicochemical properties of cell bathing aqueous medium to ELF EMF and IS and the possibility to modulate their effect by different weak environmental factors, like as light, temperature, background radiation (BGR), gas composition could serve as a powerful tool for environmental terror. Therefore, the data, obtained during the project implementation, would make possible to take precautions against such attacks. The above mentioned properties of water can be used for early detection as well as for realizing of the mechanism of human made and natural catastrophes such as earthquake, tsunami, storm etc.

Technical Approach

The project offers a hypothesis according to which water molecule dissociation serves as a universal and extrasensitive target for ELF EMF and IS effects and the latter could modulate H_2O_2 formation, through which their biological effects are realized. It was suggested that the knowledge of the mechanism of the effects of ELF EMF and IS on water molecule dissociation and H_2O_2 formation would give a chance to understand the messenger role of cell bathing aqua medium in generation of their biological effects on cell and organisms. For this purpose the frequency-dependent effects of ELF EMF and IS on specific electrical conductivity (SEC), heat fusion, gas solubility (O_2 and CO_2) and H_2O_2 formation in distilled water (DW) and physiological solution (PS) in different (temperature, background radiation light) were studied.

2. Method, Experiments, Theory.

A special setup for DW and PS SEC measurement and treatment by ELF EMF and IS, presented on figure 1, was assembled.

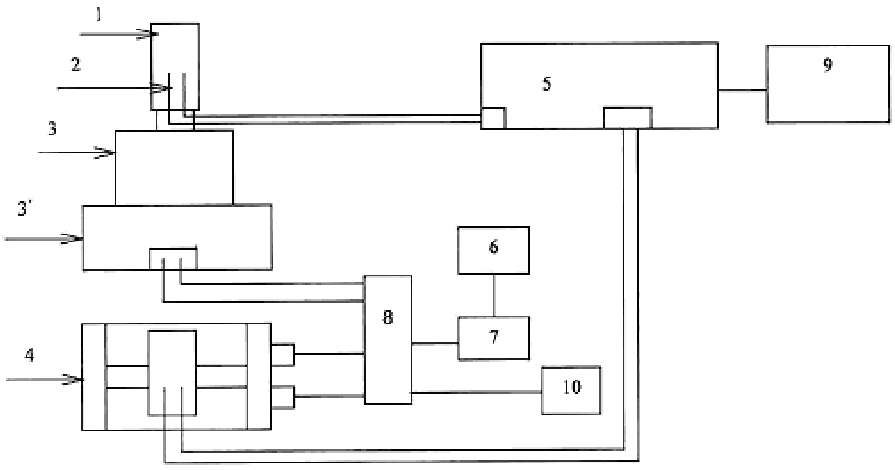


Figure 1: Setup for DW and PS treatment by EMF and IS

1. Platinum electrodes
2. Mobile part of the vibrator
3. Motionless part of the vibrator
3. The coil
4. The device for the measurement of DW SEC (conductometer)
5. Generator of sinusoid vibration
6. The low-noise amplifier
7. The switch (has 2 positions: I and II, where I- EMF and MV and II- EMF)
8. Personal Computer
9. The generator of a constant field.

SEC of DW and PS was measured by a special device "Biophys-conductometer" (production of LSIPEC, Armenia). The device has the following scheme: three glass test tubes with diameter of 10 mm and volume of 10 ml, with two platinum electrodes inside are used. Platinum electrodes- plates with the area 100 mm2, located on 5 mm distance from each other, are connected with the conductivity-measuring device capable to determine SEC of water and other liquids at currents less than the 10^{-9} A. As the conductivity of water is measured in micro power modes, the application of low-noisy voltage amplifier of alternating current in the device raises the accuracy of measurement due to exception of self-heating influence. For the continuous recording of SEC the output of a measuring device is connected to the PC through Digidata 1322A data acquisition system.

Glass test tube with 10 mm diameter and 10 ml volume was used. The vibrator (IS) is controlled by the sine-wave generator (GZ-118, Made in Russian Federation), the signal goes to the double pole switch: in position I the generator functions as EMF and IS sources, while in position II – as IS source. To obtain IS waves the vibrating device is used generating vertical vibrations by set frequency and intensity. The vibrator is constructed in the department of engineering at LSIEC on the basis of the IVCh-01 device (Russian production). To keep vibration intensity constant (30 dB) at different frequencies, a coil with a feedback amplifier system (IRPhE, Yerevan, Armenia) is used. Thus, IS is transmitted to the test tube containing DW with insignificant power dissipation. For concordance of high impedance output of generator to low impedance input of vibrator, a special power amplifier (IRPhE, Yerevan, Armenia) is used. IS frequency is controlled by a cymometer (CZ-47D, production of Russian Federation), while the intensity is measured by a measuring device (IRPhE, Yerevan, Armenia) having a sensor on the vibration table. It makes possible to keep the intensity of IS on stable level at all frequencies, including resonance frequency (more than 200Hz for the given setup).

EMF is generated by the controlled generator and low-noise amplifier on the coil. The coil has a cylindrical form with 154 mm in diameter and 106 mm in height. The coil consists of Helmholtz rings generating the homogeneous magnetic field. Rings of Helmholtz are formed by two equal ring coils located coaxially and parallel. The distance between ring coils is equal to their radius (77 mm). The magnetic field created by these rings has high homogeneity, for example, at a distance of 0.25 cm from the center of an axis strength differs from computed by formula only by 0.5 %.

$$H = 71.6 \cdot \omega \cdot \frac{I}{R}.$$

<u>Time-dependent changes of thermal capacity</u>

For studying the time-dependent changes of thermal capacity of ELF EMF- and IS-pretreated DW and PS after freezing in liquid N_2 the following method was used: the plastic tube (Vol. 1 ml) with a hermetic cup having a thermo-sensor at the bottom was fixed in another plastic tube (vol. 100ml) and was inserted into the well containing liquid N_2. After withdrawing the tube from the liquid N_2 the hermetic cup of the tube was opened and left for melting at room temperature. The temperate recording was performed by extrasensitive thermometer Biophys-TT (production of LSIPEC, Armenia), connected to the PC through Digidata 1322A data acquisition system (Axon Instruments, USA).

<u>The chemo-luminescence method of measurement of H_2O_2 content</u>

The content of H_2O_2 in aqueous solutions was measured using a sensitive assay based on enhanced chemiluminescence method in a peroxidase–luminol–p-iodophenol system. This method allows one to detect hydrogen peroxide at a concentration lower than 0.1 nM. The chemiluminescence was quantified with a Beta-1 liquid scintillation counter (Walac 1450 Finland). The efficiency of the counting of the tritium standard in this regimen is 34% against the background of 5000 cpm. Samples of solutions (each 10 ml) were treated by ELF EMF and IS at a fixed frequency in of range of 1-100 Hz for 10-20 min in 20-ml liquid scintillation counter vials (Beckman). To measure chemiluminescence after the treatments, 10 ml of a ‘‘count solution’’ containing 10 mM Tris-HCl buffer, pH 8.5, 50 µM p-iodophenol (Aldrich), 50 µM luminol (AppliChem, Germany), and horseradish peroxidase (Sigma) (1 nM for nanomolar hydrogen peroxide measurement) was added. The count solution was prepared immediately prior to measurement. Three or more samples of the solution were measured in each experiment, and the means and standard errors of the means were determined.

O_2 content and pH (CO_2 solubility) were measured by Oxometer WPIS ISO2 and pH-meter NPL-113(Russian production), correspondingly.

For studying the effects of background radiation and light the experiments were performed in normal light condition and background radiation (18-19 µRoentgen/hour), in lead box (inside less than 1 µRoentgen/hour) and in wooden dark box.

3. Results

The comparative study of temperature dependency (0-50^{0}C) of physicochemical (dissociation- SEC and pH; freezing and melting points and kinetics; thermal capacity; solubility of O_2 and CO_2), crystallographic and H_2O_2 formation properties of distilled water and physiological solutions was performed.

I. The comparative study of temperature-dependent (0-50^{o}C) changes of physicochemical crystallographic and H_2O_2 formation properties (dissociation- SEC and pH; freezing and melting points and kinetics; thermal capacity; solubility of O_2 and CO_2), of DW and PS.

1. Temperature-dependent changes of Specific Electrical Conductivity of DW and PS

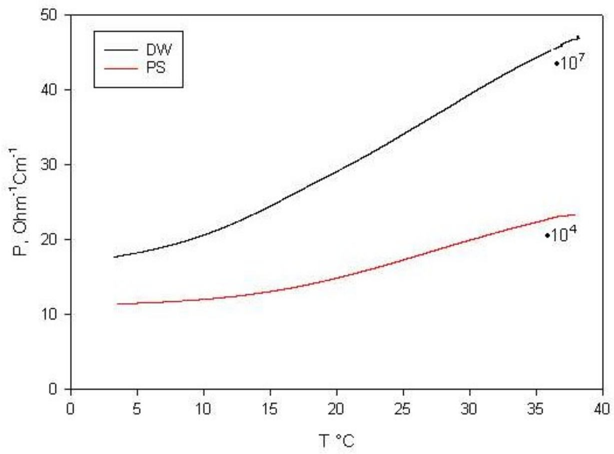


Figure 2: Temperature-dependent changes of SEC of DW and PS.

It is known that temperature elevation leads to the decrease of viscosity of aqua medium, which brings to the increase of water molecules dissociation, while the decrease of water dielectric permeability brings to the decrease of its dissociation. However, in sum of these processes the temperature elevation leads to the increase of SEC of electrolytes. At the same time it is known that by decreasing the temperature until 4^{o}C water density increases. As it can be seen from Figure 2, there is a linear dependency of SEC of DW on its temperature at 10^{o}C and higher, while in PS linear-dependent zone appears after 15^{o}C. Thus, on the basis of these data we suggest that temperature elevation could change the number of osmotic particles in water as a result of new substance formation. From literature data the candidate for new forming substances could serve reactive oxygen species (ROS), namely the H_2O_2, having long lifetime (Chaplin 2009). For testing this hypothesis the following series of experiments of the temperature effect on H_2O_2 content in water were studied.

2. The melting kinetics of DW and PS at room temperature after freezing in liquid N_2

As heat fusion rises with the increase of polarity of molecules and especially with the formation of hydrogen bond, heat fusion was suggested as a sensitive marker for the detection of the level of ion hydration in water.

A B

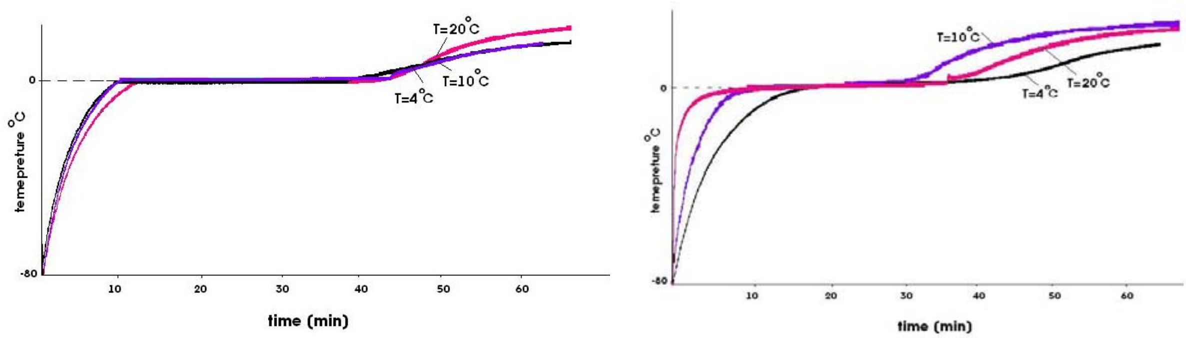


Figure 3: The melting kinetics of DW (A) and PS (B) at room temperature after freezing in liquid N_2, depending on the initial temperature before freezing.

As it can be seen in Figure 3 specific thermal capacity of frozen crystals of PS strongly depends on its initial temperature before freezing, while for DW this dependency is less pronounced. It is interesting to note that the fusion capacity of DW at initial temperature of 20^{o}C is comparatively higher than at initially colder temperatures (4 and 10^{0}C), while at PS this dependency was reversed.

3. The temperature-dependent changes of CO_2 solubility on DW and PS

The comparative study of temperature-dependent solubility of CO_2 in DW and PS was observed at 0°C, 10°C and 20°C conditions.

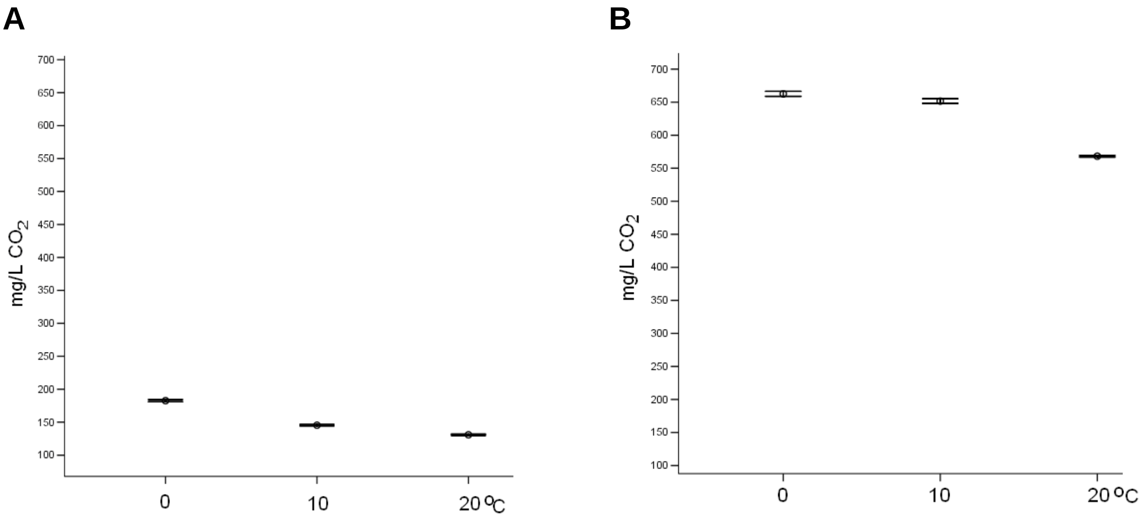


Figure 4: The temperature-dependent changes of CO_2 solubility in DW (A) and PS (B). n=10

As it is predicted temperature elevation leads to the decrease of CO_2 solubility in DW and PS. However, this dependency for DW was more pronounced in range of 0-10°C temperature, while in case of PS at 10-20°C.

4. The temperature-dependent formation of H_2O_2 in DW and PS

The concentration (in nM) of hydrogen peroxide in DW and PS was determined at 0, 4, 6, 10 and 20 ^{0}C. The obtained experimental results showed that the temperature increase had no significant effect on peroxide formation in DW (Figure 5A), while in PS the temperature-dependency exists (Figure 5B). It is interesting to note that at 4 ^{0}C, when water has maximum density the level of peroxide formation is low, which is elevated by the increase of temperature. This dependency is more pronounced in range of 4-10 ^{0}C.

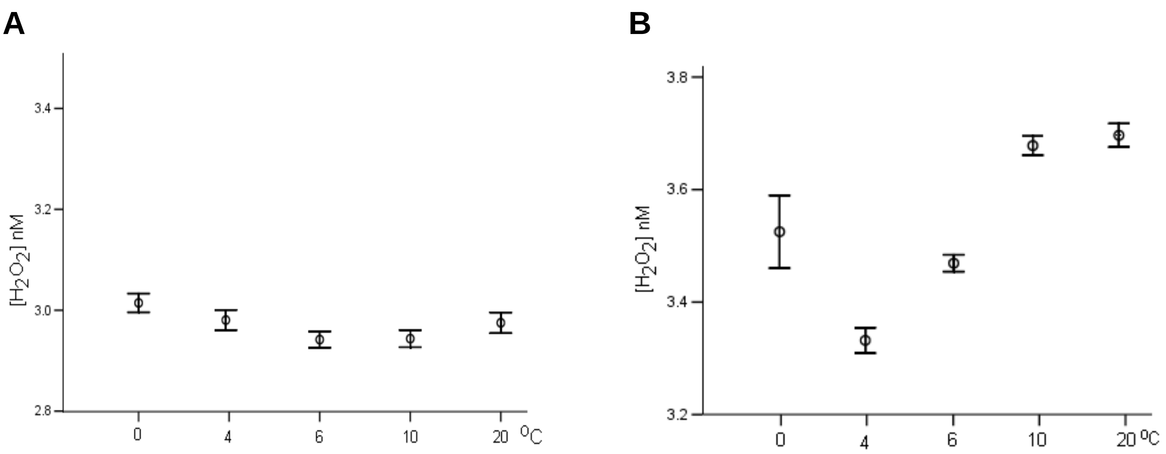


Figure 5: The temperature-dependent changes of hydrogen peroxide concentration in DW (A) and PS (B). n=10

II. The comparative study of frequency-dependent (1-100 Hz) effect of ELF EMF and IS on physicochemical, crystallographic and H_2O_2 formation properties of distilled water and physiological solution at normal background radiation (16-18 µRoentgen/hour).

1. The frequency-dependent effect of ELF EMF and IS on SEC of DW and PS

In our previous studies it was shown that ELF EMF and IS at frequencies less than 100 Hz have depressing effect on water conductivity which was accompanied by the increase of pH, as a result of the decrease of CO_2 solubility (Stepanyan et al.1999; Akopyan & Ayrapetyan 2005).

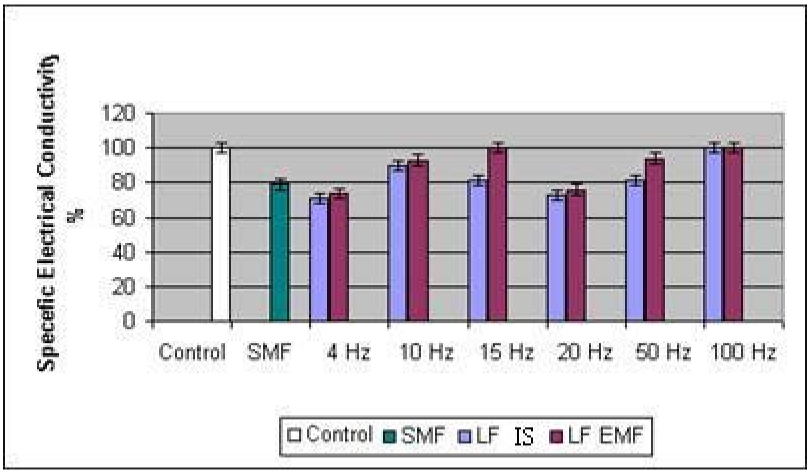


Figure 6: The frequency-dependent effect of IS and ELF EMF on SEC of DW

As it can be seen in Figure 6, the depressing effect on SEC of water is more pronounced at the frequencies of 4 and 20 Hz. It was also shown that water SEC sensitivity depends on water freshness: it is higher in fresh and lower in 3 and 6 day-DW. To find out the reason of freshness-dependency of water SEC we have studied the effect of background radiation on water SEC and its sensitivity to EMF and IS. For this purpose fresh DW samples were stored for a day in led chamber in which background radiation was less than 1 µRoentgen/hour at room temperature and then were transferred to laboratory room with background ionizing radiation of 16-18 Roentgen/hour. The time-dependent decrease of water SEC was observed after transferring the water samples from low to normal background radiation medium and this rate was significantly inhibited upon EMF (Figure 7) and IS.

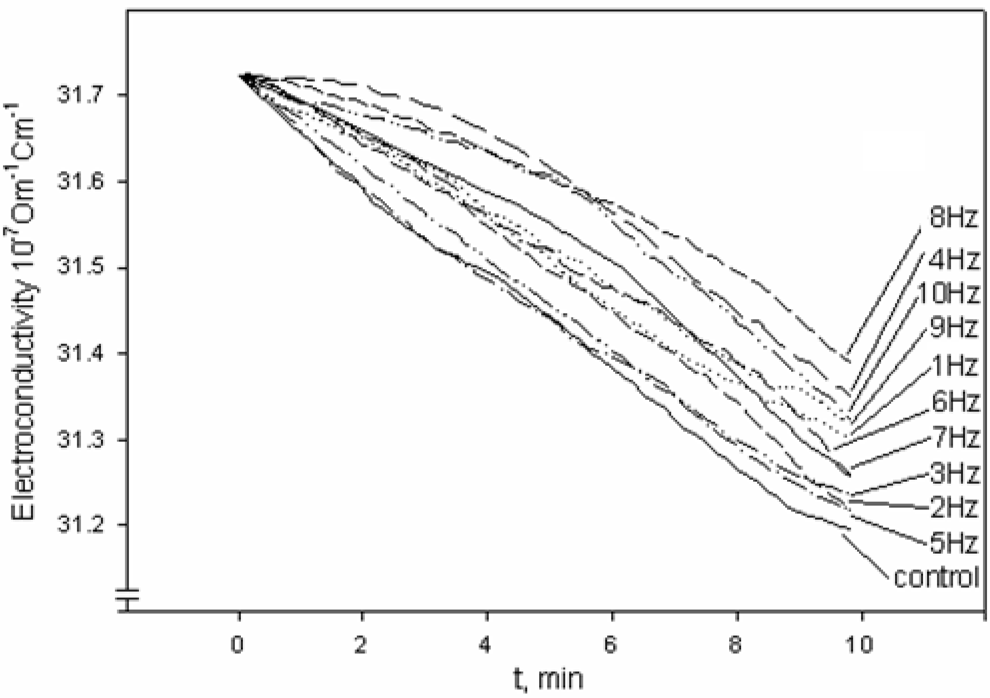


Figure 7: The frequency- and time-dependent effect of ELF EMF on SEC of DW in normal background radiation (18 µRoentgen /hour) after preliminary keeping for a day in low background radiation (less than 1 µRoentgen/hour)

As it can be seen in Figure 7 the EMF effect on the rate of SEC decrease depends on frequency and on exposure time in normal background radiation. It seems extremely interesting that during the first 5 min of incubation 4Hz EMF has more pronounced inhibitory effect on the rate of water SEC decrease, while during the following minutes of incubation such effect could be observed at 8 Hz EMF. Thus, we have two frequencies: 4 and 8 Hz EMF which had maximum inhibitory effect on water SEC in normal background ionizing radiation medium.

2. The effect of ELF EMF and IS on heat fusion of water samples frozen in liquid N_2

The next markers through which the structural changes of water can be detected are the thermal properties (heat fusion) of liquids frozen in liquid N_2 immediately after the treatment.

As the heat fusion curves are very sensitive to the initial temperature of water (see Figure 3), which is the result of temperature-dependent changes of CO_2 solubility, in order to minimize the time-dependent effect, in these series of experiments a comparatively low temperature (15^{o}C) was chosen as an initial temperature and each measurement was compared with its own control one. These studies have also shown that EMF and IS have exposure time- and frequency-dependent effect on water structure. In Figure 8 the melting curves of sham and 4Hz IS treated DW are presented. As it can be seen from Figure 8, 4Hz 30 Db mechanical vibrations of water sample even for 1 min has different kinetics of heat fusion at 18^{o}C, as compared with the sham-treated water sample.

