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Development of a Magnetic-field Stimulation System for Cell Cultures *in situ*: Simulation by Finite Element Analysis

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The effects of exposure to an extremely low-frequency magnetic field (25 Hz 20G) on animal cells have been studied. In some reports, stimulation was performed for fixed frequency and variations in magnitude; however, animal-cell experiments have established that both parameters play an important role. The present work undertook the modeling, simulation, and development of a uniform-magnetic-field generation system with variable frequency and stimulation intensity (0-60 Hz, 1-25G) for experimentation with cell cultures *in situ*. The results showed a coefficient of variation less than 1 % of the magnetic-field dispersion at the working volume, which is consistent with the corresponding simulation results demonstrating a uniform magnetic field. On the other hand, long-term tests during the characterization process indicated that increments of only 0.4°C in the working volume temperature will not be an interfering factor when experiments are carried out in *in situ* cell cultures.

Keywords : magnetic field, extremely low frequency, helmholtz coils, hall effect, pulsed magnetic field.

1. Introduction

Studies on the effects of exposure of animal cells to non-ionizing radiation of extremely low-frequency magnetic field (ELFMF), i.e. fields whose energy is not sufficient to break the molecular bonds, have been performed [1]. Some of these effects are the rise in tissue temperature produced by energy absorption [2], modifications in Deoxyribonucleic Acid (DNA), alterations in the processes of cell proliferation, and apoptosis inhibition [3-5]. Collagen-systhesis improvement due to the application of magnetic fields to animal tissue also has been reported [6, 7]. The present studies were performed using a variable magnetic-field magnitude and a fixed frequency. A number of experiments applying static magnetic fields already have been reported. For example, Fanelli *et al.* demonstrated increased cell survival by magnet-induced inhibition of apoptosis [8]. Introducing an alternative means of extremely-low-frequency magnetic-field (ELFMF) generation with permanent magnets in rotation using stepper motors, Y. Ren *et al.* generated a maximum-15 Hz rotating magnetic field. They found that the application of their procedure can affect blood pressure in the cerebral cortex [9]. As for magnetic-field exposure effects as functions of the variation of both frequency and magnitude in cell-culture stimulation [6, 7], however, there have been few reports. This line of investigation, therefore, needs to be further explored.

Factors such as temperature, humidity, concentrations of O_2 and CO_2, nutrients and light, among others, are fundamental to the care, maintenance, and development of cell cultures [10, 11]. Adequate control of those factors, therefore, must be guaranteed in order to avoid any change that leads to error in the interpretation of their effects due to the application of magnetic fields.

Our working hypothesis is that experimentation with cell cultures exposed to ELFMF variable in frequency and amplitude [6, 7] must be *in situ* and within a con-

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trolled environment so as to guarantee that the effects observed were due only to the stimulus of the magnetic field. In this sense, it is important to develop a system for generation of magnetic fields variable in magnitude and frequency that yet preserves normal cell-culture conditions.

This paper presents the modeling, simulation, and development of a system for generation of uniform magnetic fields variable in magnitude and frequency for compatibility with *in situ* cell cultures within an incubator environment.

2. Materials and Methods

2.1. Magnetic-field generator: Simulation and design criteria.

To guarantee a uniform magnetic field in a working volume, it is necessary to establish some configurations or arrangements of coils such as the square coil systems (Merritt, Alldred & Scollar, and Rubens) and circular coil systems like those of Lee-Whiting and Helmholtz [12, 13]. Moreover, these systems should be able to work with both direct and alternate current since magnetic-field stimulation is needed for *in situ* cell cultures.

The Helmholtz configuration was chosen to generate a uniform magnetic field due to its simplicity, efficiency, and small size because this generator will be housed in an incubator whose volume is relatively small, 0.184 cubic meters (TC 230, Thermo Scientific Forma, Thermo Fisher Scientific, USA). This configuration consists of a pair of coils of equal size placed on the same axis and separated by a distance equal to its radius.

The magnitude of the magnetic field of this Helmholtz coil arrangement is directly proportional to the circulating current I and the number of turns N of each of them, and inversely proportional to the separation of the coils; in this case, the same as radius R. According to Biot-Savart's law, the magnetic field B can be calculated based on the current in the coils, as given by Eq. (1) [14].

$$B(z) = \frac{1}{2}\mu_0 NIR^2\left\{\left[R^2 + \left(z + \frac{R}{2}\right)^2\right]^{-\frac{3}{2}} + \left[R^2 + \left(z - \frac{R}{2}\right)^2\right]^{-\frac{3}{2}}\right\} \tag{1}$$

Where:

z is the distance from the center of the coils to a point where a uniform field should be observed.

μ_0 is the permeability (Tm/A).