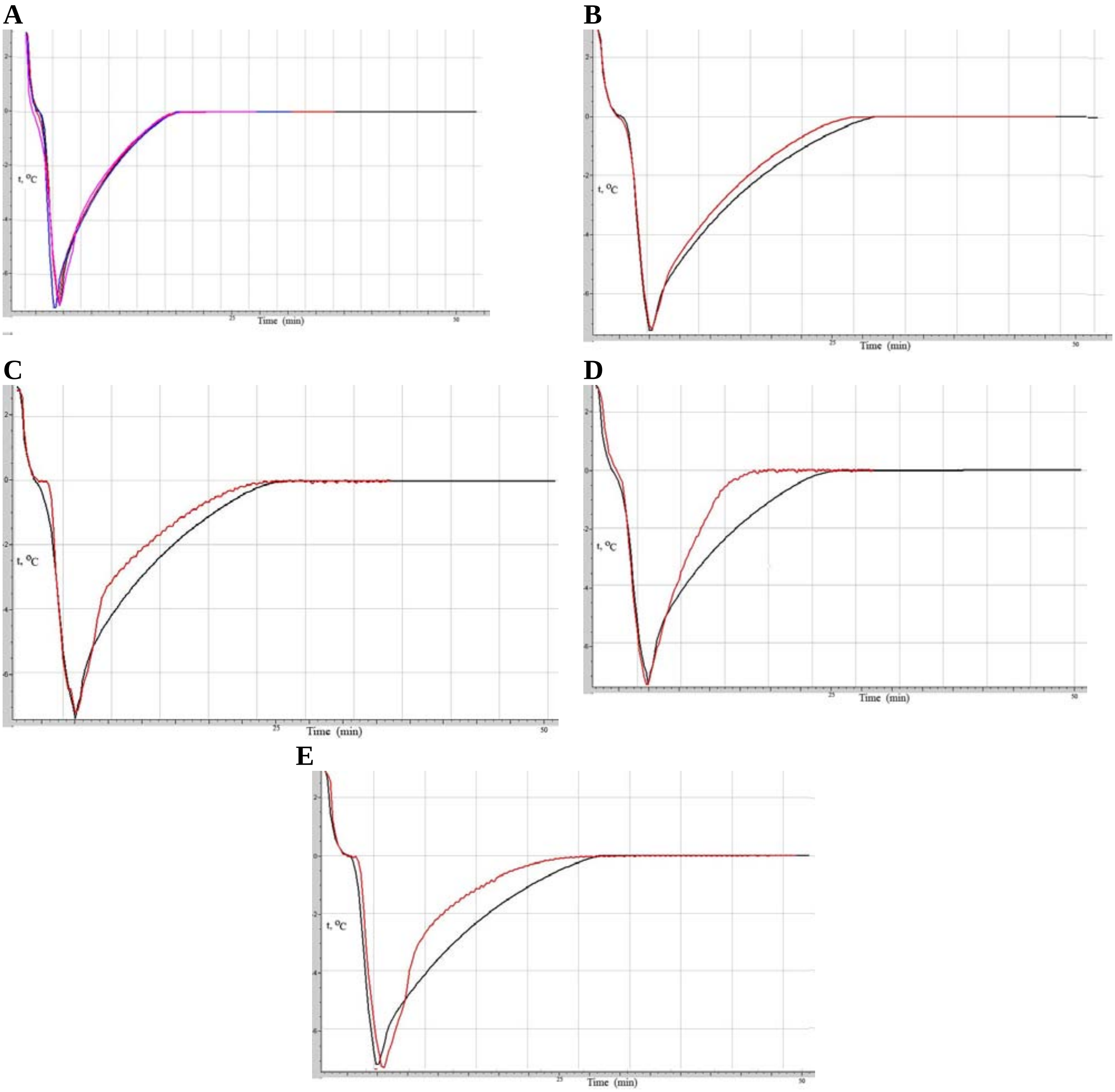


Figure 8: The kinetics of heat fusion of water exposed to 4 Hz 30 Db mechanical vibrations at room temperature (18^{o}C): sham (A), 1 min (B), 5 min (C), 15 min (D) and 30 min (E) IS pretreated and frozen water samples.

By studying the frequency-dependent effect of IS and ELF EMF on heat fusion of DW, more pronounced effects were observed at 5, 9 and 10 Hz frequencies (Figure 9), while in case of PS we also observed a significant modulation effect on melting processes at 1, 2 and 3 Hz, as compared with the sham-exposed group(Figure 10).

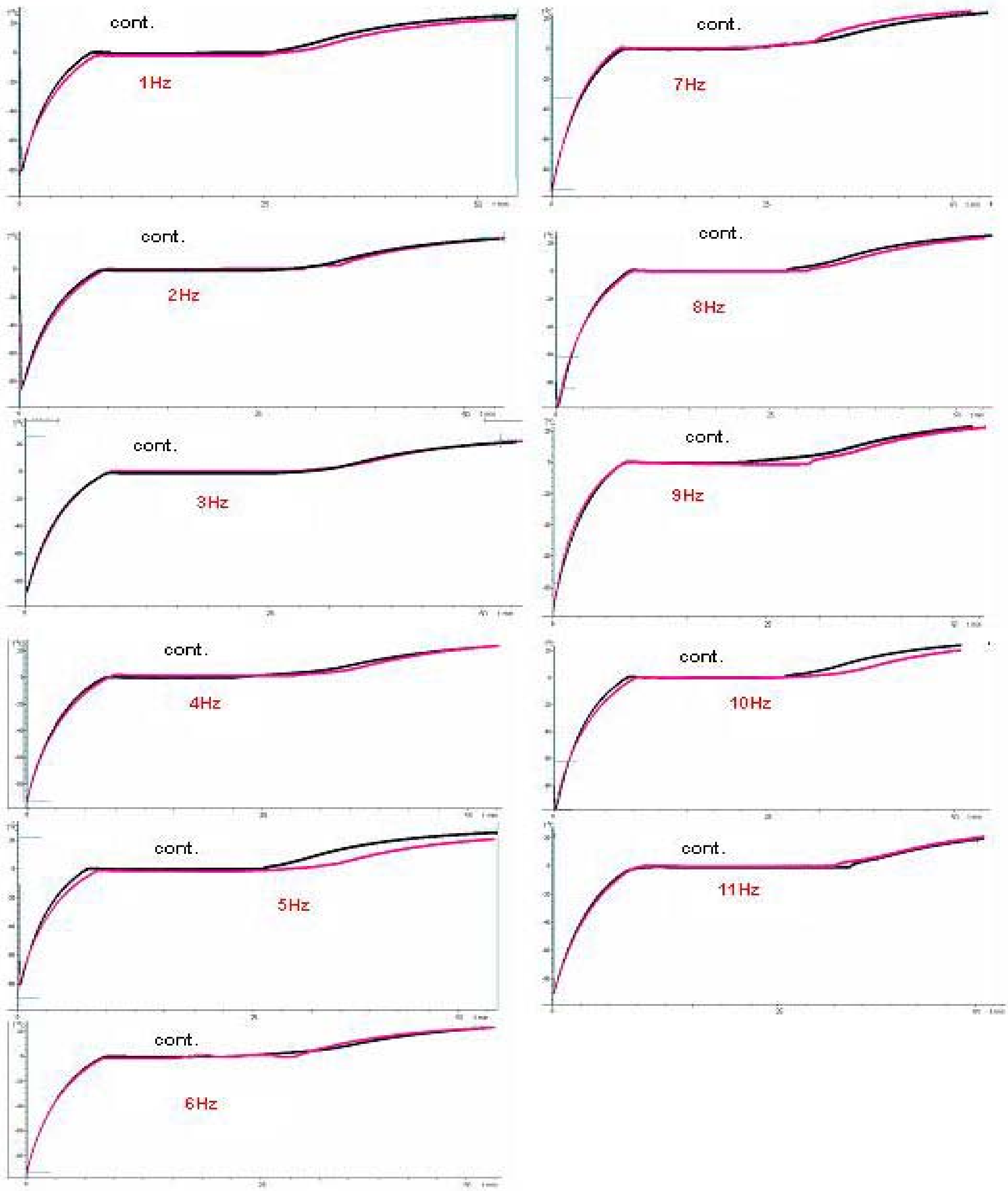


Figure 9: The frequency-dependent effect of IS (10 min exposure) on melting kinetics of DW at room temperature after freezing in liquid N_2. A typical cure of 5 experimental results.

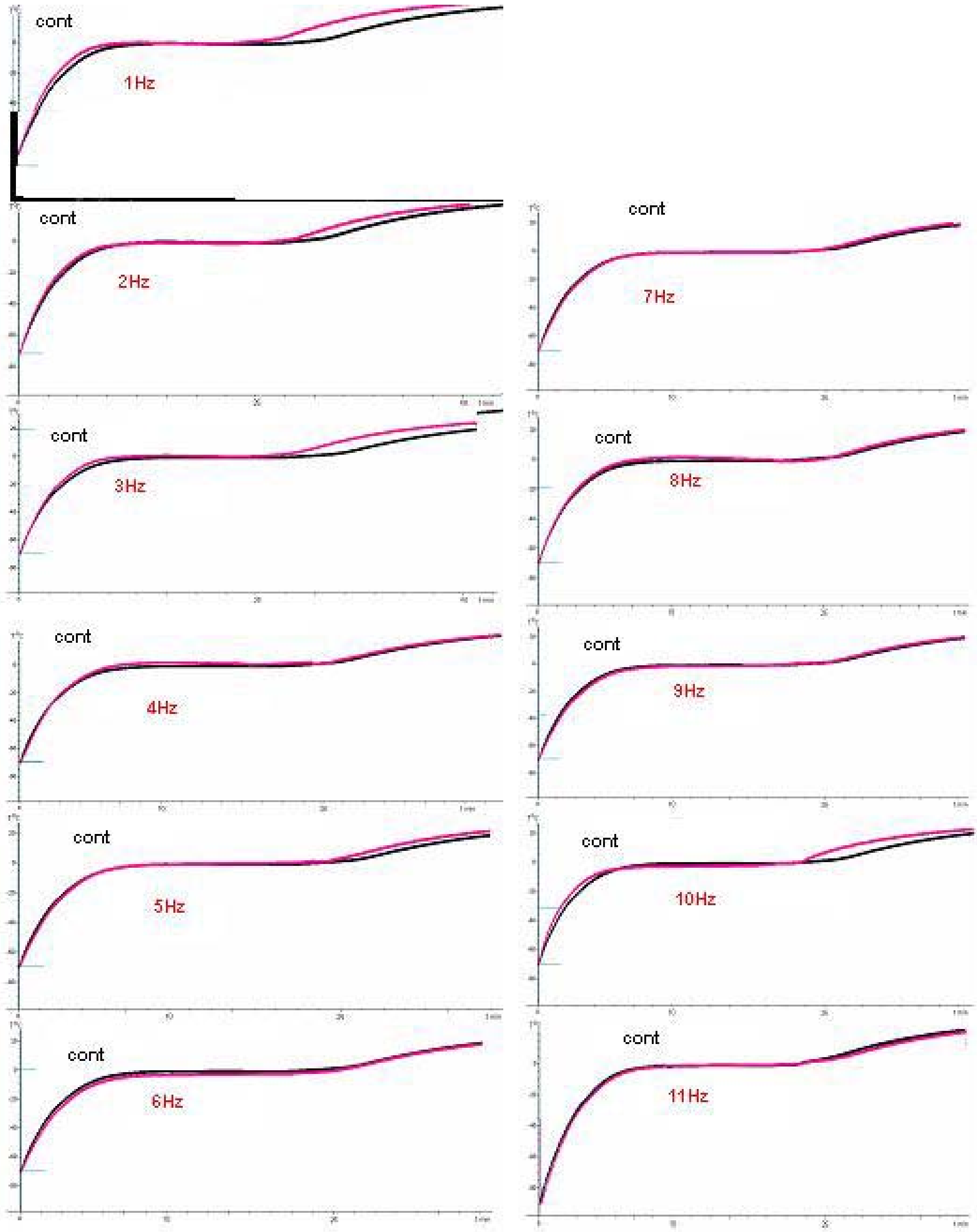


Figure 10: The frequency-dependent effect of IS (10 min exposure) on melting kinetics of PS at room temperature after freezing in liquid N_2. A typical cure of 5 experimental results.

In case of EMF-pretreated DW samples, more pronounced effect on melting process was observed at 2, 4 and 9 Hz (Figure 11), while on PS samples, like in case of IS-exposure, 1, 2 and 3 Hz also had significant effect on water melting process (Figure 12). It is interesting to note that in case of EMF 50 and 100 Hz exposure had significant effect on DW melting process (Figure 11), while in case of PS 20 Hz had pronounced effect on it and there was no effect at 50, 100 Hz (Figure 12).

Figures 11 and 12 represent the melting kinetics of DW (Figure 11) and PS (Figure 12) exposed to EMF at different frequencies at room temperature after freezing in liquid N_2.

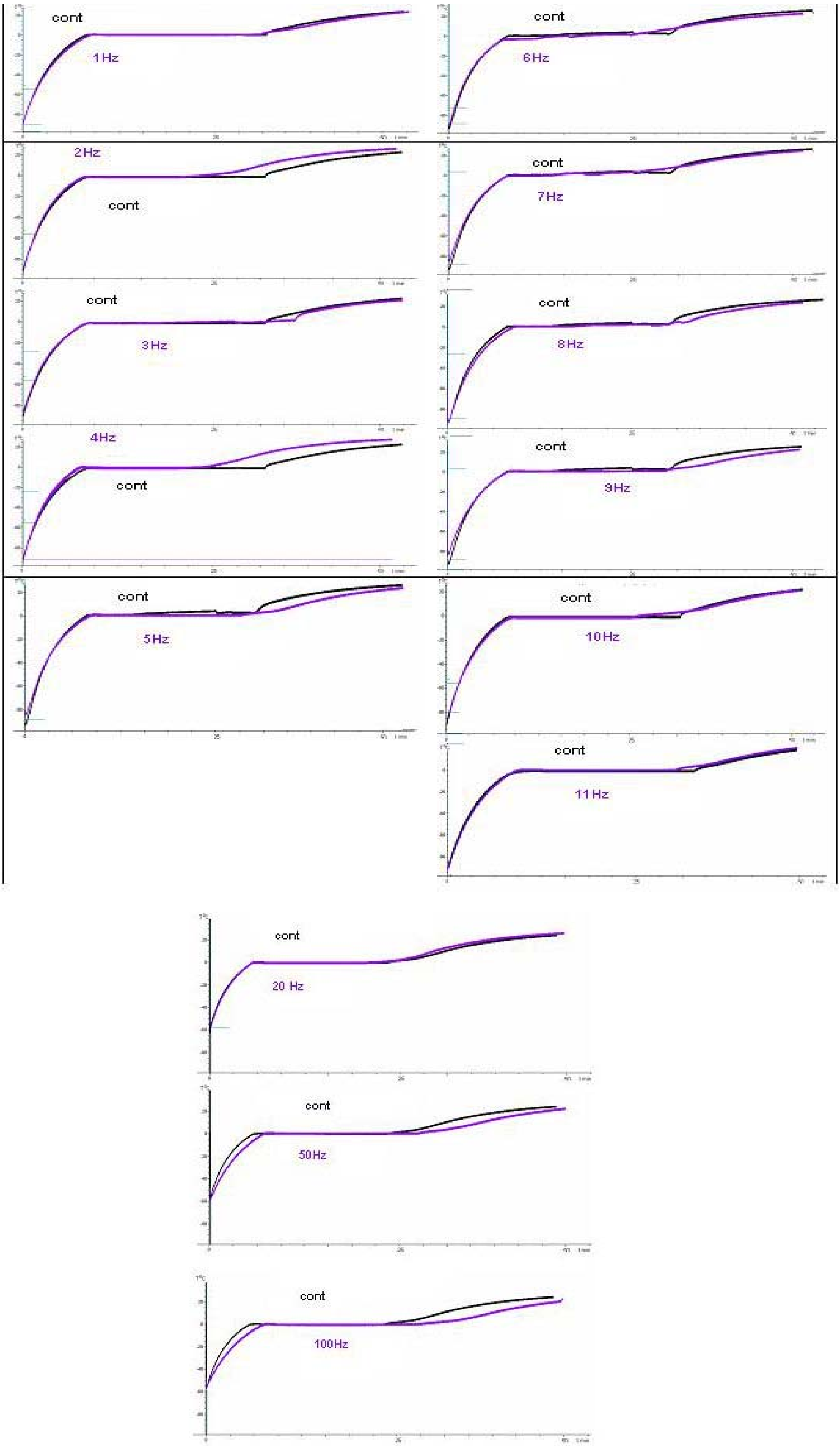
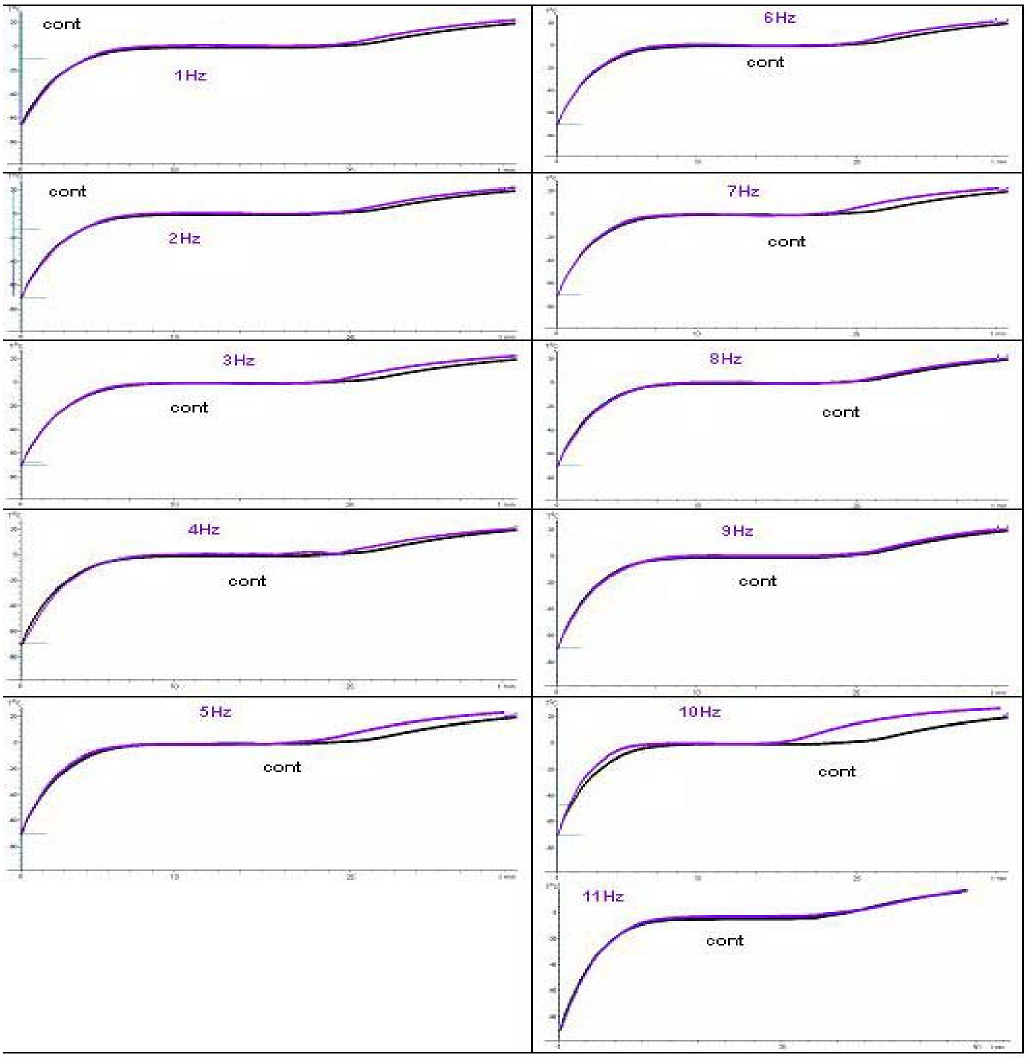


Figure 11: The frequency-dependent effect of EMF (10 min exposure) on melting kinetics of DW at room temperature after freezing in liquid N_2. A typical cure of 5 experimental results.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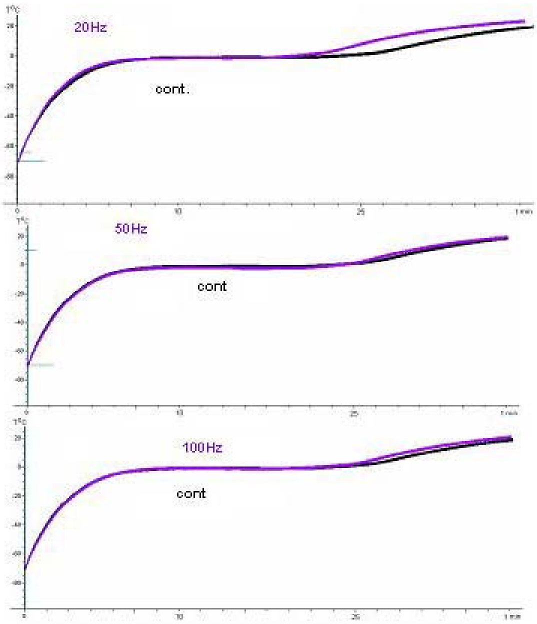
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Figure 12. The frequency-dependent effect of EMF (10 min exposure) on melting kinetics of PS at room temperature after freezing in liquid N_2. A typical cure of 5 experimental results.

3. The effect of IS and EMF exposure on H_2O_2 content in DW and PS

As H_2O_2 content depends on water structure, in the present series of experiments the frequency-dependent effect of IS and EMF on H_2O_2 content in DW and PS was studied.

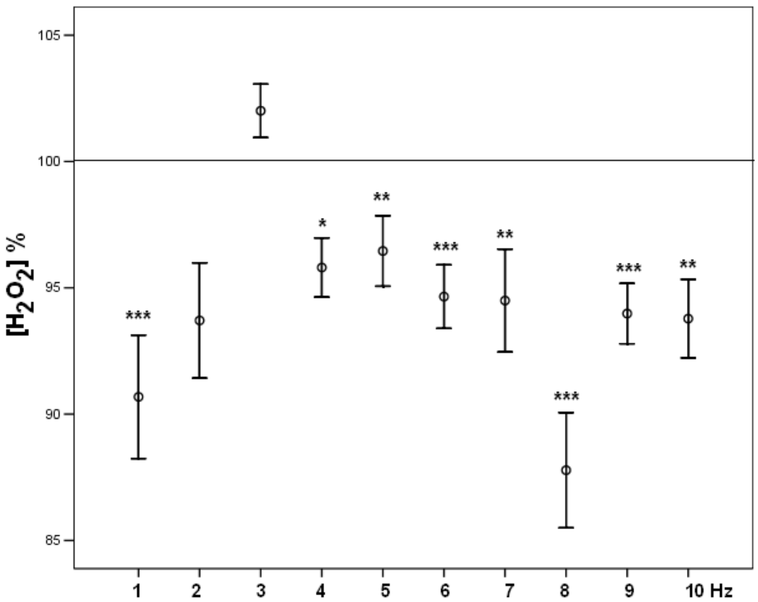


Figure 13: The content of H_2O_2 in DW exposed to 1-10Hz 30dB IS (during 10 min) at 15^{o}C

As it can be see in Figure 13 IS at the frequency of 3 Hz stimulates the H_2O_2 formation in DW, while at 8 Hz IS has depressing effect on it.

In figure 14 all frequencies had depressing effect on H_2O_2 formation in PS, however in case of 5, 6 and 10 Hz this depression was less pronounced.

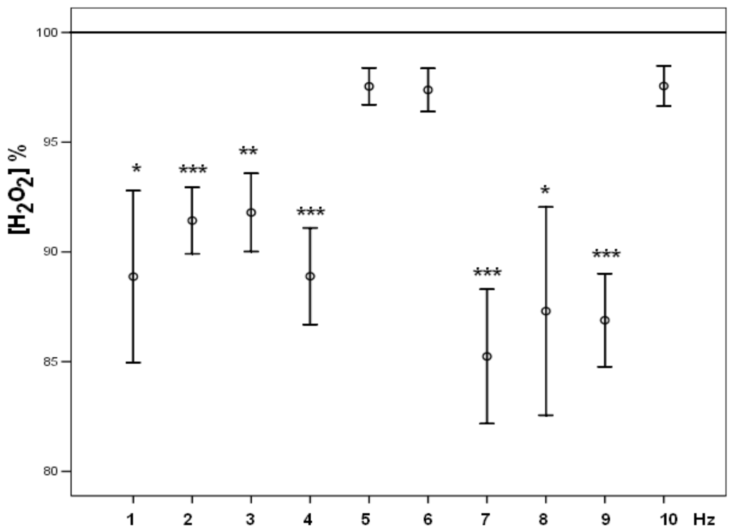


Figure 14: The content of H_2O_2 in PS exposed to 1-10 Hz 30dB IS (during 10 min) at 15^{o}C

In case of EMF effect on H_2O_2 formation all frequencies less than 50 Hz, except 4Hz, depressed the H_2O_2 formation in DW, while at higher frequencies (60-100 Hz) we observed the activation of H_2O_2 formation (Figure 15).

The activation of H_2O_2 formation in PS was observed only in case of 10 Hz EMF; the rest of observed frequencies showed the decrease of H_2O_2 content and the most pronounced depressing effect was observed at 8 Hz (Figure 16).

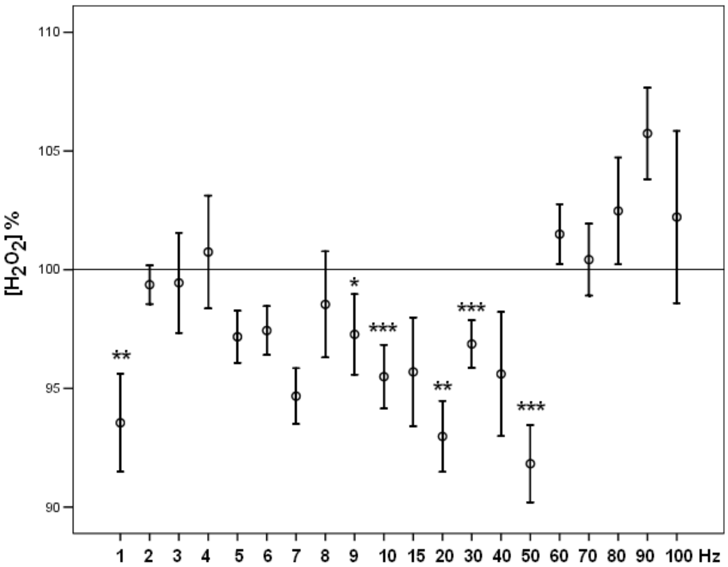


Figure 15: The content of H_2O_2 in DW exposed to 1-100 Hz EMF (during 10 min)

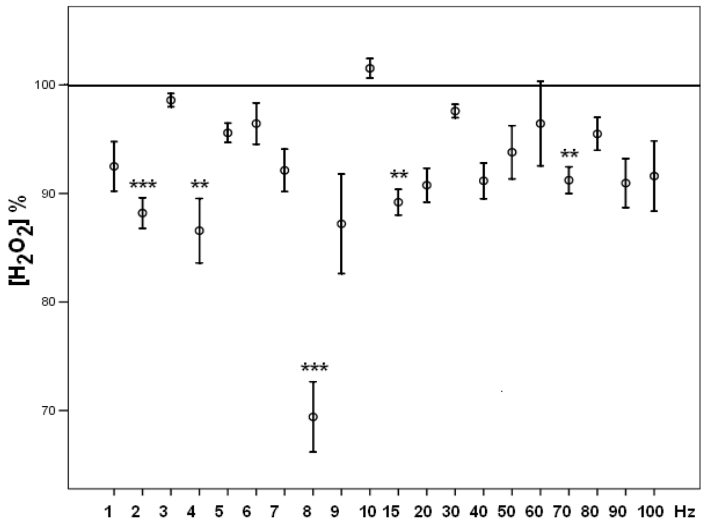


Figure 16: The content of H_2O_2 in PS exposed to 1-100 Hz EMF (during 10 min)

All the obtained data show that there are pronounced frequency “windows” of IS and ELF EMF effects on SEC and heat fusion of DW and PS, as well as on the formation of H_2O_2 in water and PS.

III. The comparative study of the most effective ELF EMF and IS frequency windows effect on physicochemical, crystallographic and H_2O_2 formation properties of DW and PS at low background radiation (1-2 µRoentgen/hour).

The study of ELF EMF and IS effect on physicochemical properties of DW and PS at low background radiation (LBGR) was performed in lead chamber, in which IS frequency vibration stand and ELF EMF –generating coil were constructed (Figure 17). In this chamber with the closed door the background radiation was 1 micro Roentgen/hour, while the background radiation in the room (with the open door) was 18 µRoentgen/hour.

Figure 17: The experimental lead chamber with IS frequency vibration stand and ELF EMF–generating coil constructed in it

1. The frequency-dependent effect of ELF EMF and IS on SEC of DW and PS at LBGR

Before studying the effects of ELF EMF and IS on SEC of DW and PS, the effect of LBGR (1 µRoentgen/hour) on SEC was studied. For this purpose the electrochemical cell with water sample was located in lead chamber and the SEC was recorded with the chamber door open and closed, i.e. at normal (NGBR=18 µRoentgen/hour) and low (LBGR=1 µRoentgen/hour) BGR, correspondingly.

The gradual decrease of water SEC at room temperature at NBGR was shown previously. Only after 3 days this process was stabilized. It can be explained by the decrease of CO_2 solubility (Stepanian et al., 1999). To make this changes of SEC more pronounced, the present experiments were performed in comparatively cold conditions (at 15^oC), at which CO_2 solubility increased and became 0.1970%, as compared to the data of normal room temperature (20^oC)- 0.1688%.

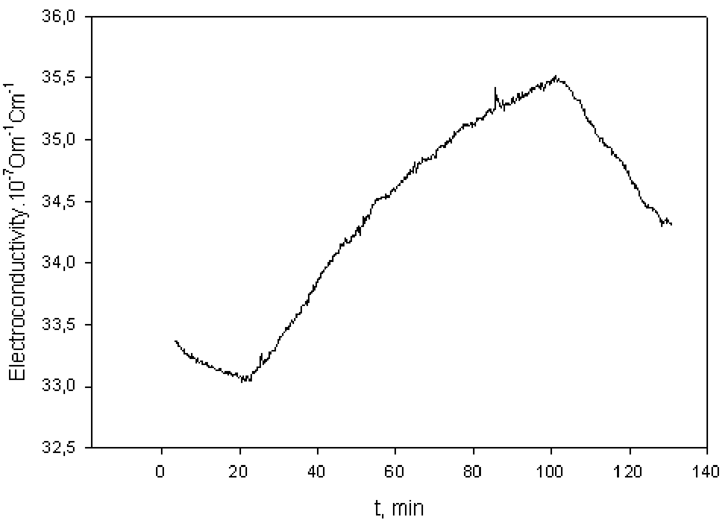


Figure 18: The effect of LBGR and NBGR on DW SEC at room temperature of 15^oC at LBGR.

As it can be seen from Figure 18, at 20-100 min LBGR (when the chamber door was closed) the rate of water SEC was gradually increased and then stabilized, while at NBGR during 0-20 and 100-130 min (opening the chamber door) the water SEC was decreased.

The study of the frequency-dependent effect of ELF EMF on DW SEC showed that EMF at low frequencies depressed the SEC of DW. This effect was more pronounced at 4 and 8 Hz frequency (Figure 19). Such similarity of the effects of 4 and 8Hz on water SEC seems extremely interesting and could be a subject for more detailed investigation.

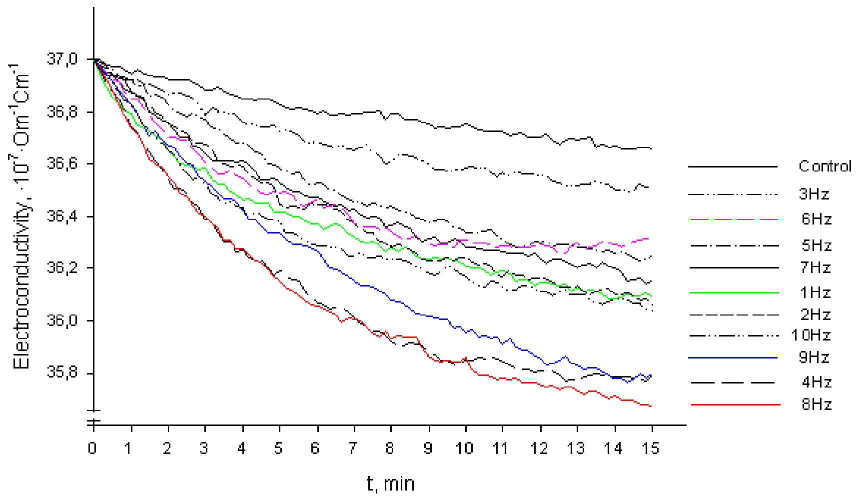


Figure 19. The effect of different frequency EMF on time-dependent changes of ELF EMF on SEC of DW at LBGR. A typical record of 5 experiments.

The family of curves on time-dependent changes of SEC of PS exposed to EMF at LBGR is presented in Figure 20. As it can be seen from this figure, we observed the time-dependent decrease of PS SEC at LBGR, like as in case of DW SEC. However, in case of PS more pronounced effect was observed only at 9 Hz frequency, while in DW these frequency windows appeared at 4, 8 and 9 Hz.

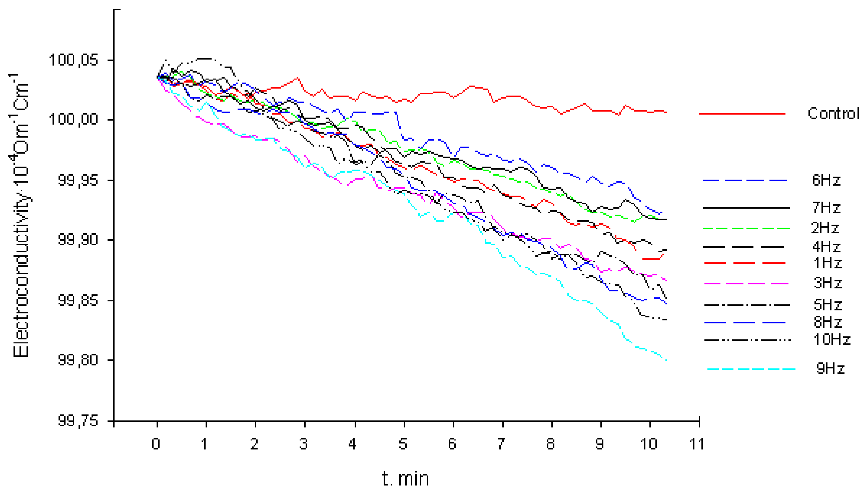


Figure 20: The frequency-dependent effect of ELF EMF on SEC of PS at LBGR. A typical record of one of 5 experiments.

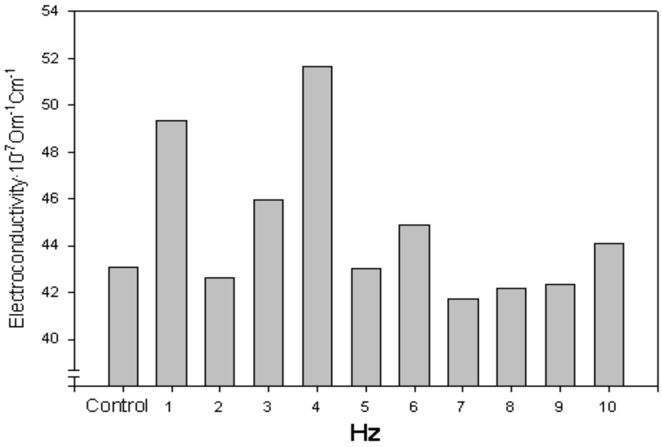


Figure 21: The frequency-dependent effect of IS on SEC of DW at LBGR. A typical record of 5 experiments.

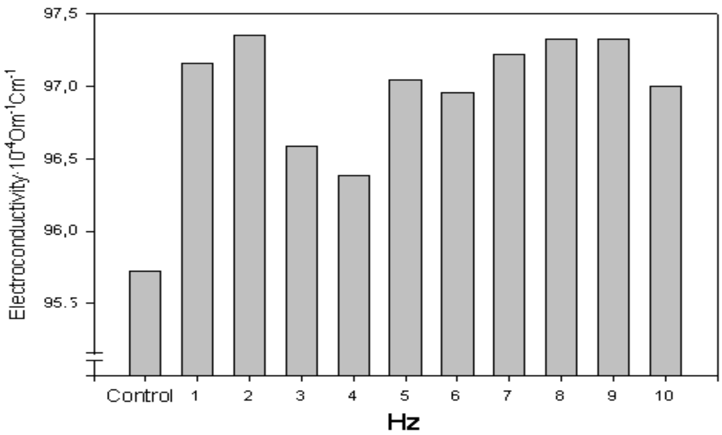


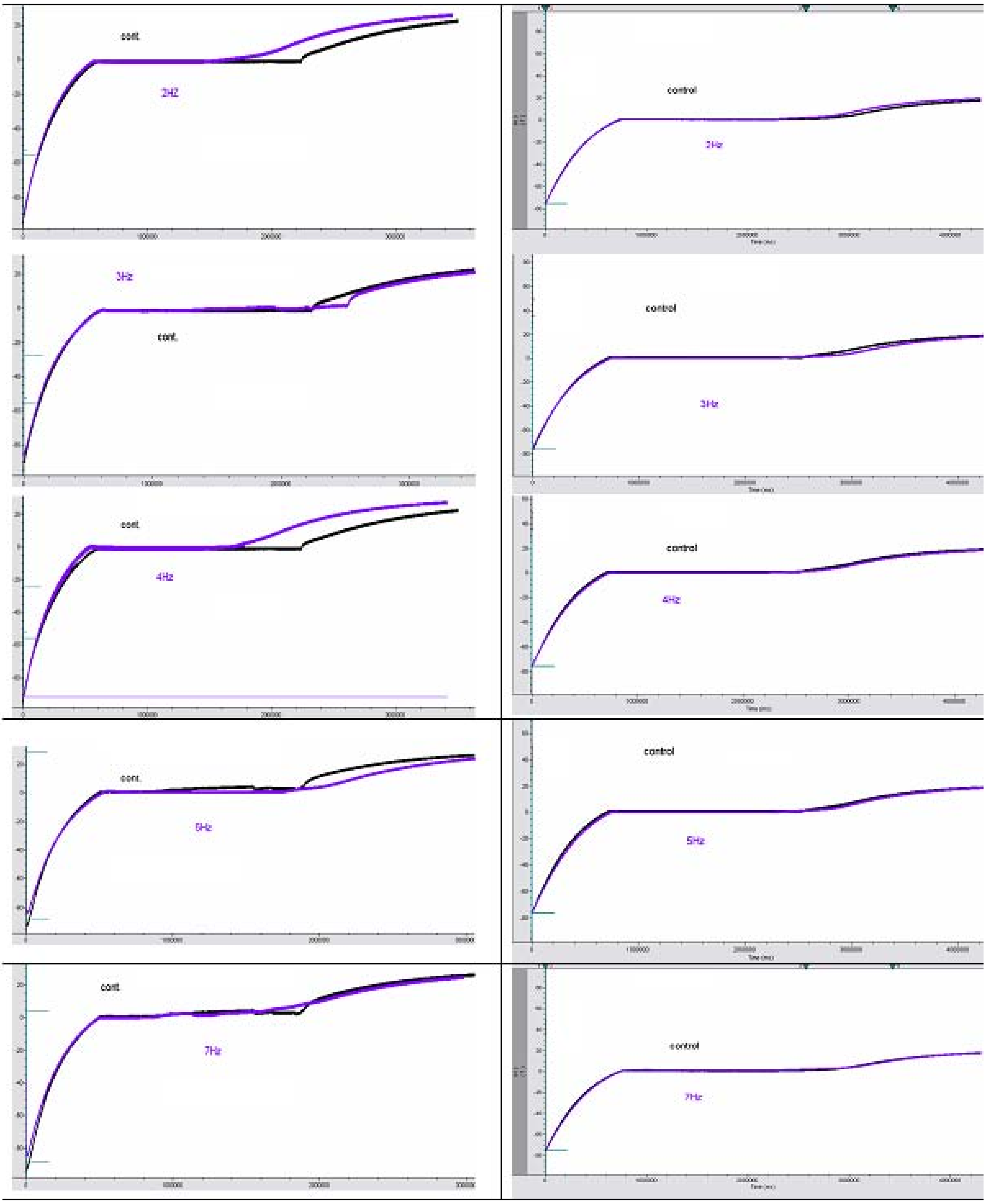
Figure 22: The frequency-dependent effect of IS on SEC of PS at LBGR. A typical record of one of 5 experiments.

IS has elevation effect on SEC of PS at 1-10 Hz frequency. This effect was less pronounced at 4Hz.

2. The effect of ELF EMF and IS at LBGR on heat fusion of water samples frozen in liquid N_2

The studies of previous tasks showed that EMF effect on melting process of DW at NBGR was more pronounced at 2, 4 and 9 Hz frequencies, while in case of PS samples- at 1, 2 and 3 Hz.

As it can be seen from figure 23 the effect of EMF treatment on melting process of DW was significantly depressed (or disappeared) at 2-100 Hz at LBGR. Thus, LBGR has depressing effect on EMF-induced heat fusion changes, as well as delays the 50 and 100Hz EMF-induced DW melting process.


cont.
2HZ
control
2Hz
3Hz
cont.
control
3Hz
cont.
4Hz
control
4Hz
cont.
5Hz
control
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cont.
7Hz
control
7Hz

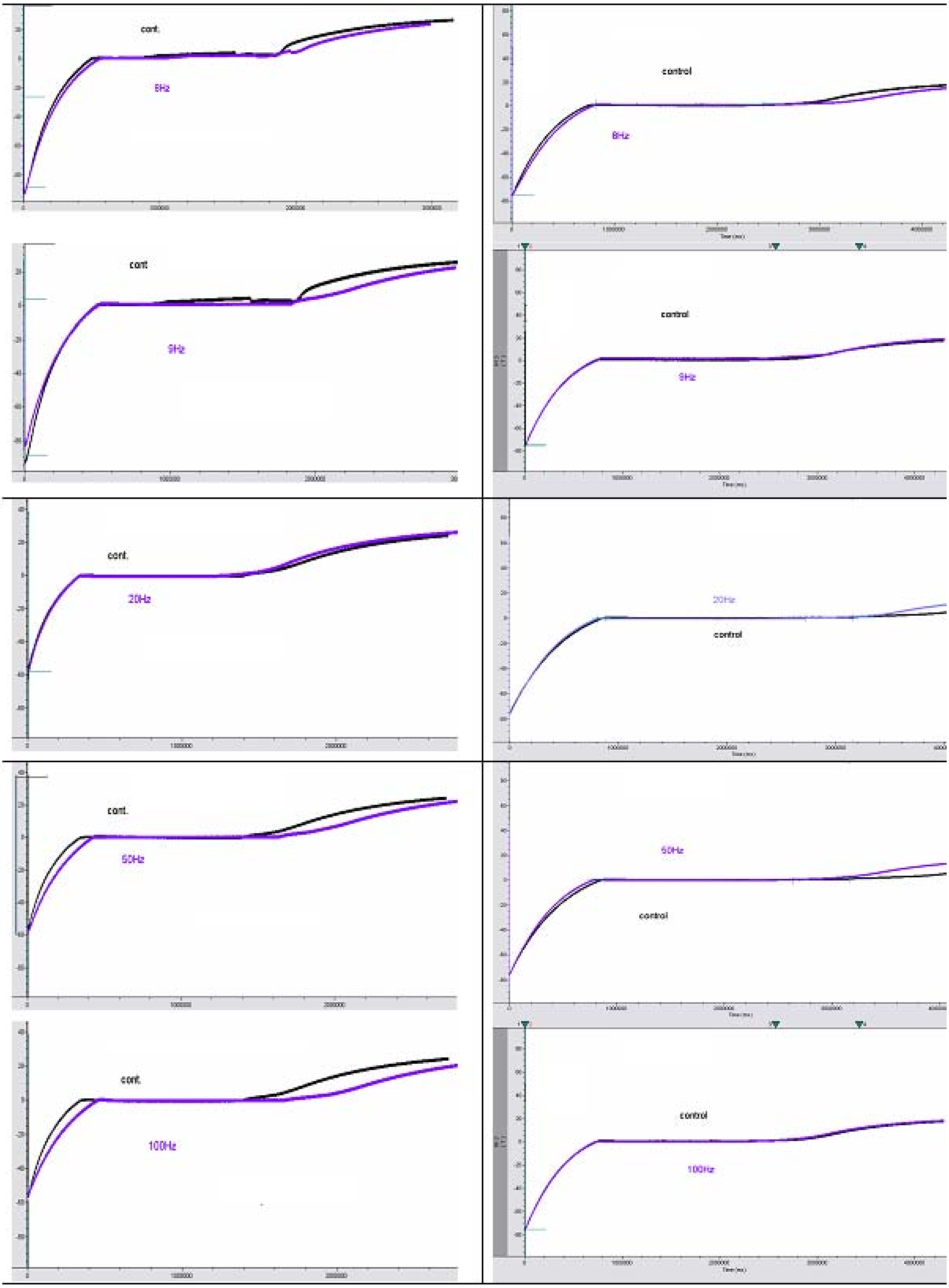


Figure 23: The melting kinetics of DW exposed to EMF at different frequencies at NBGR (left column) and LBGR (right column) at room temperature after freezing in liquid N_2. Typical curves of 5 experimental results at each frequency of EMF

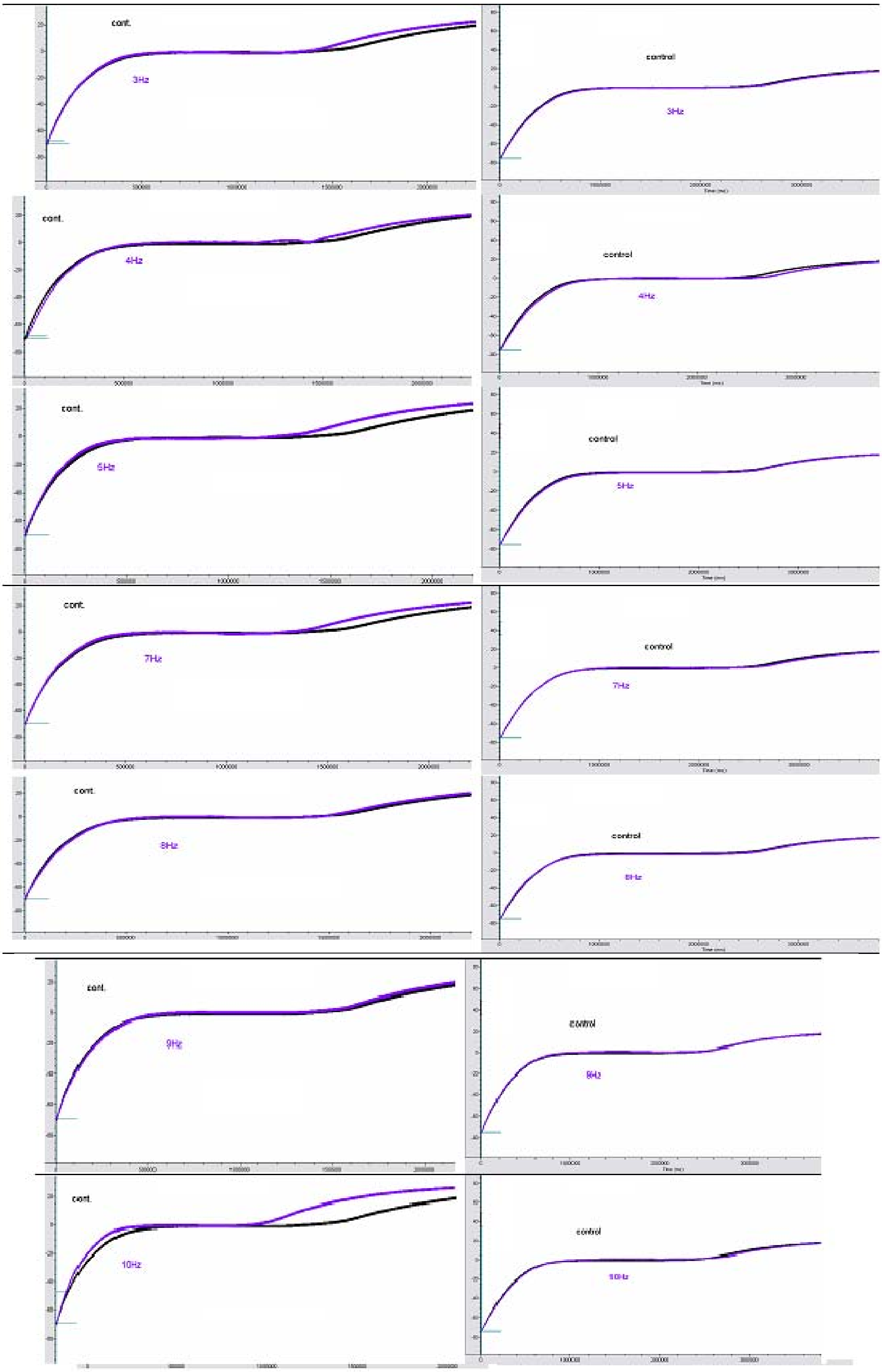


Figure 24: The melting kinetics of PS exposed to EMF at different frequencies at NBGR (left column) and LBGR (right column) at room temperature after freezing in liquid N_2. Typical curves of 5 experimental results at each frequency of EMF.