The magnetic field distribution is considered to be uniform at the center of the coils, i.e., z = 0.

Thus Eq. (1) is reduced to Eq. (2)

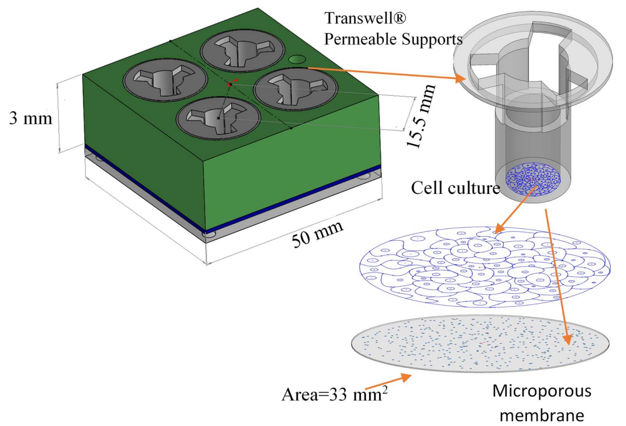

Fig. 1. (Color online) Chamber designed and built specifically for the placement of cell cultures.

$$B(0) = \frac{16}{5\sqrt{5}}\frac{1}{2}\frac{\mu_0 NI}{R} = 1.43\frac{\mu_0 NI}{D} \tag{2}$$

Eq. (2) shows a relatively simple way to define the parameters of a magnetic field generator based on Helmholtz configuration.

2.2. Design parameters of the magnetic field generator

2.2.1. Definition of working volume

Because the experimental protocol to be implemented contemplates an arrangement of four experimental samples of cell cultures to observe the effects of the magnetic field radiation, a chamber with four independent wells for the placement of these cultures was designed and built, see Fig. 1. To define the working volume of the Helmholtz configuration, the characteristics and physical dimensions of the chamber were considered. It is in these wells where the permeable inserts, which would contain the culture cells, would be placed.

2.2.2. Simulation and Modeling

A computer software based on finite element analysis called COMSOL Multiphysics® was used to simulate and model different excitation conditions of magnetic radiation at the border of cell cultures seeded in the permeable inserts to avoid uncertainty in the design and operation of the physical model of the magnetic-field generator. This simulation allowed to experiment with the physical and electrical characteristics of the field generator to obtain the uniformity of the working volume required for the conditions of the experimental protocol.

The data obtained from COMSOL Multiphysics® allowed us to implement, with a good approximation, the physical design of the generator as well as to define the best placement of the chamber which contains the experimental cell cultures.

2.2.3. Considerations for the design of the magnetic field generator for the simulation

The design of the magnetic field generator took into consideration the results of several simulated tests, the working volume required due to the physical characteristics of the chamber for the cell cultures, and that cell cultures were going to be exposed to the radiation of a magnetic field variable in frequency. The Helmholtz coil array model was designed with a CAD program in a 3D environment compatible with COMSOL Multiphysics®.

The generated three-dimensional model was exported to COMSOL Multiphysics®, using the "AC-DC, Magnetic fields, stationary study" module, to observe the magnetic field conditions in the working volume.

Two important aspects considered during the simulation were the determination of the boundary conditions, achieved by the subdivision of the model of interest, called mesh; and the choice of materials according to the environment in which the system was going to work.

An extra fine meshing was assigned to the working region to provide a better resolution of the simulation parameters of the chamber which contained the filters, while a thick mesh was chosen for the surrounding areas to avoid a high computational cost since the information in these regions was not relevant.

2.3. Physical prototype

The parameters considered in the model to design and build the physical coils prototype were 900 turns of copper wire AWG 17, 60 mm radius, and 32.7 mm of the separation between them.

Acrylic was chosen to build the support for the coils because it does not have ferromagnetic characteristics that could interact directly with the magnetic field, it has an adequate mechanical resistance, and it is light and easy to clean. Figure 2 shows the physical prototype of Helmholtz coils.

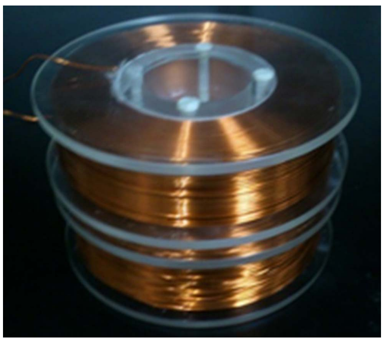

Fig. 2. (Color online) The physical arrangement of Helmholtz coils, acrylic structure.

Probable increments of temperature were considered for the design of the coils because of two reasons: first, experiments with cell cultures may involve prolonged times, and second, the inherent changes in the wire current and impedance as consequence of the increment of both the frequency and the magnitude of the magnetic field during the tests. Since the impedance of the coils was modified, it was necessary to compensate with voltage increments to sustain the current that defined the magnetic field, as shown in Eq. (2). For cell culture research protocols, these temperature increments should not go beyond to what cells can tolerate without suffering stress, which is 0.5° above 37°C.