In previous tasks we showed that in DW treated by 1, 5, 8 and 9Hz IS the temperature rises in ice until the melting point, while in liquid state temperature rising is slightly delayed, which can be explained by the IS-induced increase of heat capacity in both states. As it can be seen from figures above, the LBGR depresses this effect at most of the observed frequencies, while at 1 and 9Hz this effect was reversed in LBGR.

3. The effect of EMF and IS on hydrogen peroxide content in DW and PS at LBGR

As in the lead chamber (door closed), beside the background radiation, the light is also absent, before starting the study of LBGR effect on H_2O_2 content, the effect of light on it was studied. For this purpose the sham-exposed samples were also kept in dark wooden box. As it can be seen from figure 25, in dark wooden box the hydrogen peroxide (H_2O_2) content in DW (exposure time- 20 hours) was higher than in the box in normal light conditions. While in led box the H_2O_2 content is much higher compared with the data of the wooden box. These data allow us to suggest that light and normal background radiation destroyed H_2O_2 molecules.

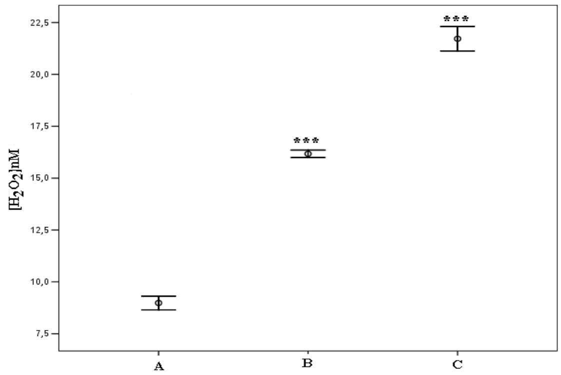


Figure 25: The content of hydrogen peroxide in DW at normal light conditions (A), NBGR in wooden box (B) and in led chamber (C).

As it was shown in the previous task, the ELF EMF at the frequency of less than 50 Hz (except 4 Hz) in NBGR decreases the H_2O_2 level, while at 60-100 Hz it has an elevation effect on H_2O_2 content in DW. The study of the effect of 1-100 Hz EMF on H_2O_2 content at LBGR (Figure 28B) shows the big differences from the previous results obtained on NBGR (Figure 26A): at NBGR the level of H_2O_2 in DW was minimal at 1, 7, 20 and 50 Hz and maximal at 4 and 90 Hz, while at LBGR we had reversed picture; the H_2O_2 content at 1, 7, and 20 Hz were closer to the control level and 50 Hz EMF had the maximal elevation effect on H_2O_2 formation in DW. At 4 Hz where the H_2O_2 level exceeded the control level at NBGR (Figure 26A), in case of LBGR the H_2O_2 level was much less than in control (Figure 26B).

A

B

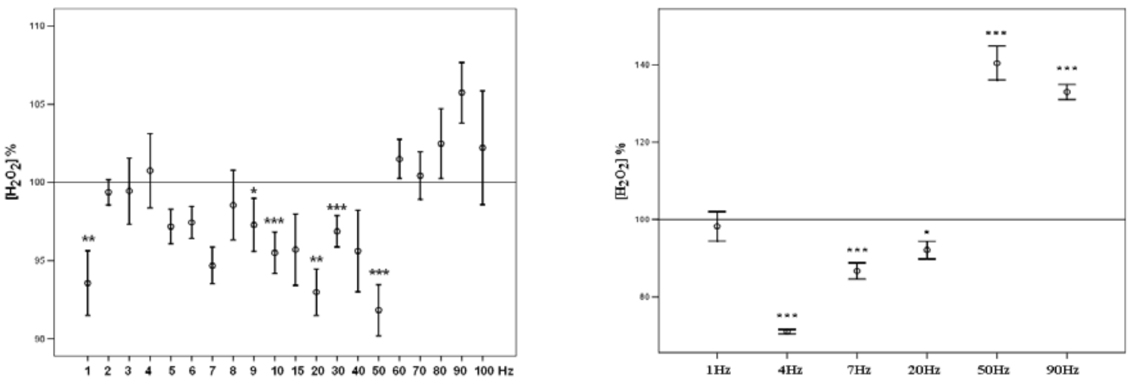


Figure 26: The content of H_2O_2 in DW exposed to 1-100 Hz EMF (during 10 min) at NBGR (A) and LBGR (B) at 15^0C.

The same protocol of experiments was performed on EMF effect on PS exposed to 1-100 Hz EMF at LBGR and NBGR.

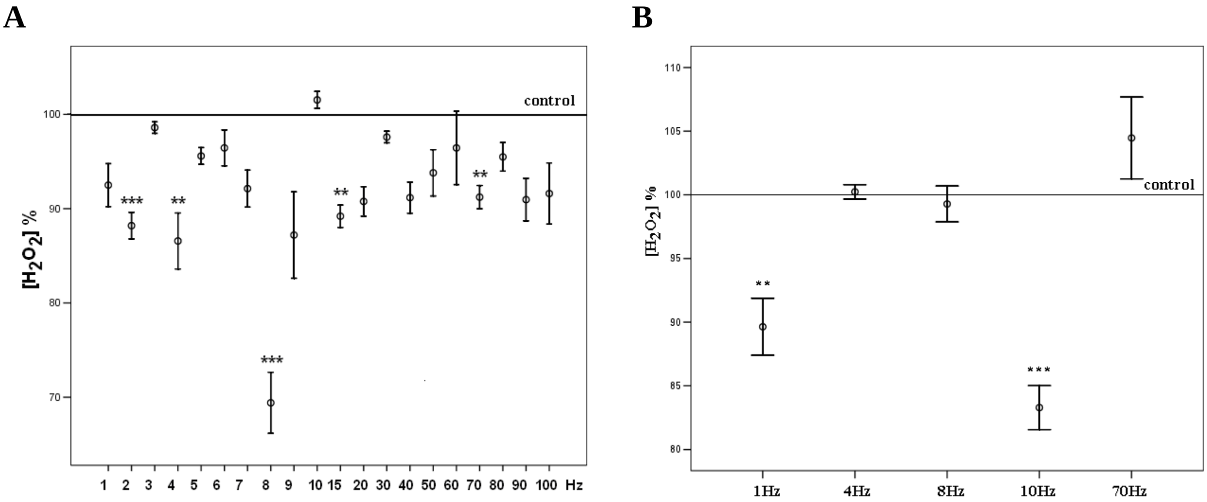


Figure 27. The content of H_2O_2 in PS exposed to 1-100 Hz EMF (during 10 min) at NBGR (A) and LBGR (B) at 15^0C.

The presented data show that as in case of DW, in PS LBGR reverses the EMF-induced changes of H_2O_2 content compared with NBGR.

It is worth to note that EMF effect on H_2O_2 content is also exposure time dependent. As it can be seen in Figure 28, 1 Hz EMF exposure of PS during 10 minutes at LBGR depresses the H_2O_2 content as compared with the control one, while 20-minute-EMF-exposure at the same frequency has elevation effect on it.

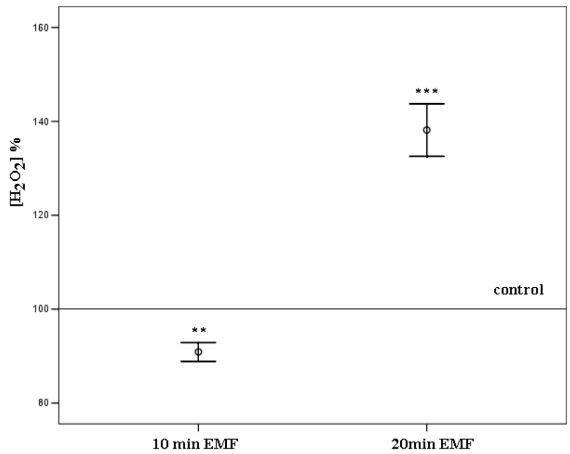


Figure 28: The H_2O_2 content in PS exposed to 1 Hz EMF during 10 and 20 min at LBGR and at 15^0C.

The similar experiments were performed on IS effect on H_2O_2 content in DW and PS at LBGR and the obtained data were compared with the data of NBGR.

The data presented below show that as in case of EMF exposure, LBGR reverses the IS-induced effect on DW and PS: 3 Hz IS has elevation effect on H_2O_2 formation in NBGR while in LBGR it has strong depressing effect on it.

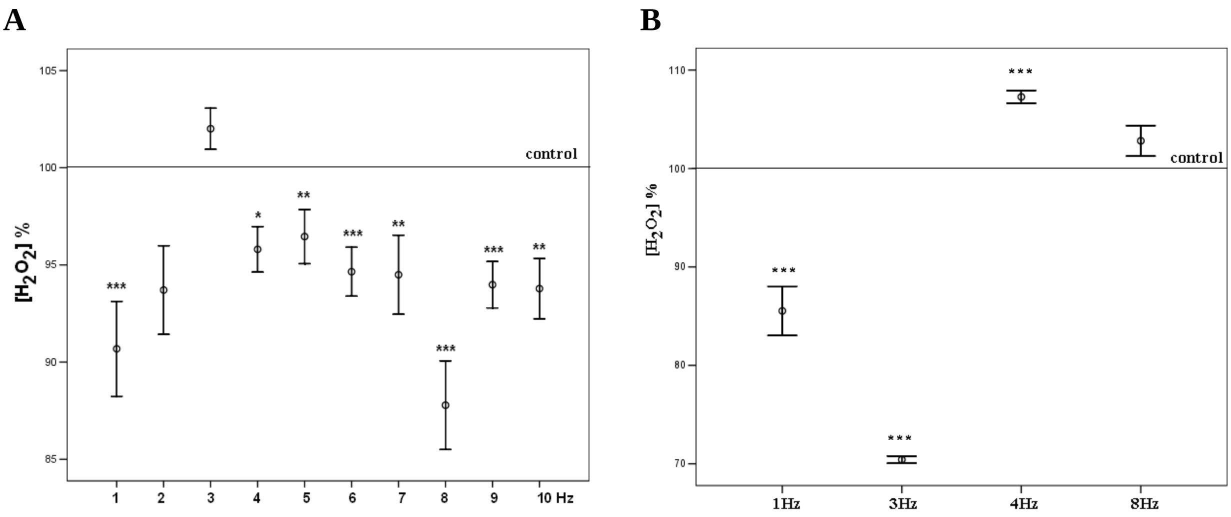


Figure 29: The content of H_2O_2 in DW exposed to 1-10 Hz IS (during 10 min) at NBGR (A) and LBGR (B) at 15^0C.

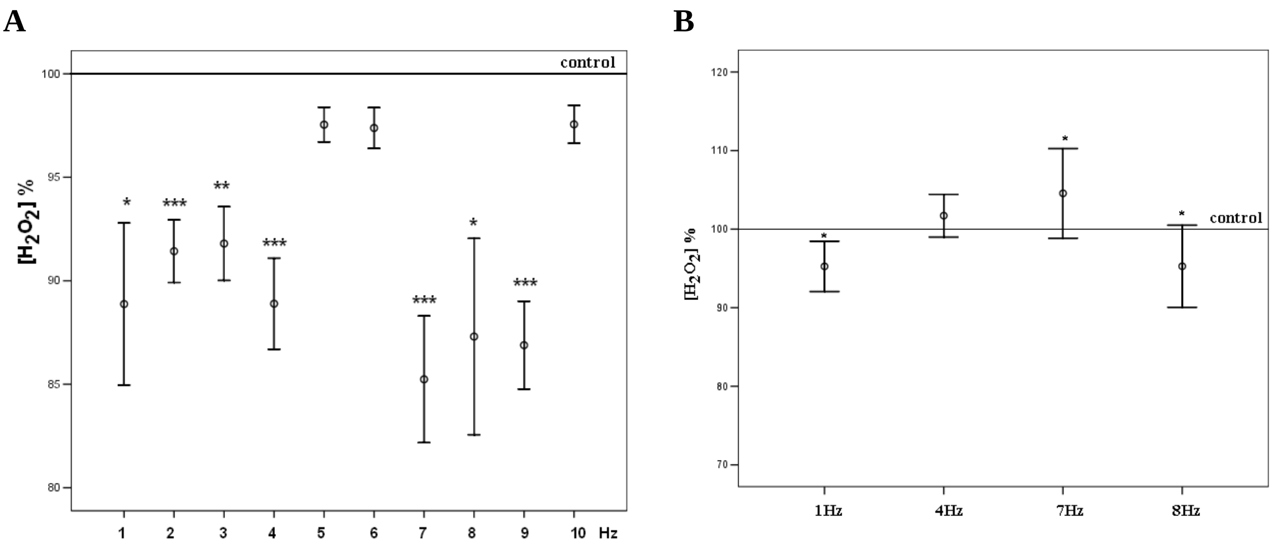


Figure 30: The content of H_2O_2 in PS exposed to 1-10 Hz IS (during 10 min) at NBGR (A) and LBGR (B) at 15^0C.

Thus, the obtained data show the strong modulation effect of background radiation on EMF- and IS-induced changes of physicochemical properties of water.

IV. The comparative study of the dependency of 4 Hz ELF EMF and IS effect on SEC, heat fusion, O_2 and CO_2 solubility and H_2O_2 content of DW and PS at normal background radiation and light condition (NBGR+light), NBGR-light and low background radiation-light (LBGR-light) at 4^oC and 15^oC

In previous study a number of new phenomena connected with the light effect on physicochemical properties of water were discovered, as well as light, ionizing background radiation, temperature and ionic composition dependency of EMF- and IS-induced effect on water properties. Thus, we have decided to prolong the final task for a month to make additional and summary experiments on project.

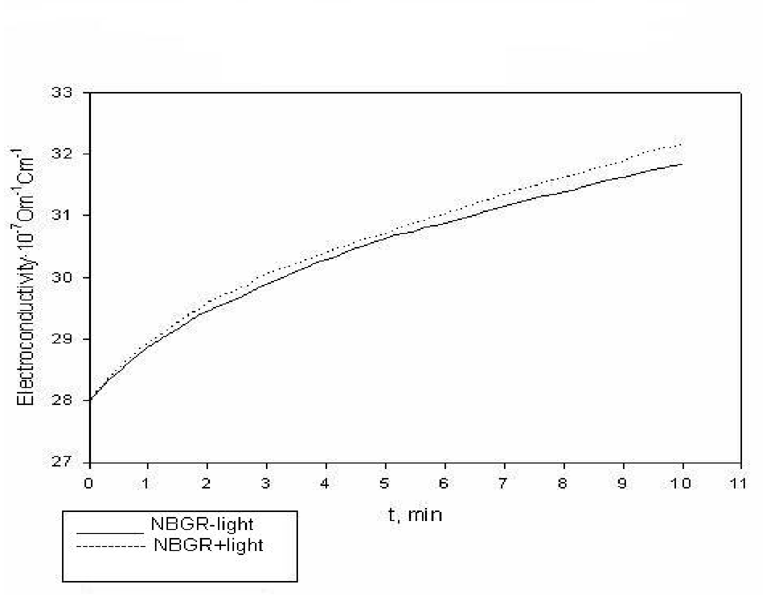
For this purpose serial experiments on light effect on physicochemical properties of water (out of initial tasks of this project) were performed. Below are presented these data.

4.1 Light effect on physicochemical properties of DW and PS

4.1a Light effect on DW and PS SEC in normal and CO_2 saturated medium

As it can be seen from figure 31 in normal light room conduction DW SEC at 4°C was higher than in dark medium (A) and this effect was more pronounced at 15°C (B).

A.



B.

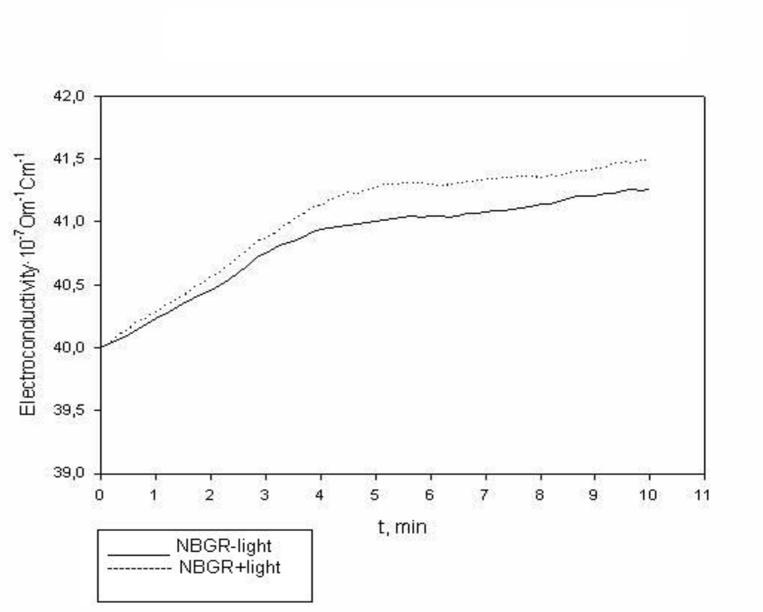
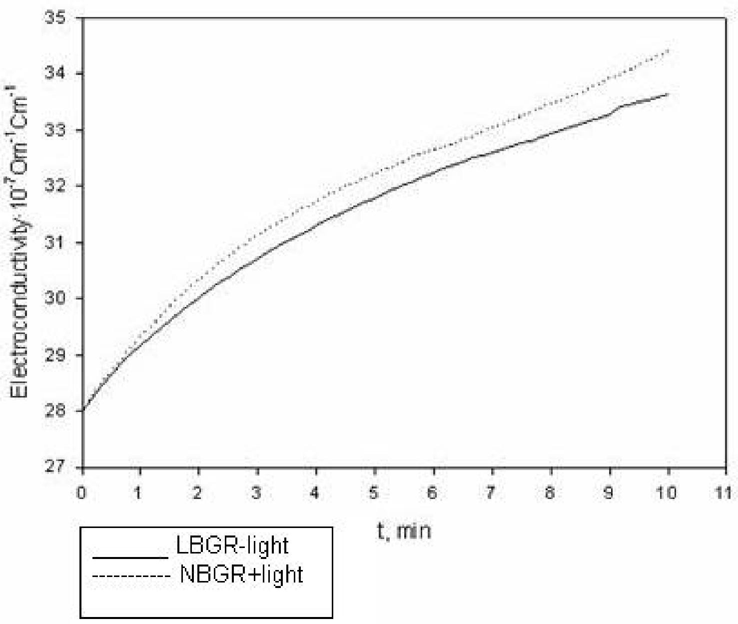


Figure 31: The time-dependent changes of DW SEC at 4^{O}C (A) and 15 ^{O}C (B) in light and dark conditions. The mean data of five experiments are presented.

The time-dependent increase of DW SEC in lead box in dark (LBGR-light) and light (NBGR) medium at 4^{O}C (A) and 15^{O}C (B) is showed in figure 32. As it can be seen from the presented data we have light-induced increase of DW SEC at 4 ^{O}C, while at 15 ^{O}C light has depressing effect on it. From these data we can conclude on the existence of BGR-induced modulation effect on DW SEC.

A.



B.

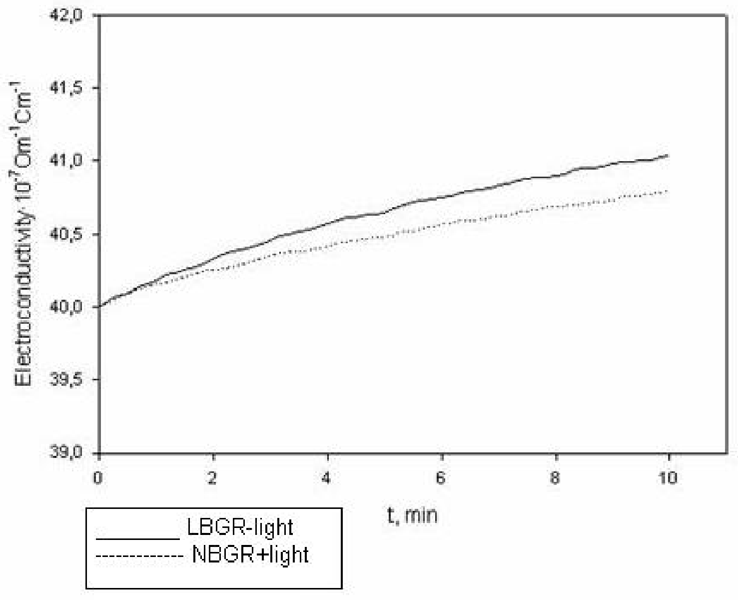


Figure 32: Time-dependent changes of DW SEC at 4^{O}C (A) and 15 ^{O}C (B) in LBGR-light and NBGR+light. The mean data of five experiments are presented.

4.1 b Light effect on heat fusion of DW and PS after freezing in liquid N2

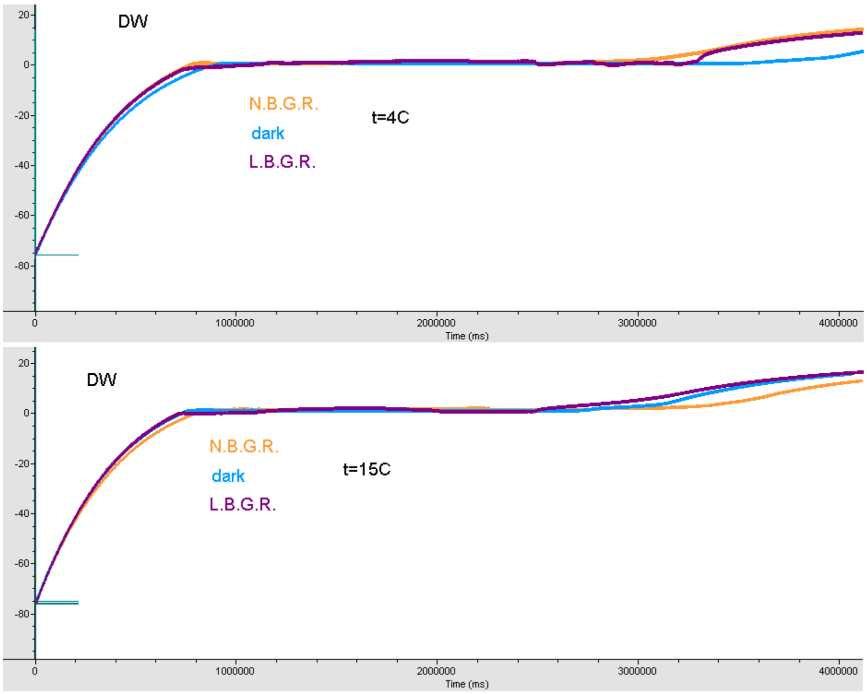


Figure 33: Time-dependent changes of melting processes of frozen DW in liquid N_2 at NBGR+light, NBGR-light and LBGR-light at 4 ^{O}C and 15 ^{O}C.

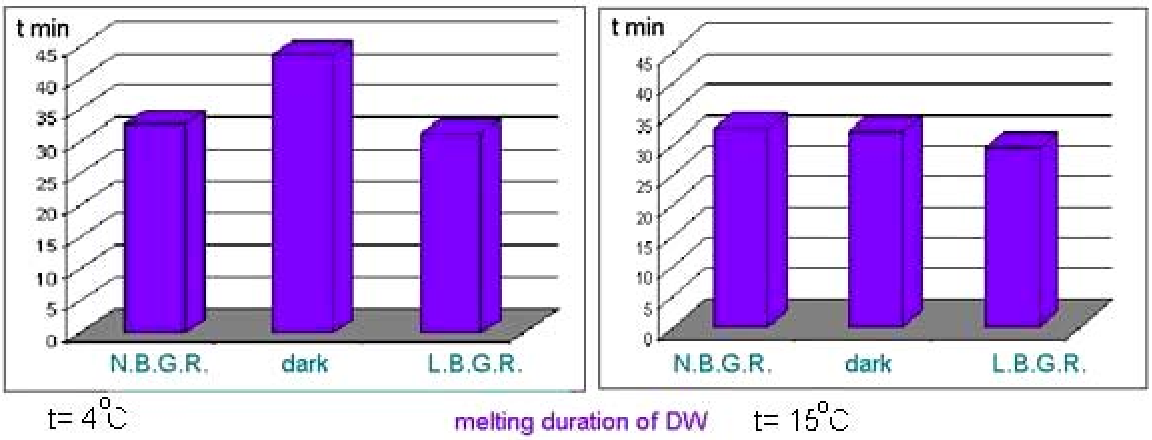


Figure 34: The duration of DW after freezing in liquid N_2 at NBGR+light, NBGR-light and LBGR-light at 4^{o}C and 15 ^{O}C.

The presented data on figures 33 and 34 show that heat fusion of DW, which was pre-incubated in dark condition at 4^{o}C for 30 min, was higher than the heat fusion of DW incubated in normal light condition. This increasing effect was disappeared at 15^{o}C.

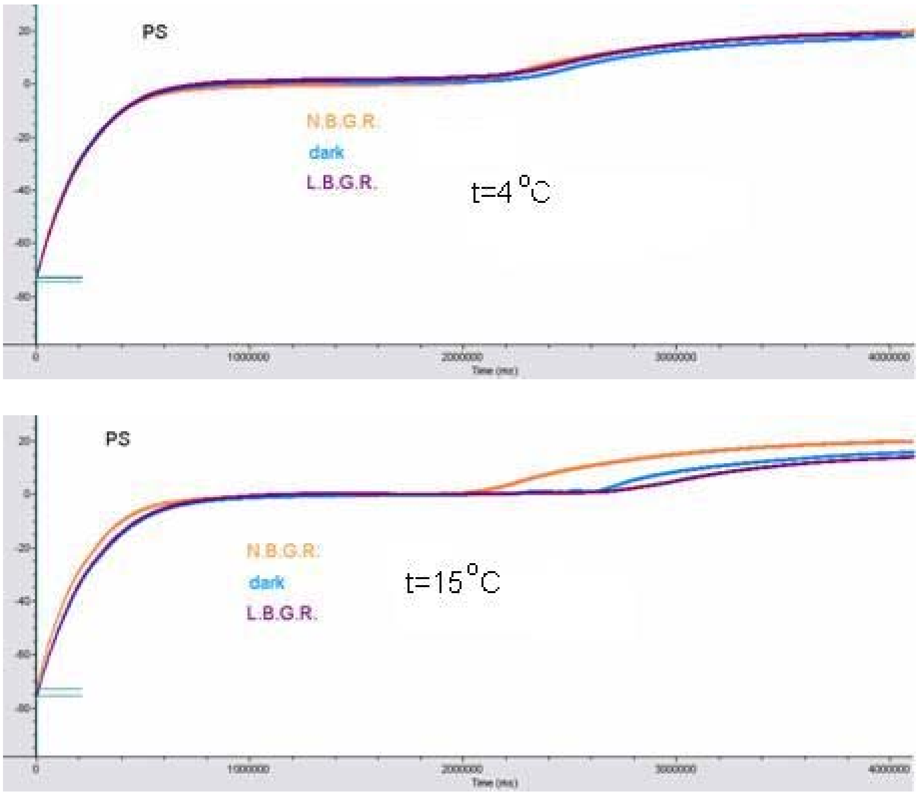


Figure 35: Time-dependent changes of melting processes of frozen PS in liquid N_2 at NBGR+light, NBGR-light and LBGR-light at 4 ^{O}C and 15 ^{O}C.

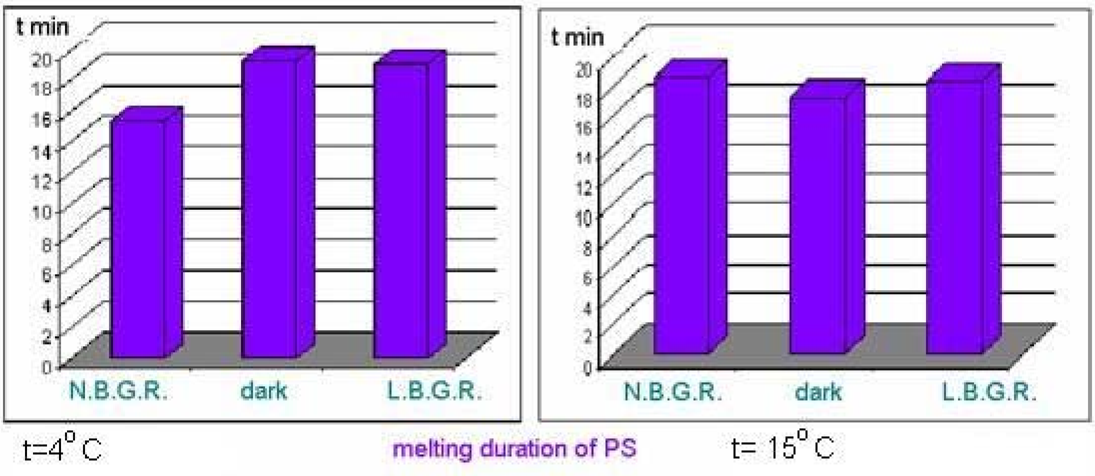


Figure 36: The duration of frozen PS fusion in liquid N_2 at NBGR+light, NBGR-light and LBGR-light at 4 ^{O}C and 15 ^{O}C.

As in case of DW the heat fusion of PS was also increased after 30 min. pre-incubation at dark and 4^{o}C medium, while at 15^{o}C this effect was reversed.

4.1c Light effect on O_2, CO_2 content and H_2O_2 formation in DW and PS

In figure 37 is demonstrated light and BGR effect on O_2 content in DW at 4 and 15^{O}C. As it can be seen from the presented data O_2 content was increased in dark condition and in dark lead box. This effect was more pronounced at 15^{O}C. From these data we can conclude that light and BGR have depressing effect on O_2 solubility in DW.

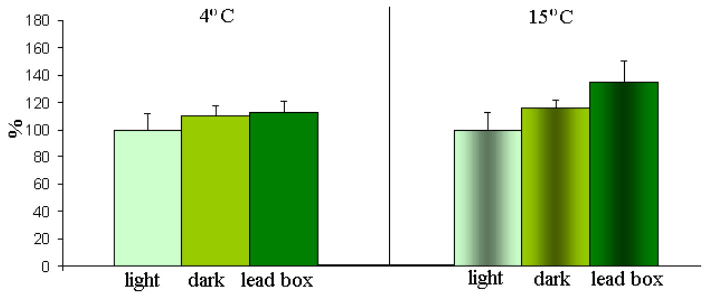


Figure 37: The effect of light and BGR on O_2 content in DW at 4 and 15^{O}C

From figure 38 it can be seen that BGR has more strong elevation effect on O_2 solubility than light has. We have more pronounced effect at 15^{O}C again like in case of DW. Light and BGR also have modulation effect on pH of DW at 4 and 15 ^{O}C while in PS only at 4^{O}C.

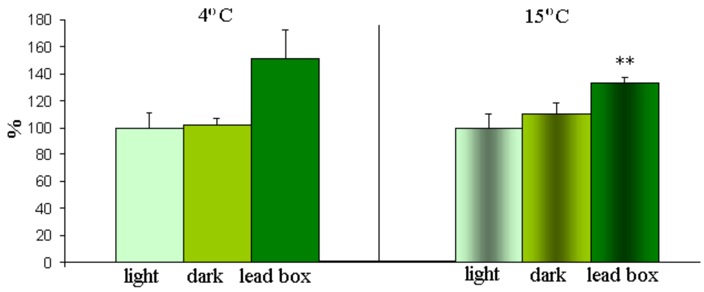


Figure 38: The effect of light and BGR on O_2 content in PS at 4 and 15^{O}C

Light and LBGR have depressing effect on pH at 4^{O}C, while at 15^{O}C the effect of dark condition was increased as compared with the effect of light and NBGR.

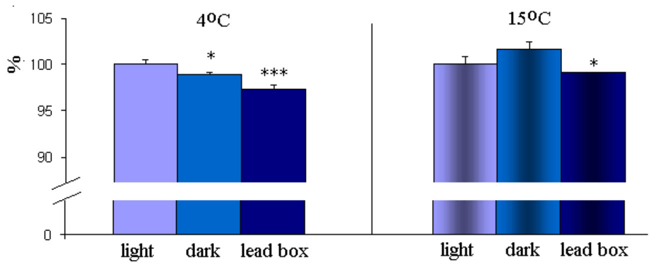


Figure 39: The effect of light and BGR on pH (CO_2 content) in DW at 4 and 15^{O}C

The disappearance of the effect of dark condition at LBGR on pH of PS at 15^{o}C can be explained by the buffer properties of PS, which was depressed at 4^{o}C.

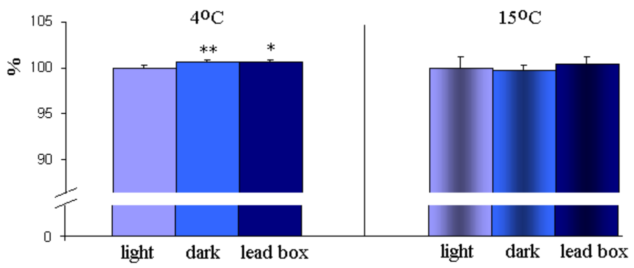


Figure 40: The effect of light and BGR on pH (CO_2 content) in PS at 4 and 15^{O}C

Thus, the above presented data clearly show that gas solubility and SEC (water dissociation) of DW and PS are sensitive to both light and BGR level.

The study of light and BGR effect on DW and PS has shown that at 4^{o}C dark condition and LBGR have statistically significant depressing effect on H_2O_2 content as compared with the control one. The same but less pronounced effect was obtained at 15^{o}C.

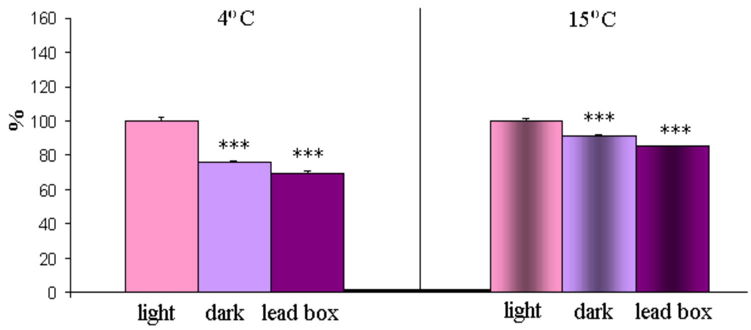


Figure 41: The effect of light and BGR on H_2O_2 content in DW at 4 and 15^{O}C

However, in PS dark condition and LBGR have the opposite effect on H_2O_2 content: in dark condition it was significantly decreased, while at LBGR there was a strong elevation effect on H_2O_2 content as compared with the control one.

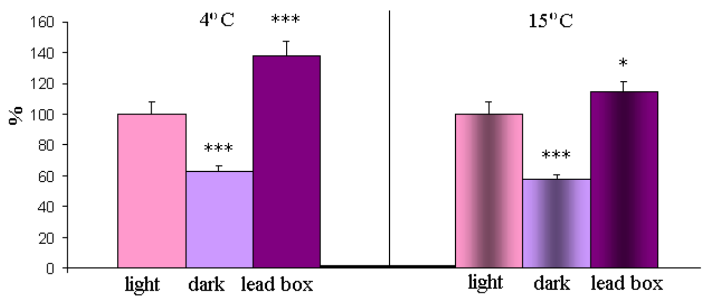


Figure 42: The effect of light and BGR on H_2O_2 content in PS at 4 and 15^{O}C

As H_2O_2 serves as a strong modulator for different chemical reactions and cell metabolism, the obtained data seems to be extremely important from practical point of view for understanding the role of light and background ionizing radiation in regulation of functional activity of cells and organisms.

4.2. IS effect on SEC of physicochemical properties of DW and PS.

4.2. a IS effect on SEC of DW and PS at NBGR+light ; NBGR-light and LBGR-light

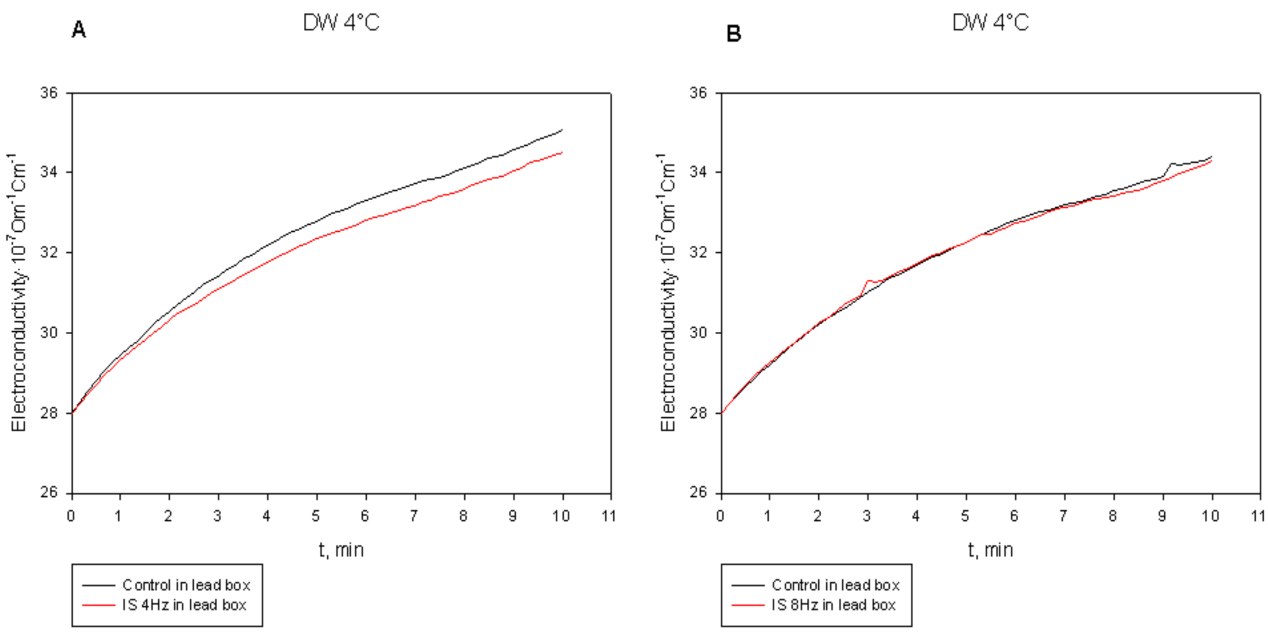


Figure 43: 4 (A) and 8 Hz (B) IS effect on DW SEC at LBGR-light, NBGR+light and NBGR-light at 4^{O}C

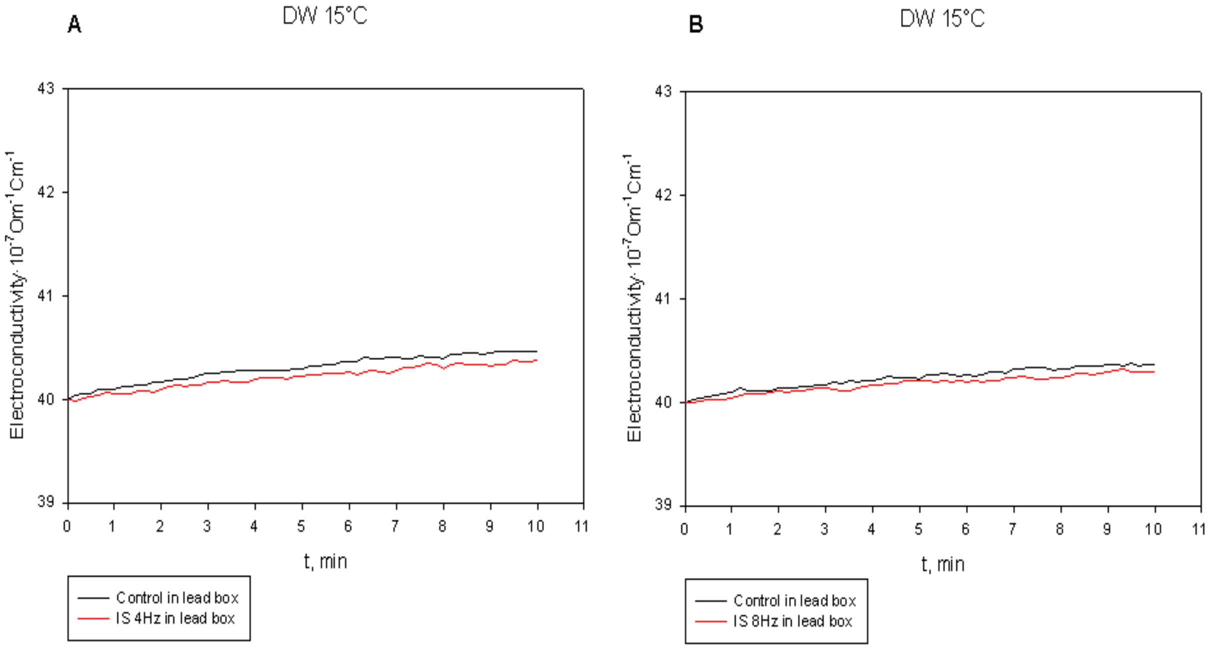


Figure 44: 4 (A) and 8 Hz (B) IS effect on DW SEC at LBGR-light, NBGR+light and NBGR-light at 15^{O}C

As can it be seen on figure 43, 4Hz IS at LBGR and 4^{o}C has depressing effect on SEC of DW (A), however 8Hz has no effect on it (B), while at 15^{o}C 4Hz-induced effect on SEC was disappeared (Figure 44 A and B).

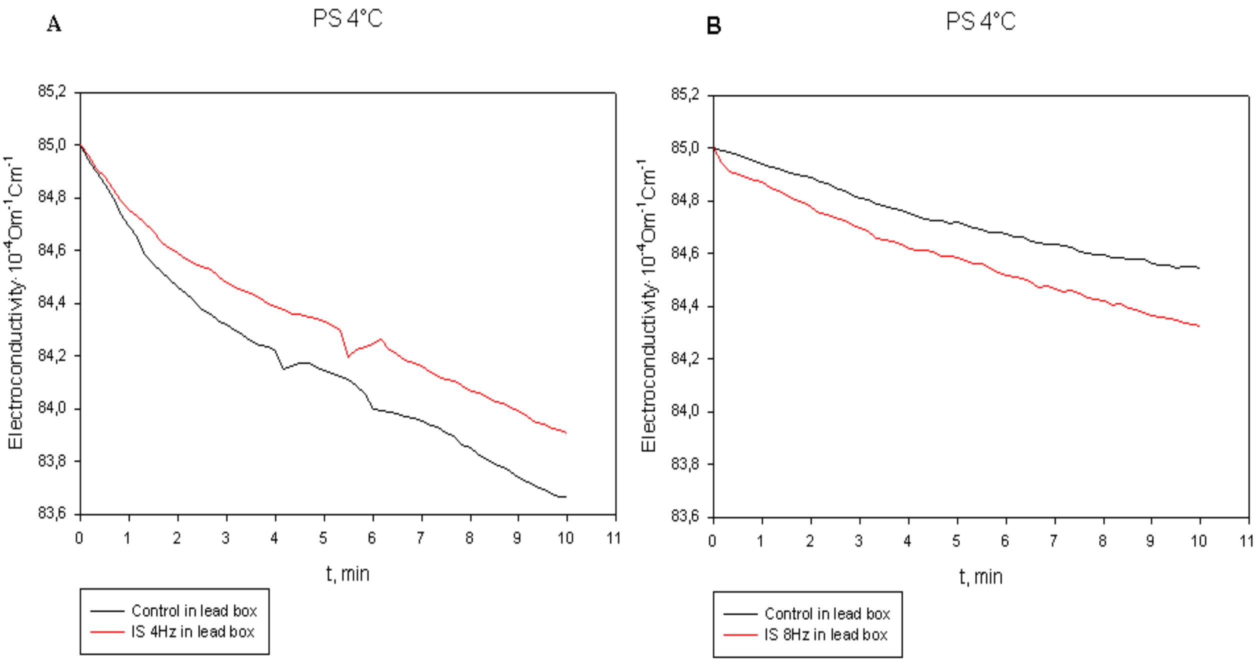


Figure 45: 4 (A) and 8 Hz (B) IS effect on PS SEC at LBGR-light at 4^{O}C

It is interesting that 4Hz IS has increasing effect on SEC of PS in LBGR at 4^{o}C when water has maximum density, (figure 45 A) while 8Hz IS has depressing effect on it (B), while at 15 ^{o}C IS at both 4 and 8 Hz frequencies has decreasing effect on PS SEC (Figure 46 A and B).

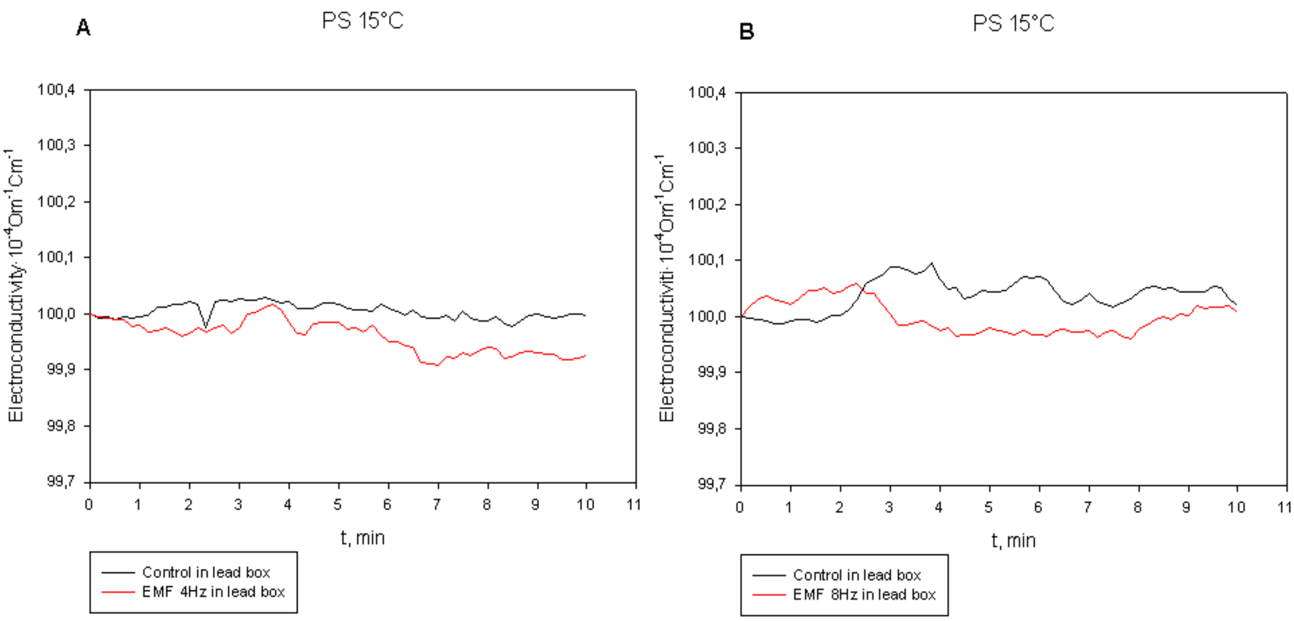


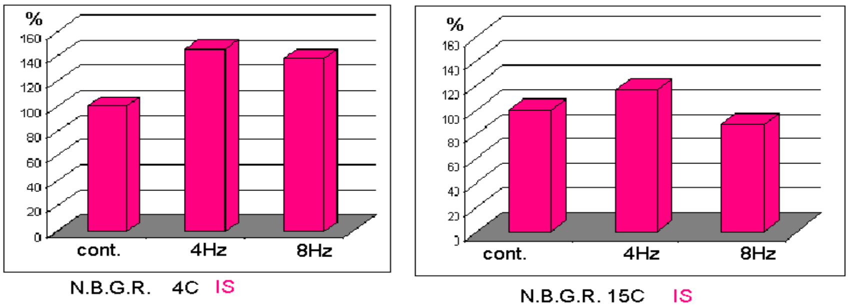
Figure 46: 4 (A) and 8 Hz (B) IS effect on PS SEC at LBGR-light at 15^{O}C

4.2. b IS effect on heat fusion of DW and PS at NBGR +light ;NBGR -light and LBGR-light

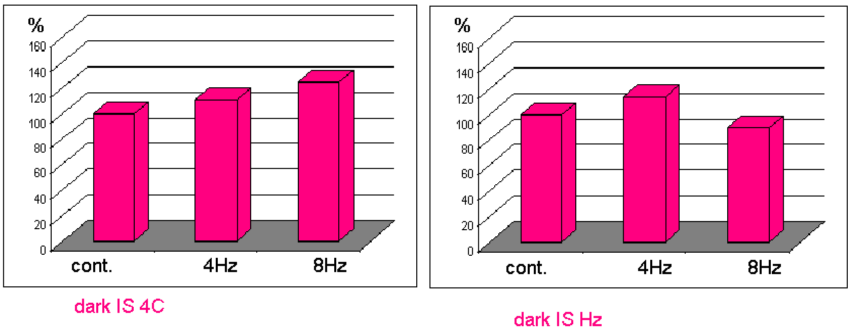
4 and 8 Hz IS has elevation effect on heat fusion of DW at 4^{o}C in NBGR, while at 15^{o}C 8Hz IS has depressing effect on it. As it can be seen from figure 47 (B) in NBGR and dark condition 4 and 8 Hz IS has the same effect on heat fusion as in light condition (A), while in LBGR this effect was reversed and it has depressing effect on heat fusion at 4^{o}C, but in 15^{o}C 8Hz has elevation effect on it.

From these data we can conclude that 8Hz IS effect on heat fusion depends on DW density: 8Hz IS at 4°C has depressing effect, while at 15°C it has elevation effect on heat fusion.

A.



B.



C.

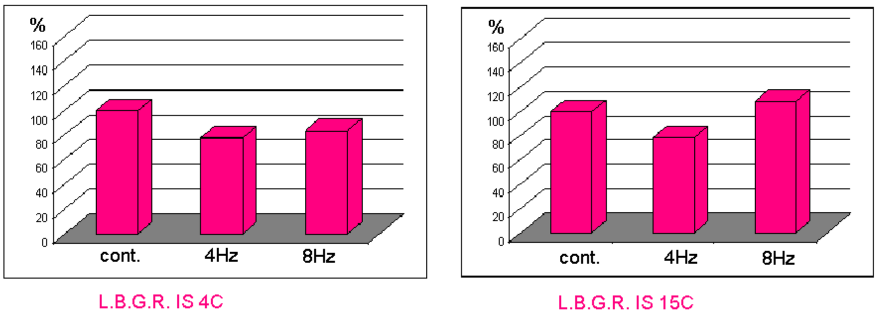
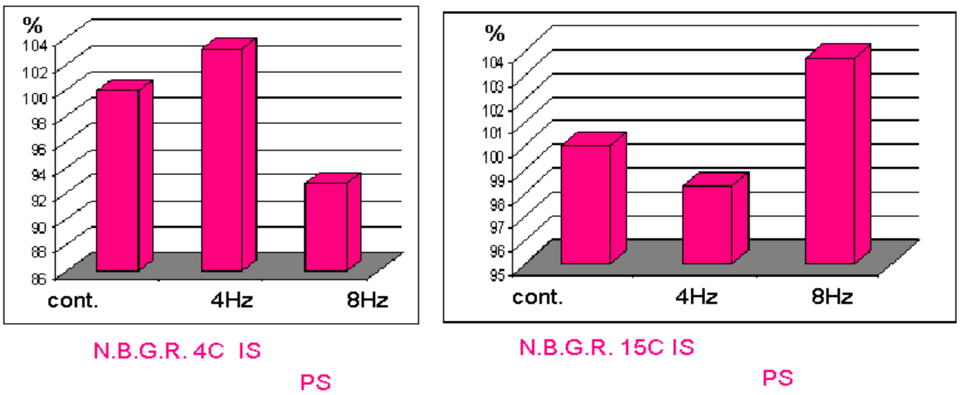
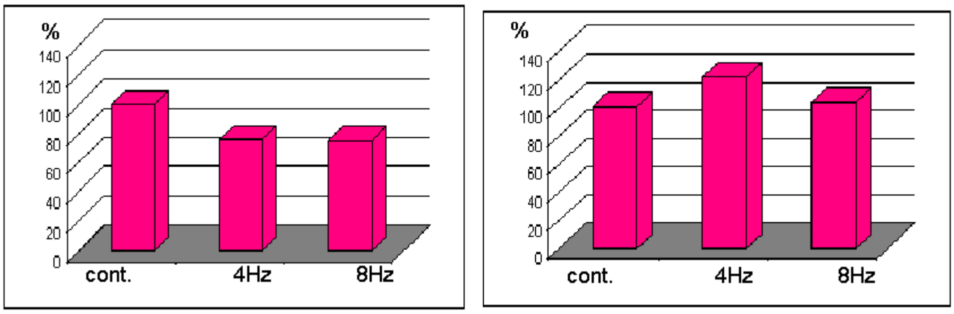


Figure 47: The effect of 4 and 8Hz IS on heat fusion of DW in room temperature (18°C), after freezing in liquid N_2 at NBGR +light (A); NBGR –light (B) and LBGR-light (C) at initial temperature 4 and 15°C. Results of one of five typical experiments are presented.

A.



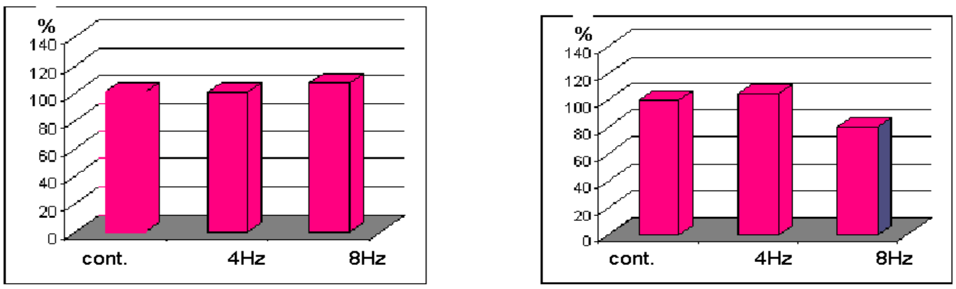
B.



IS dark PS 4C

IS dark PS 15C

C.



IS L.B.G.R. 4C PS

IS L.B.G.R. 15C IS

Figure 48: The effect of 4 and 8Hz IS on heat fusion of PS in room temperature (18°C), after freezing in liquid N_2 at NBGR+light (A); NBGR-light (B) and LBGR-light (C), at initial 4 and 15°C temperature. Results of one of ten typical experiments are presented.

The effect of 4 and 8Hz IS on heat fusion of PS was dramatically different from the effect of DW. 4Hz IS has elevation and 8Hz depressing effect on heat fusion of PS at 4°C, while at 15°C these effects were reversed: 4Hz has depressing and 8Hz elevation effect on heat fusion in NBGR+light compared with the control one. 4 and 8Hz IS has depressing effect on heat fusion of PS at 4°C and elevation effect at 15°C in NBGR and dark condition. In LBGR 4 and 8Hz IS has increasing effect at 4°C, while 8Hz at 15°C has decreasing effect on heat fusion. These data bring us to the conclusion that the effect of IS on heat fusion of PS depends on frequency, temperature, BGR and light.

4.2c IS effect on O_2, CO_2 content and H_2O_2 formation in DW and PS at NBGR+light; NBGR -light and LBGR-light

IS has insignificant effect on O_2 content of DW at NBGR and light condition, while in dark medium at 15°C 4Hz IS has depressing effect on it.

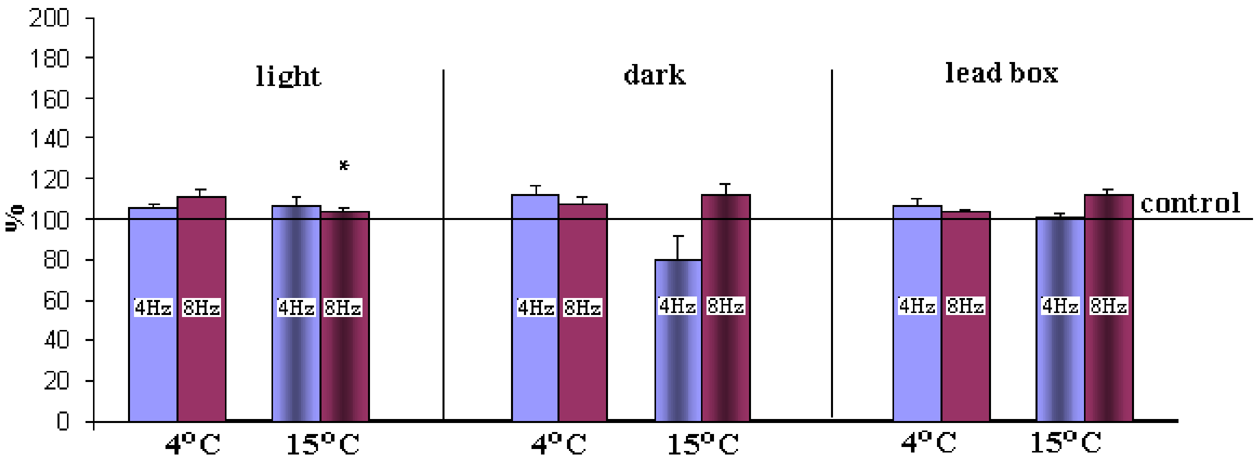


Figure 49: The effect of 4 and 8 Hz IS on O_2 content in DW at 4 and 15 ^oC in NBGR+light, NBGR-light and LBGR-light conditions

As it can be seen from figure 49, 4 and 8 Hz IS has elevation effect on O_2 content in PS at 4^OC, while at 15^OC 8 Hz has insignificant effect on it as compared with control one at NBGR and light condition.

It is interesting that in dark medium and at 15^oC 8Hz has a strong elevation, while 4Hz has significant depressing effect on O_2 content.

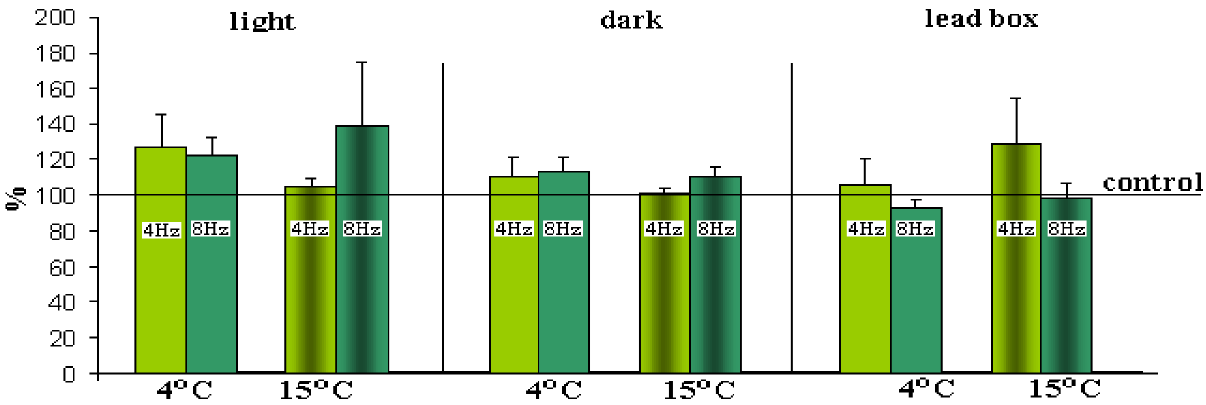


Figure 50: The effect of 4 and 8 Hz IS on O_2 content in PS at 4 and 15 ^oC in NBGR+light, NBGR-light and LBGR-light conditions

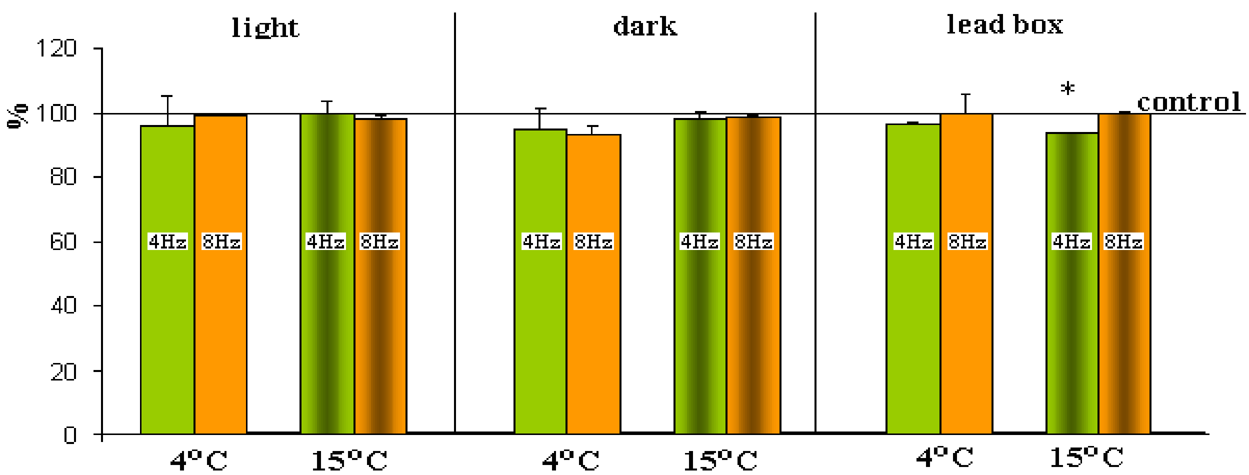


Figure 51: The effect of 4 and 8 Hz IS on pH of DW at 4 and 15 ^oC in NBGR+light, NBGR-light and LBGR-light conditions

Only 4 Hz IS at 15^OC has significant depressing effect on pH (CO_2 content) of DW in lead box (LBGR) (Figure 51).

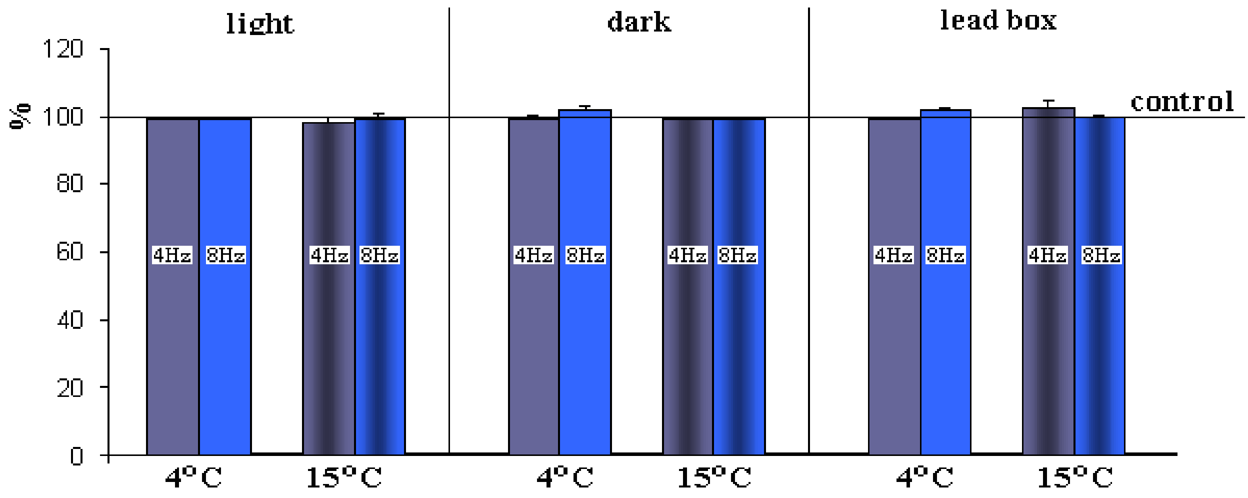


Figure 52: The effect of 4 and 8 Hz IS on pH of PS at 4 and 15 ^oC in NBGR+light, NBGR-light and LBGR-light conditions

As it was predicted IS has insignificant effect on pH of PS in above mentioned conditions because of the buffer properties of PS.

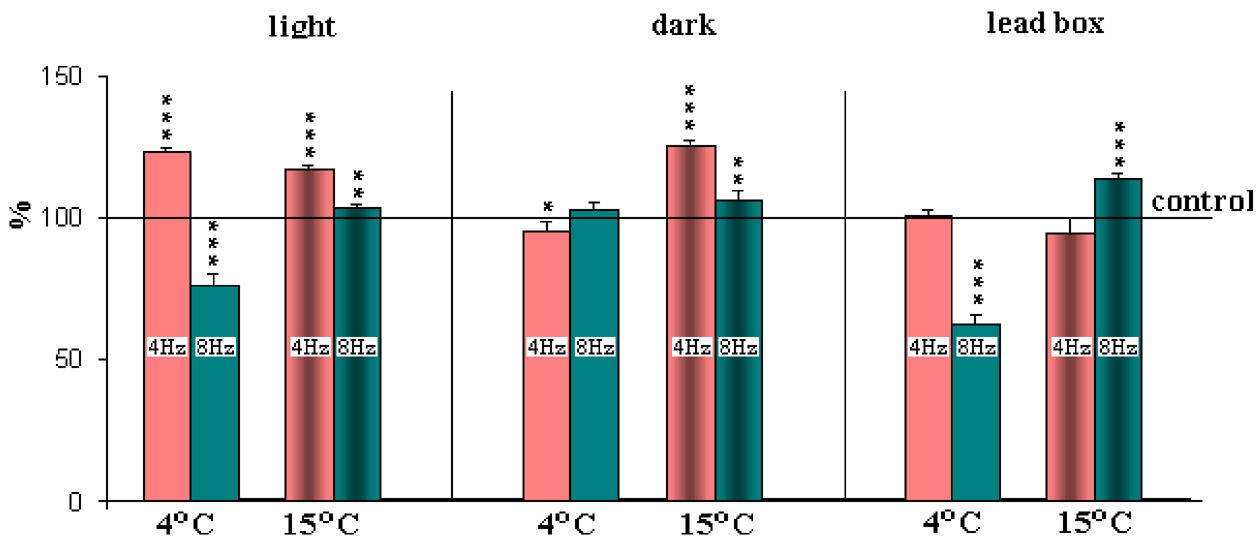


Figure 53: The effect of 4 and 8 Hz IS on H_2O_2 content in DW at 4 and 15 ^oC in NBGR+light, NBGR-light and LBGR-light conditions

As it can be seen from figure 53 H_2O_2 content has significantly changed upon the effect of IS in all- light, dark and lead box conditions: 4 Hz IS has elevation effect in NBGR and light condition at 4 and 15^OC, while 8 Hz has strong depressing effect at 4^OC and slight increasing effect at 15^OC as compared with control one; at NBGR and dark condition the mentioned effect of 4 and 8 Hz IS was disappeared at 4^OC, but at 15^OC this effect remains the same as the effect of NBGR in light. 4 Hz IS has no effect on H_2O_2 content at 4 and 15 ^{O}C, while 8 Hz IS has significant depressing effect at 4 ^{O}C and elevation effect at 15 ^{O}C. These data clearly demonstrate the variability of H_2O_2 content in DW depending on frequency of IS, temperature, light and BGR.

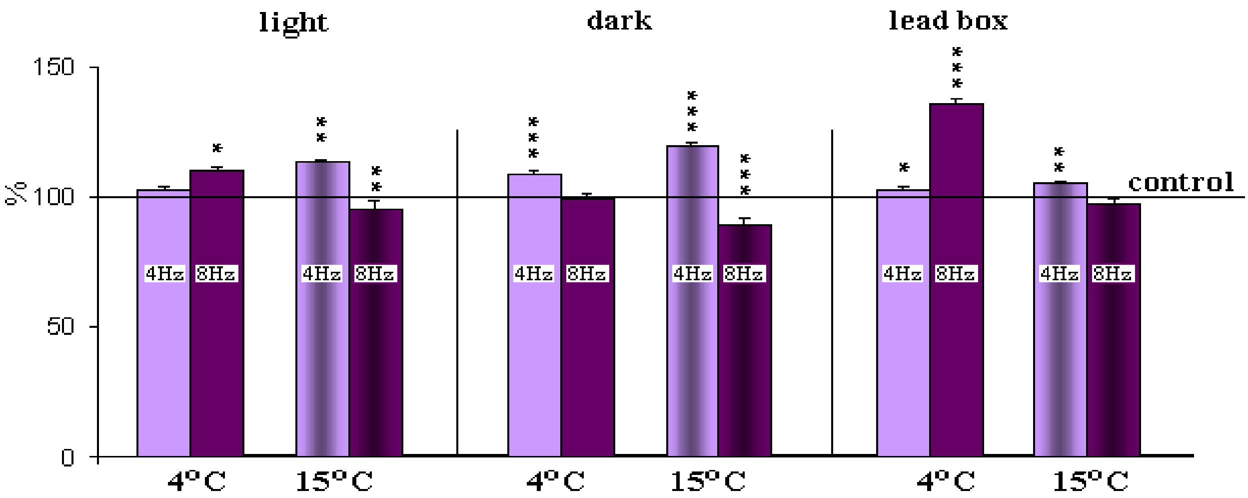


Figure 54: The effect of 4 and 8 Hz IS on H_2O_2 content in PS at 4 and 15^{O}C in NBGR+light, NBGR-light and LBGR-light conditions

It is interesting to note that at 15^{o}C 4 Hz IS has increasing, while 8 Hz has depressing effect on H_2O_2 content, while at 4 ^{o}C 4 and 8 Hz IS effect on H_2O_2 content was different in NBGR+light, NBGR-light and LBGR-light conditions: in NBGR and lead box 4 Hz has insignificant changes, while 8 Hz has significant elevation effect on H_2O_2 content, but in dark condition 4 Hz has elevation effect and 8 Hz has no effect on H_2O_2 content as compared with control one. Thus, these data clearly show that IS could change water clusters at 4 ^{o}C when water has maximal density.

4.3. ELF EMF effect on SEC of physicochemical properties of DW and PS.

4.3a EMF effect on SEC of DW and PS at NBGR +light; NBGR-light and LBGR-light

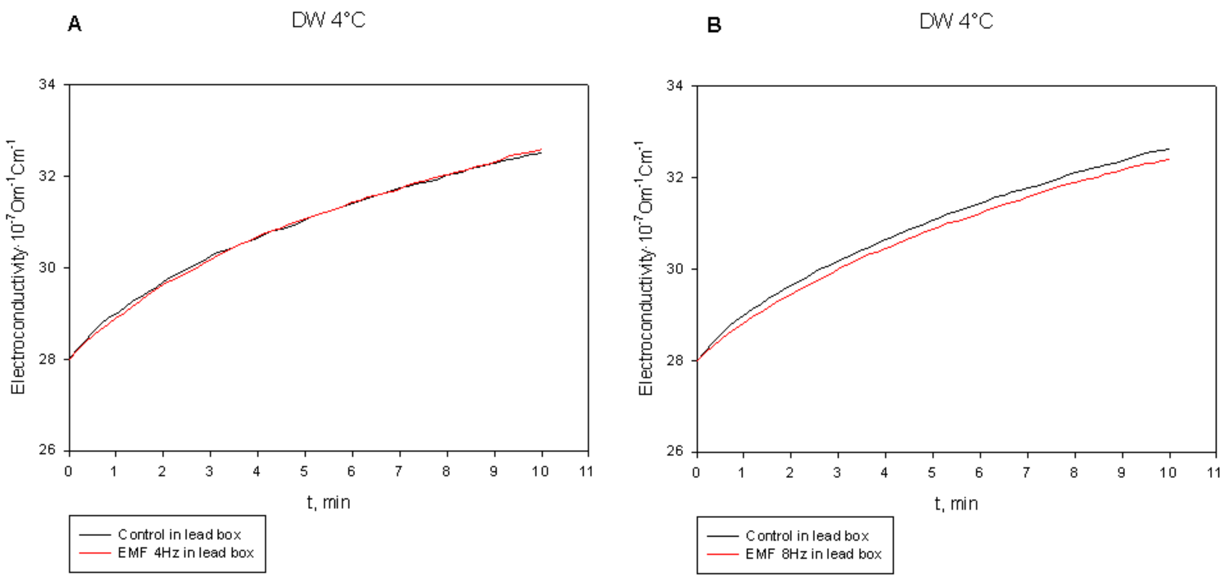


Figure 55: The effect of 4 (A) and 8 Hz (B) EMF on SEC of DW at NBGR +light; NBGR-light and LBGR-light at 4^{o}C

As it can be seen from figure 55 (A and B) 4 and 8 Hz EMF has insignificant effect on SEC of DW at 4°C, while in the same condition IS has strong modulation effect on it. These differences indicate on different mechanism of EMF- and IS-induced changes of water SEC. If EMF-induced changes of SEC can be explained by the valentic angle changes in water molecules, IS-induced effect on SEC is realized through water molecule (clusters) vibration-induced breaking of hydrogen bonds.

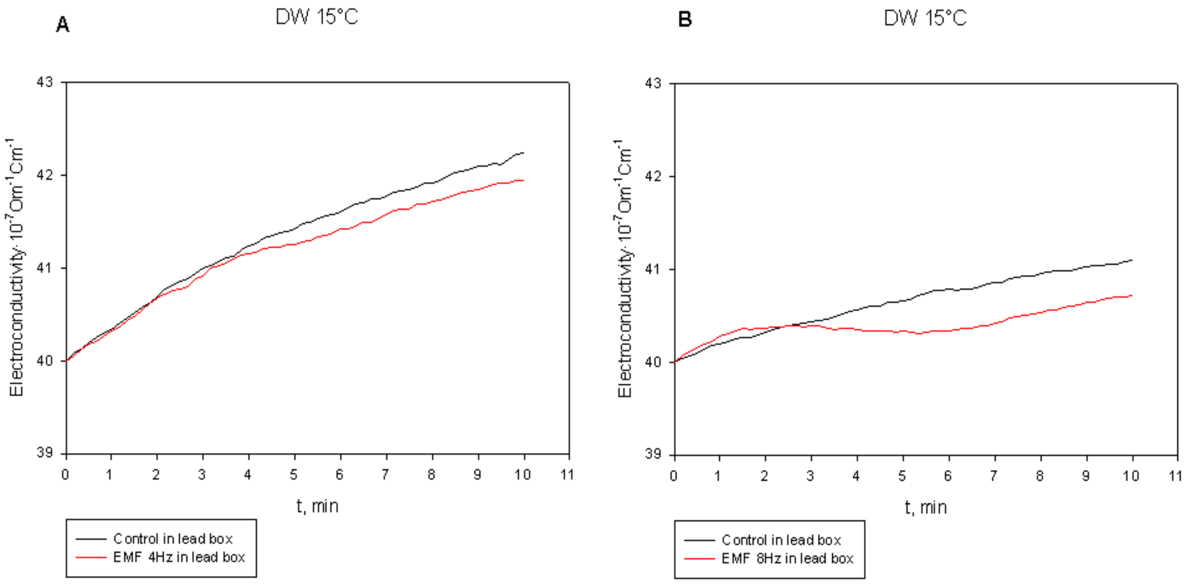


Figure 56: The effect of 4 (A) and 8 Hz (B) EMF on SEC of DW at NBGR +light; NBGR-light and LBGR-light at 15°C

At 15°C, when the water density is less than at 4°C, in result of which the valentic angle in water molecules is variable, 4 and 8 Hz EMF has depressing effect on SEC of DW. As the presented data show this effect was more pronounced at 8 Hz EMF.

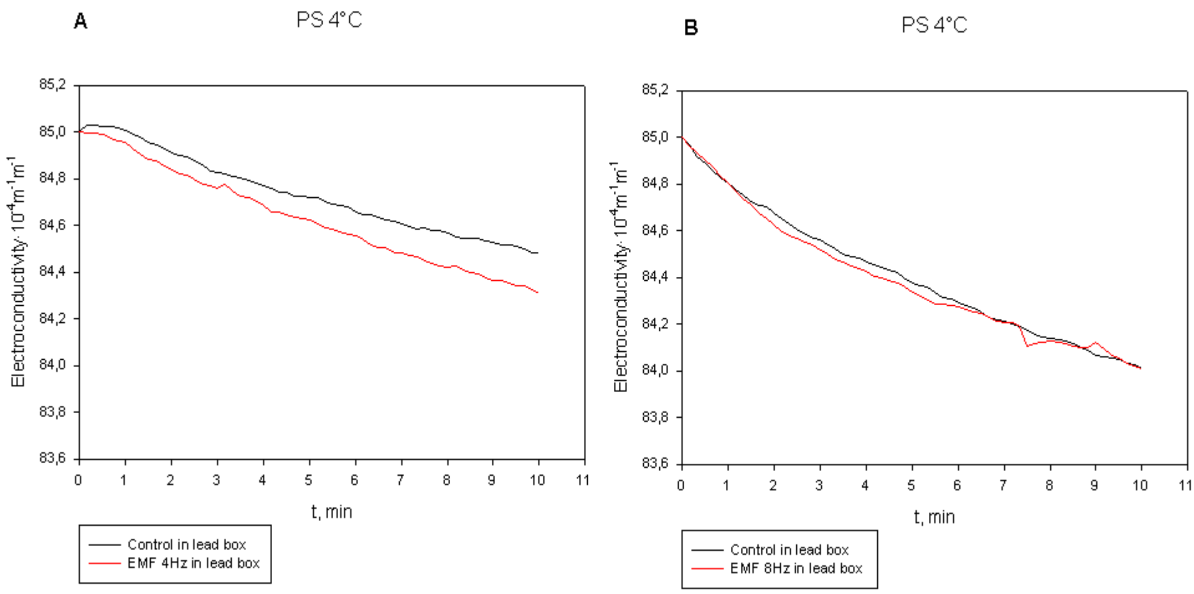


Figure 57: The effect of 4 (A) and 8 Hz (B) EMF on SEC of PS at NBGR +light; NBGR-light and LBGR-light at 4°C

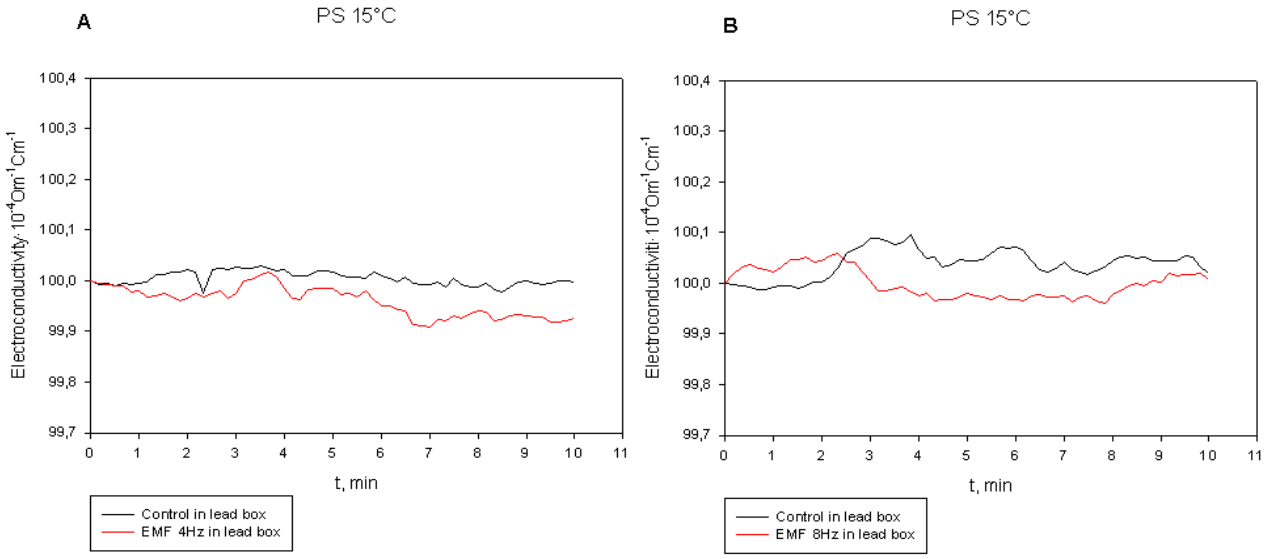


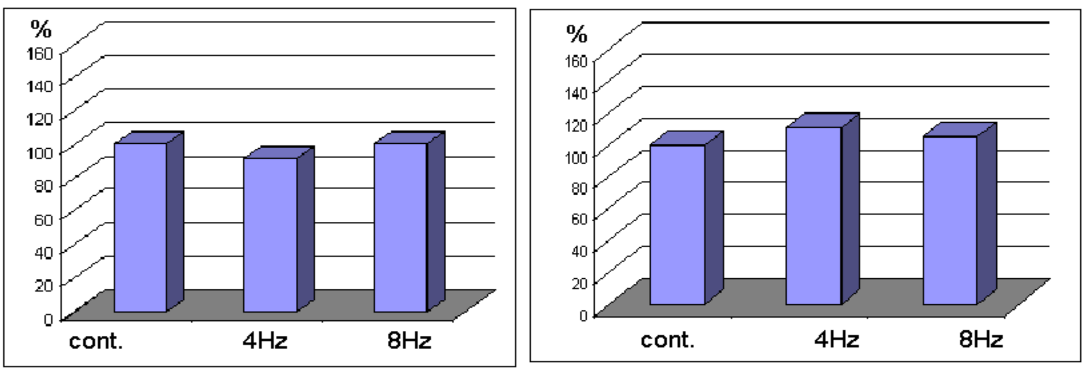
Figure 58: The effect of 4 (A) and 8 Hz (B) EMF on SEC of PS at NBGR +light; NBGR-light and LBGR-light at 15°C

4 Hz EMF has more pronounced effect on SEC of PS than 8 Hz at 4°C (figure 57 A and B). These data correspond with early biological experiments of our Center and South Korean group on functional activity of muscles and neuronal cells (Ayrapetyan et al. 2004, Park et al. 2008). As in case of 4 °C at 15°C also 4 and 8 Hz EMF has depressing effect on PS SEC (figure 58 A and B).

4.3b EMF effect on heat fusion of DW and PS at NBGR+light; NBGR-light and LBGR-light

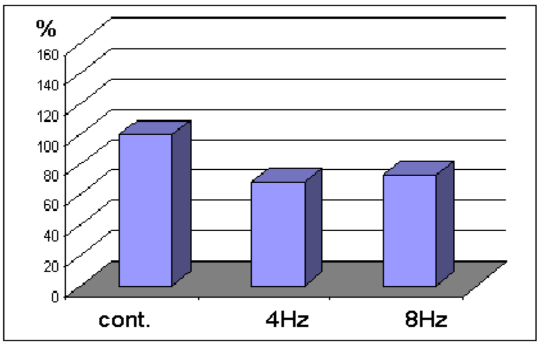
The data presented on figure 59 show that 4Hz EMF has depressing effect at 4°C and elevation effect at 15°C on heat fusion of DW, while 8Hz has insignificant effect on it at 4 and 15 °C. 4 and 8Hz EMF has strong inhibitory effect at 4°C, while at 15°C 4Hz has increasing effect and 8Hz has no effect on heat fusion in NBGR and dark condition. 4 and 8Hz EMF has slight increasing effect at 4°C, while at 15°C only 8Hz has increasing effect on heat fusion in LBGR and dark condition.

A.

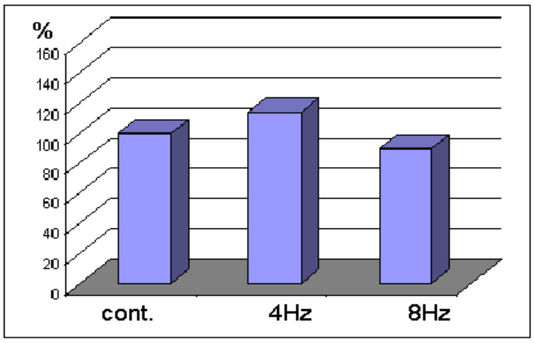


EMF N.B.G.R. 4C EMF N.B.G.R. 15C

B.

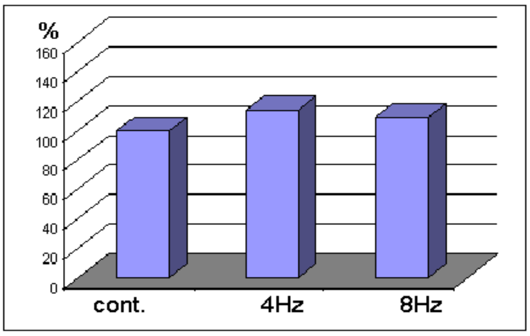


dark EMF 4C

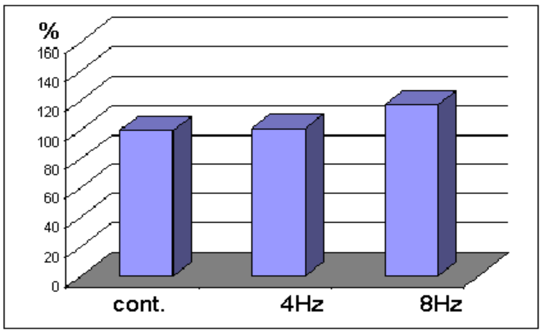


dark EMF 15C

C.



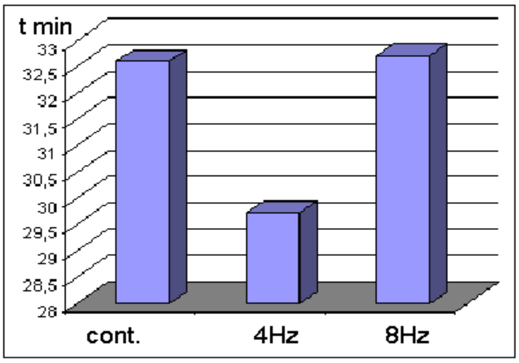
EMF L.B.G.R. 4C



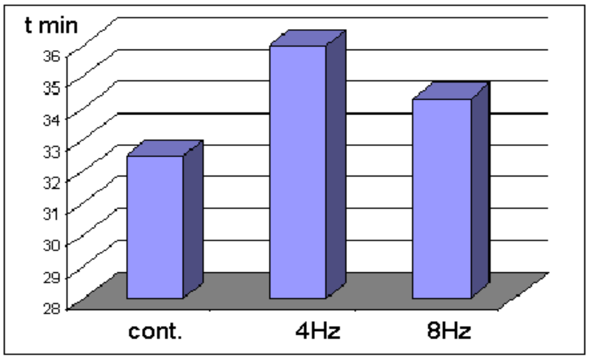
EMF L.B.G.R. 15C

Figure 59: EMF effect on heat fusion of DW at NBGR +light (A); NBGR–light (B) and LBGR-light (C) at 4 and 15°C

A.

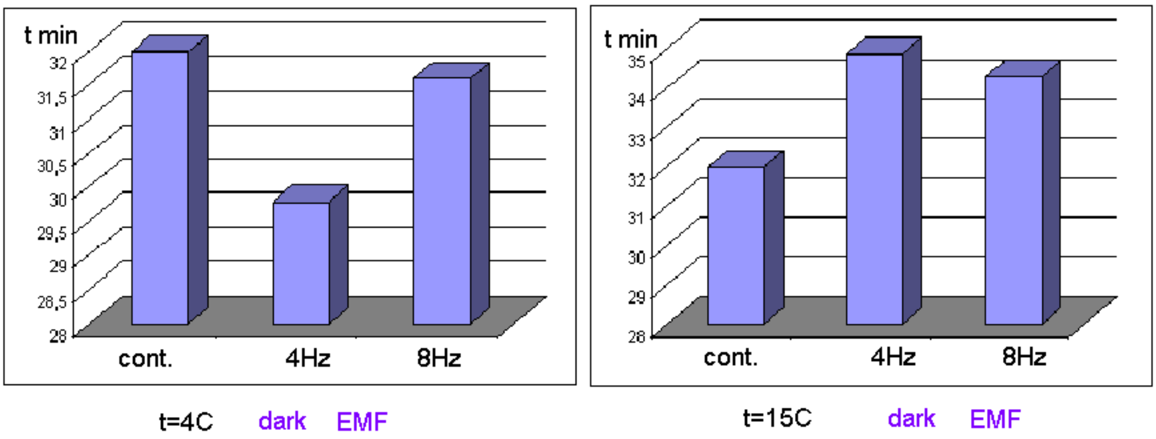


t=4C N.B.G.R. EMF



t=15C N.B.G.R. EMF

B.



C.

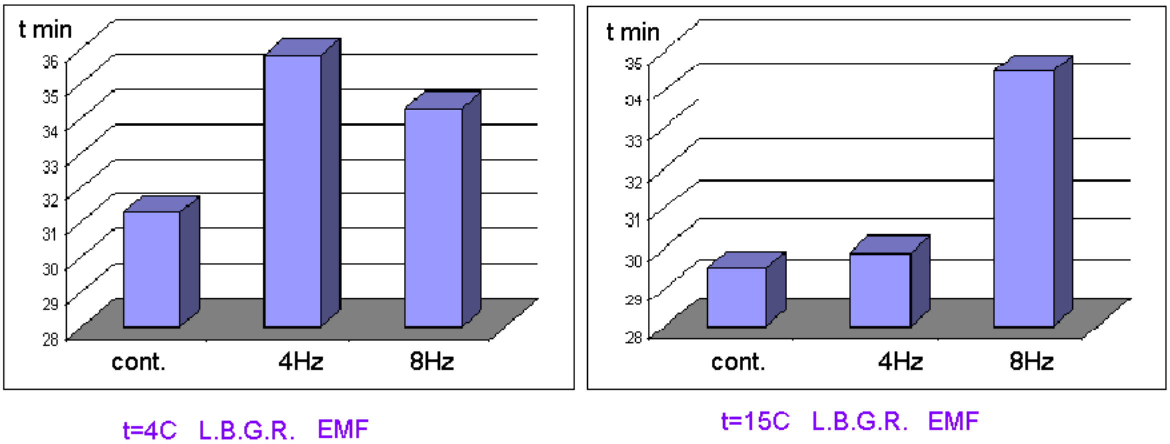


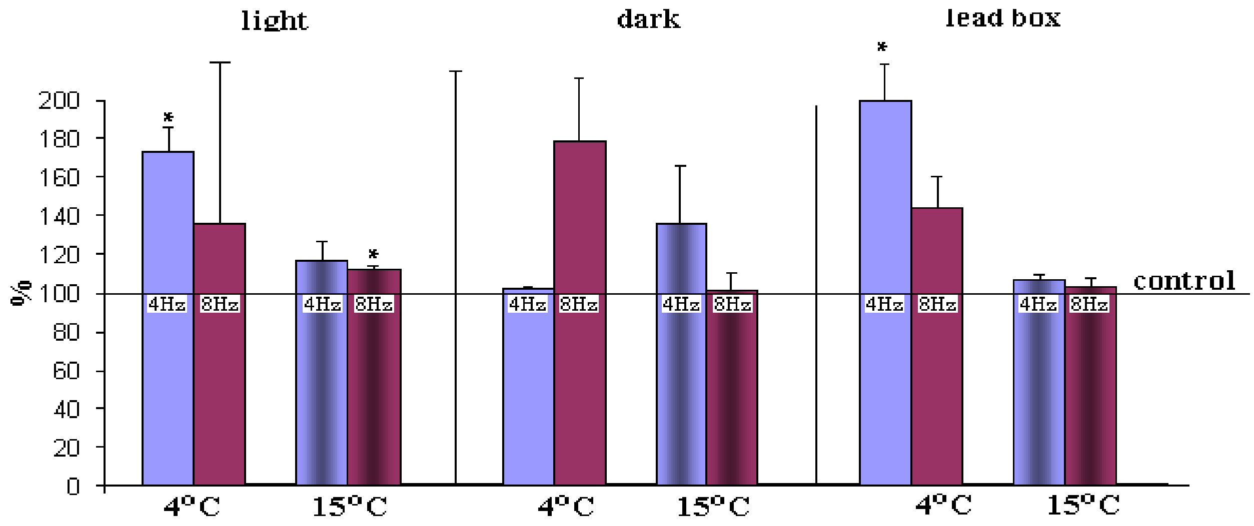
Figure 60: EMF effect on heat fusion of PS at NBGR +light (A); NBGR –light (B) and LBGR-light (C) at 4 and 15°C

The sensitivity of PS heat fusion to EMF was more pronounced than IS sensitivity of PS. 4Hz EMF has strong inhibitory effect on heat fusion and 8Hz has no effect on it at 4°C, while at 15°C 4 and 8Hz EMF has elevation effect on heat fusion in NBGR and light condition. The same effect of 4 and 8Hz EMF was obtained at 4 and15°C in NBGR and dark condition as in NBGR and light condition, while 4 and 8Hz has strong increasing effect on heat fusion at 4°C, but at 15°C 4Hz has slight and 8Hz strong increasing effect on it in LBGR. Thus, EMF effect on PS heat fusion highly depends on frequency, temperature and BGR and is comparatively non-sensitive to light.

4.3c EMF effect on O_2, CO_2 content and H_2O_2 formation in DW and PS at NBGR +light; NBGR-light and LBGR-light

The study of EMF effect on O_2 solubility in DW has shown that at 4°C 4Hz has elevation effect at NBGR and LBGR. However, at 15°C this effect was negligible, while in dark condition at 4°C only 8Hz has strong increasing effect on O_2 solubility (figure 61 A). The EMF-induced increasing effect of O_2 solubility in PS was observed only in LBGR at 8Hz frequency at 4°C (figure 61 B).

A.



B.

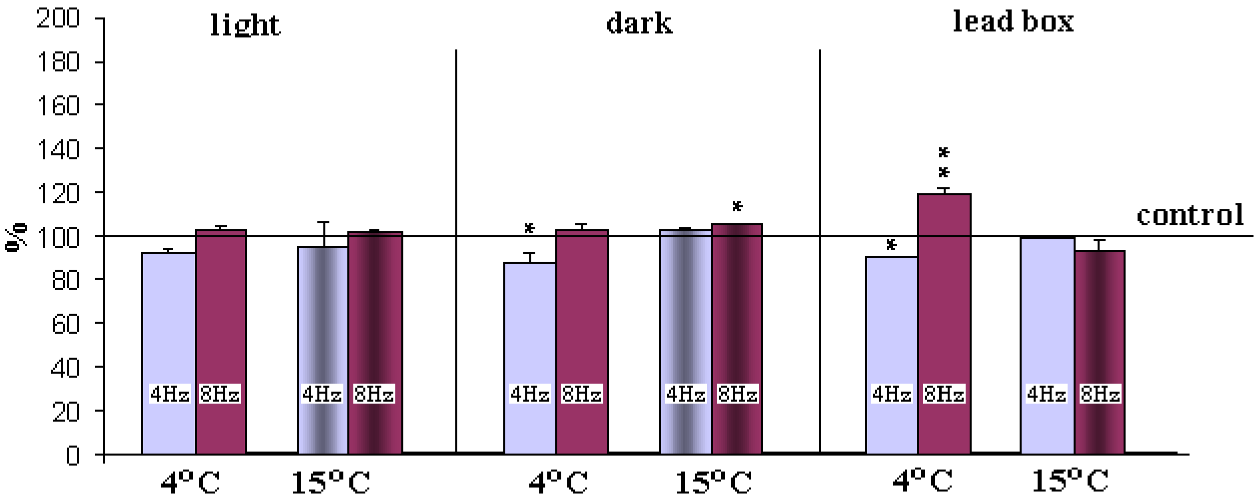
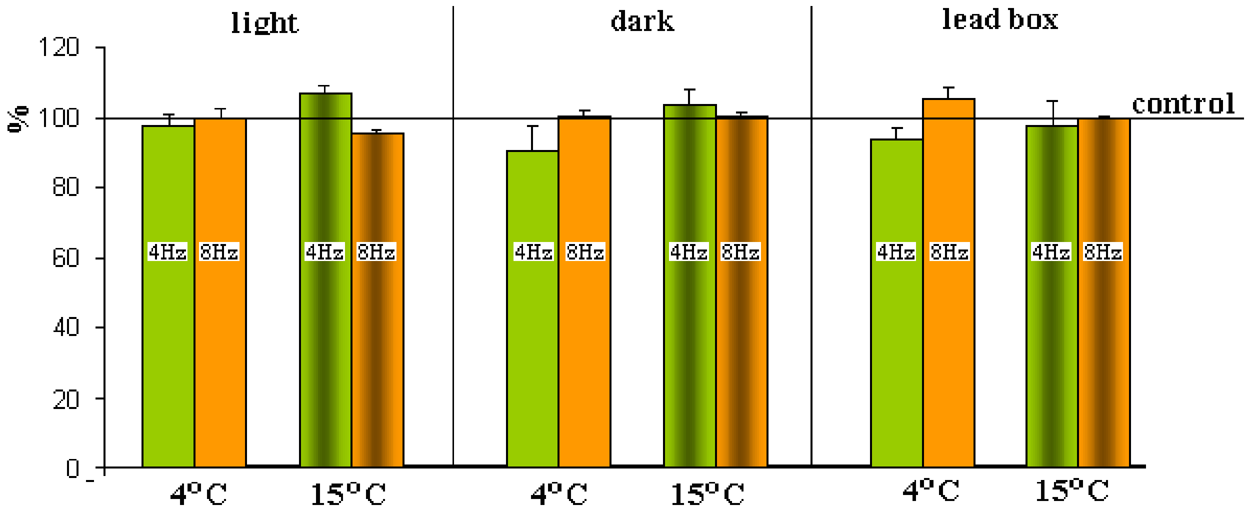


Figure 61: The EMF effect on O_2 content in DW (A) and PS (B) at NBGR +light; NBGR -light and LBGR-light at 4 and 15°C

EMF has no significant effect on CO_2 solubility in DW and PS (figure 62 A and B).

A.



B.

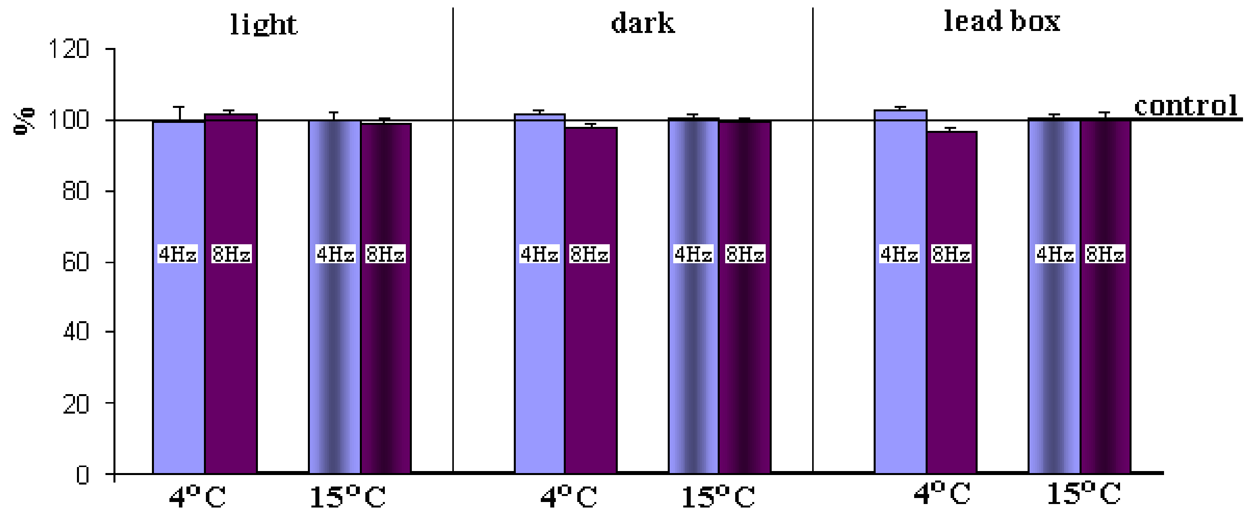


Figure 62: The EMF effect on pH (CO_2 solubility) in DW (A) and PS (B) at NBGR +light; NBGR - light and LBGR-light at 4 and 15°C

As it can be seen from the presented data H_2O_2 content was increased after treating DW by 4Hz EMF at 4 and 15°C in light and NBGR, but 8Hz EMF has depressing effect at 4°C and no effect at 15°C in the same conditions, while in NBGR and dark condition all these effects were reversed: 4Hz has depressing and 8Hz increasing effect on H_2O_2 content as compared with the control one. 4Hz EMF has depressing effect on H_2O_2 content also in dark lead box. These data indicate strong light dependency of 4 and 8Hz EMF-induced H_2O_2 content changes in DW.

In PS 4Hz has depressing effect on H_2O_2 content at 15°C in NBGR and light condition, while in dark condition 4Hz has increasing effect on H_2O_2 content at 15°C, but 8Hz has decreasing effect on it. In lead box 4Hz has depressing effect and 8Hz has no effect on it. Thus, EMF effect on H_2O_2 content in PS also depends on frequency, temperature, BGR and light.

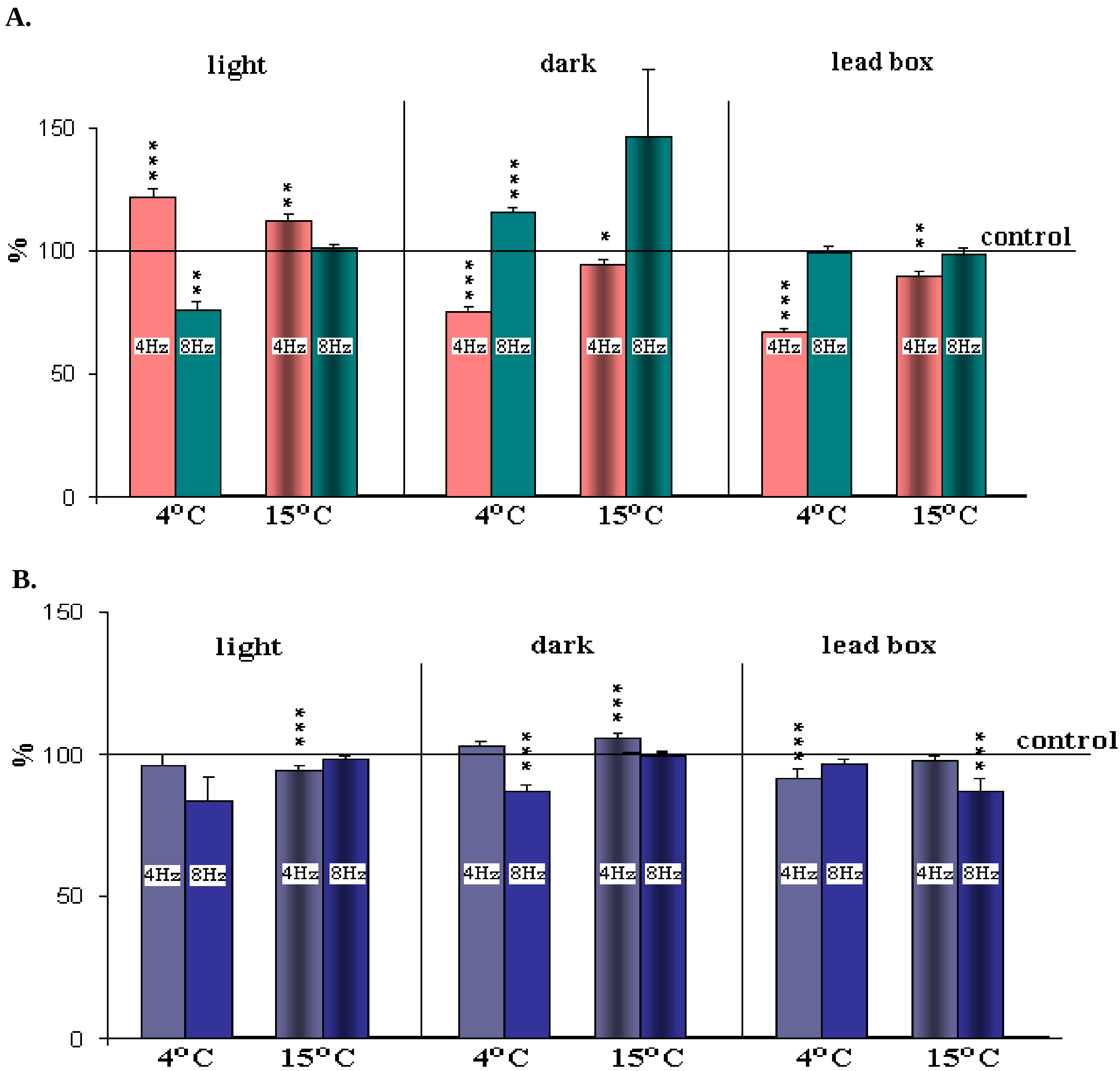


Figure 63: The EMF effect on H_2O_2 content in DW (A) and PS (B) at NBGR +light; NBGR -light and LBGR-light at 4 and 15°C

4. Conclusion

1. Temperature dependency of SEC in DW is higher than in PS. If the temperature dependency of DW SEC could be considered as a result of the increase of water molecules dissociation, in case of PS, SEC depends on ion hydration and velocity.
2. Heat fusion and melting rate of DW and PS depends on their initial temperature. This dependency is higher in case of PS than in DW.
3. The temperature-dependent decrease of CO_2 solubility in DW is more pronounced in range of temperature at 0-10°C , while in case of PS at 10-20°C.

4. The temperature dependency of H_2O_2 formation in DW was not pronounced while in case of PS H_2O_2 formation took place in range of 4-10^oC temperature. It is worth to note that at 4 ^oC, when PS has maximum density, the H_2O_2 content in PS was less than at 0 ^oC.
5. At LBGR during the first 30 min the rate of water SEC decay was gradually increased and then stabilized, while after transferring the samples at NBGR the water SEC started to decrease.
6. The ELF EMF has frequency-dependent depressing effect on SEC of DW and this effect was more pronounced at 4 and 8 Hz frequencies. Such similarity of the effects of 4 and 8Hz on water SEC seems extremely interesting and could be a subject for more detailed investigation.
7. At LBGR we observed the time-dependent decrease of PS SEC, like as in case of DW SEC. However, in case of PS more pronounced effect was observed only at the frequency of 9 Hz, while in DW these frequency windows appeared at 4 and 8 Hz.
8. The frequency-dependent effect of IS on SEC of DW at LBGR shows the pronounced elevation effect at 1 and 4 Hz, while in case of PS 9Hz IS has maximum depressing effect on it.
9. The 1-10 Hz IS has elevation effect on SEC of PS at LBGR. This effect was less pronounced at 4Hz.
10. The effect of EMF treatment on melting process of DW at LBGR was significantly depressed (or disappeared) at 2-100 Hz. LBGR has depressing effect on EMF-induced heat fusion changes, as well as delays the 50 and 100 Hz EMF-induced DW melting process.
11. At NBGR EMF exposure leads to the decrease of H_2O_2 content in DW at 1, 7, 20 and 50 Hz and to the increase at 4 and 50 Hz. At LBGR we had reversed picture: the H_2O_2 content at 1, 7, and 20 Hz was closer to the control level and 50 Hz EMF had the maximal elevation effect on H_2O_2 formation in DW
12. The effect of EMF on H_2O_2 level in PS is time –dependent: 1 Hz EMF exposure of PS during 10 minutes at LBGR depresses the H_2O_2 as compared with the control one, while 20-minute-exposure at the same EMF frequency has elevation effect on it.
13. As in case of DW, EMF has opposite effect on H_2O_2 content in PS at LBGR, as compared with NBGR, except 1 and 10 Hz frequency at which the H_2O_2 level is equal to the control one.
14. The above presented data show that 3Hz IS has elevation effect on H_2O_2 formation at NBGR while at LBGR it has strong depressing effect on it.
15. There are frequency "windows "(4 and 20 Hz) of ELF EMF and IS effects on water SEC. The EMF and IS effect on water SEC is decreased as a result of background radiation. The depressing effect of the latter was delayed at 4 and 8Hz EMF.
16. 16.1 The IS pretreated DW samples have high heat fusion at 5 and 10 Hz ,while the PS has low heat fusion at 1, 3 and 10 Hz frequencies as compared with non-treated DW and PS, correspondingly.

    16.2 The EMF-pretreated DW samples have low heat fusion at 2 and 4 Hz, while the PS samples, like as in case of IS-exposure, at 1, 3 and 10 Hz than control samples.
17. 17.1 Hz IS has stimulation while 8 Hz depressing effect on H_2O_2 formation in DW, while in case of PS IS has depressing effect on H_2O_2 formation at all frequencies. However in case of 5, 6 and 10 Hz this depression was less pronounced.

    17.2 EMF has depressing effect on H_2O_2 formation in DW at less than 50 Hz frequency, except 4Hz, while at higher frequencies (60-100 Hz) it has elevation effect on H_2O_2 formation , but in PS, the activation effect was observed only in case of 10 Hz EMF. The rest of observed frequencies showed the decrease of H_2O_2 content. The most pronounced depressing effect was observed at 8 Hz.

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Attachment 1

List of published papers and reports with abstracts

Ayrapetyan G., Dadasyan E., Hayrapetyan H., Ayrapetyan S. 2008. *Exogenous Hydrogen Peroxide as a messenger for stimulation effect of magnetized physiological solution on heart contractility*. Bioelectromagnetics, 29(7): 549-558

Abstract

The dual effect of magnetized physiological solution (MPS) on snail heart muscle contractility (muscle relaxation and stimulation of heartbeat) was shown previously. The MPS-induced relaxation of the heart muscle has been explained by activation of cGMP-dependent Ca2þ effluxes from the muscle; however, the mechanism of the stimulating effect of MPS on heartbeat remains unclear. As in the presence of paramagnetic oxygen molecules, magnetic fields could generate the exogenous reactive oxygen species, such as hydrogen peroxide (H2O2), we hypothesize thatH2O2 may play a role as the possible messengers through which the activation effect of MPS on heartbeat is realized. To test this hypothesis, the dose-dependent effect of exogenous H2O2 on heart muscle contractility and 45Ca uptake were studied. Here we compared the obtained data with the previous results of the effects of MPS on heart muscle contractility and 45Ca uptake. We found that exogenous H2O2 and MPS have similar effects on Naþ–Kþ pump-induced transient inhibition of muscle contractility and 45Ca uptake.

The Naþ–Kþ pump-induced depression of H2O2-sensitivity of muscle contractility is determined by activation of Ca2þ efflux from the cell. On the basis of these data we suggest the exogenous H2O2 as a possible messenger through which the stimulation effect of MPS on heart muscle is realized.

Ayrapetyan G., Hayrapetyan H, Dadasyan E, Barseghyan S, Baghdasaryan N., Mikayelyan E., Ayrapetyan S. *The non-thermal effect of weak intensity millimeter waves on physicochemical properties of water and water solutions***.** Electromagnetic Biology and Medicine (in press).

Abstract

The comparative study of the effects of 5,8 mW/cm2 Millimeter Waves (MMW) and near Infrared (IR) irradiation on thermal properties, specific adsorption rate (SAR), specific electrical conductivity (SEC) and hydrogen peroxide (H2O2) content of distilled water (DW) and physiological solutions (PS) was performed. The thermal effect of MMW irradiation appeared only after the first minute irradiation, while the IR heating started from the first min of irradiation. The heat fusion of frozen MMW-treated DW and PS was significantly less than sham and IR-treated DW and PS. MMW irradiation had time-dependent elevation effect on water SEC and SAR, which was accompanied by the increase of H2O2 formation in it. We suggest that the MMW-induced vibration of water dipole molecules caused the non-thermal changes of physicochemical properties of DW and PS, which promote the formation of H2O2 in water.

Musheghyan G., Deghoyan A., Ayrapetyan S. *The brain tissue dehydration as a mechanism of analgesic effect of hypertonic physiological solution in rats.* Anesthesia & Analgesia (in press)

Abstract

In previous our work, performed on single isolated neurons, we have showed that the neuronal shrinkage in hypertonic solution causes the decrease of the number of functionally active protein

molecules in the membrane that have channel forming, receptor and enzyme properties. On the basis of these data we have suggested that the hypertonic solution-induced pain-relieving effect on brain trauma could be explained by cell dehydration-induced depression of neuromembrane functional activity. For testing this hypothesis the correlation between brain tissue hydration, number of ouabain receptors (marker for membrane proteins) in the membrane and pain threshold to the hot plate was studied in rats, interperitoneally injected with physiological solution (PS) of different tonicities as well as in rats, which were given *ad libitum* access to distilled water (instead of regular drinking water) during 3 and 5 days.

Baghdasaryan N., Ayrapetyan S. *The frequency-dependent infrasound effect on bull sperm velocity*. Journal of Reproduction (in press)

Abstract

In present work the frequency (1-8 Hz), intensity (10-50 dB) and exposure time (1-9 minutes) dependency effect of infrasound (IS) on bull sperm velocity, 45Ca uptake, and intracellular cyclic nucleotide contents were studied. Sperm velocity was estimated by digital video-microscopic recording method by means of a special computer program. For 45Ca uptake and for intracellular level measurements of cyclic nucleotides the isotope method was used. It was shown that there were frequency and exposure time-dependent and intensity-less-dependent effects of IS on sperm velocity. 5 min. exposure of IS at 2Hz frequency on sperm velocity had more pronounced activation effect on it (38±4%, *** $p<0.001$), which was accompanied by the decrease of the intracellular level of cGMP (26±8% *: $p<0.05$) and by the elevation of the intracellular level of cAMP (43.5±7%, *: $p<0.05$) and by the activation of 45Ca-uptake by sperm (285±25%, *: $p<0.05$). It was suggested that the frequency window (2Hz) effect of IS on functional activity of sperm was due to the activation of mechanosensors in sperm membrane.

Attachment 2

List of presentations at conferences and meetings with abstracts

UNESCO/ONRG Seminar:
"Electromagnetic Fields: Mechanisms of Action and Health Effect"
24-26 October 2008
Yerevan, Armenia

Sinerik Ayrapetyan (Armenia) "*Cell hydration as a marker for detection of biological effect of EMF"*

Abstract

There is more and more use of electricity and RF communication frequencies in both civilian life and for military purposes. The clarification of cellular and molecular mechanisms of their biological effects is extremely important in order to rationally evaluate their harmful effects from the point of public health. Moreover, the possibility to using EMF-induced modulations makes EMF potentially a powerful instrument for environmental terror. The most obvious danger is use of electromagnetic pulses to disrupt communication. However, EMF also poses direct threats to human health through mechanisms that are poorly understood. These involve different physical and chemical factors, as well as multiple effects on biological systems including humans. Therefore, this problem is an appropriate and important subject for consideration under the Environmental Antiterrorism Program.

Naira Baghdasaryan, Erazik Mikayelyan & Sinerik Ayrapetyan *"The influences of different physical factors on hydrogen peroxide formation in distillated water and physiological solution"*

Abstract

It is known, that weak intensity physical signals such as static magnetic field (SMF), electromagnetic field (EMF), infrasound (IS), temperature variation and so on, could have some biological effect on various type of cells. On the basis of many data it was suggested that cell buffering medium could be served as a target through witch the biological effect of this weak physical factors could realized. However the nature of the messenger through witch the factor induced changing of this physical signals take place is not clear yet.

At the same time it is well known, that upon the influence of this weak physical signals the formation of H_2O_2 in water and physiological solution takes place.

Erna Dadasyan & Gayane Ayrapetyan *"The comparative study of the effects of 4Hz Electromagnetic Fields-, Infrasound-treated, and H_2O_2-containing physiological solution on heart muscle contractility"*

Abstract

The aim of the present work is to investigate the comparative study of 4 Hz extremely low frequency Electromagnetic Fields (ELF EMF), Infrasound (IS) and hydrogen peroxide (H_2O_2) on Na+ – K+ pump-induced transient inhibition of heart muscle contractility. It was documented that EMF-treated and H2O2-containing physiological solution has synergic depressing effect on Na+–K+ pump-induced inhibition of muscle contractility, while the IS has elevation effect on it. On the basis of the obtained data the H2O2 could be suggested as the messenger through which the stimulation effect of EMF on heart muscle is realized as it is known that the magnetization of aqua solutions leads to the elevation of the concentration of hydrogen peroxide (H2O2), while the relaxing effect of IS on heart contractility is due to the decrease of CO2 solubility of muscle bathing aqueous medium.

Anush Deghoyan, Gohar Musheghyan, Sinerik Ayrapetyan *"Magnetic Field Sensitivity of Organism Depends on the Level of Cell Hydration*

Abstract

In early works of our laboratory it was shown that there are close correlation between cell hydration and impedance characteristics of cell membrane (Carpenter et al 1992). Therefore, the overall aim of the present work is to find out more sensitive component of impedance after the influence by magnetic field and the age-dependent changes of impedance characteristics (in young and adult rats group). For this purpose the effects of distillated water as a drinking water and 15 min SMF exposure on brain tissue hydration and impedance in both adult and young rats were studied by means of determination of tissue hydration - (wet-dry)/dry, and active and reactive components of tissue impedance at low and high frequencies. Based on the obtained data we came to the conclusion that the reactive component at high frequency is the most sensitive impedance component. In adult animals 15 min SMF exposure decreases the brain hydration level and after the irrigation of the animals by DW this characteristic is increased. While in the group of young animals we observed the opposite effect. The same data were shown in case of active and reactive components of impedance at low and high frequencies.

Attachement 3

Information on patents and copy rights

1. The method of tumor inhibition by the use of 4 Hz EMF-treated DW **(Armenian patent № 2149 A2)**
2. The method of stimulation of bull sperm functional activity by the use of 2 Hz infrasound treatment **(Armenian patent AM 20080 148)**
3. The method of water purification from microbes **(Armenian patent AM 20080 155).**