2.3.1. Instrumentation

In order to evaluate the physical model developed, five Hall effect sensors UGN3503 (Allegro MicroSystems, USA) were used to measure the magnetic field distribution. Also, an LM35 sensor (Texas Instruments, USA) was used to measure the temperature. The information from the sensors was digitalized using a NI-USB 6218® data acquisition card (National Instruments, USA), of 16 bits to 250 kS/s. The acquisition and processing of data, the configuration and excitation of the coils, and the storage and visualization of the information were made using a graphic interface developed in the visual platform LabVIEW®.

2.4. Characterization of magnetic field sensors

The five Hall effect sensors were characterized to observe the performance of the magnetic field generator on the working volume. Each sensor was placed parallel to a reference sensor, Gauss/Tesla Meter 5180 (FW BELL, USA), at the center of the Helmholtz coil array, thereby ensuring the same magnetic-field radiation conditions. This reference sensor measures the magnetic field in a range of 0.1 G to 30 kG with a bandwidth of 0-20 kHz

Table 1. Mathematical model for adjustment of the sensors data.

Y= aX+b	Sensor 1	Sensor 2	Sensor 3	Sensor 4	Central Sensor
a	525	582.7	579.9	531.3	767.5
b	−1247	−1403	−1416	−1279	−1874
R^2	0.9998	0.9999	0.9999	0.9998	0.9998
*Corr	0.9999	0.9999	0.9999	0.9999	0.9999

*Corr correlation of reference and each sensor and an accuracy of 1.1 %. For this procedure, the information of three magnetic field magnitude scans was recorded in the range 1.5 to 40 G.

A model of linear regression adjustment was applied to the data obtained from the measurements of each of the Hall effect sensors compared with the reference sensor; the results are shown in Table 1. Additionally in this table, the correlations of the reference and each sensor are presented. The results showed that the responses of all sensors are lineal and they have a good correlation with the reference sensor.

2.5. Evaluation of the physical system

Experiments with direct and pulsed current were carried out for this evaluation because the protocol of experimentation intended to be implemented with cell cultures requires frequencies from 0 to 60 Hz. The graphical interface developed in LabVIEW® was used in conjunction with the Helmholtz configuration to generate the desired magnetic field.

2.5.1. Tests with direct current

The uniformity of the magnetic field was verified using an array of four Hall effect sensors according to the distribution shown in Fig. 3 and the position of the wells for the cell cultures. The information obtained was validated by the commercial reference FwBell, labeled as Ref.

Ten series of measurements of the magnetic field were carried out for twenty intensities, from 1.5 to 40 G. Both the mean and the standard deviation of the ten series of measurements made for the 4 Hall effect sensors were determined.

The coefficient of variation (CV) was computed to offer the measure of statistical dispersion between all Hall effect sensors. The CV is the ratio of the standard deviation

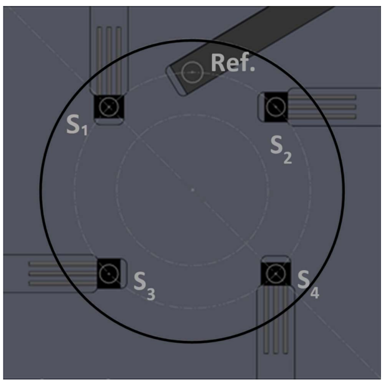

Fig. 3. (Color online) Sensor distribution in the working region for the simultaneous recording of the magnetic field. S1-S4: Hall effect Sensors, Ref.: Gauss Sensor/Tesla Meter.

and the mean of the magnetic field given by the four sensors corresponding to each well, eq. (3), this parameter was determined for the twenty intensities of each magnetic field magnitude. A low CV value indicates a uniform distribution of the magnetic field in the working volume.

$$cv(\%) = \frac{STD}{Mean} \times 100 \quad (3)$$

2.5.2. Tests with pulsed magnetic fields

In order to observe the behavior of the magnetic field related to the frequency and temperature changes, tests with the pulsed magnetic field in two conditions were performed; the first was at room temperature (24.6°C) and the second was inside the commercial incubator TC 230 (36.7°C). It should be mentioned that the cell cultures within an incubator should commonly be kept at a temperature of 36.5°C ± 0.5°C [10, 11]. For these tests, a Hall effect sensor was used in the central region of the coils. A magnetic field with sinusoidal waveform and amplitude of 25 G was generated at an initial frequency of 0.5 Hz at room temperature of 24.6°C. The frequencies of 0.5, 1, 2, 5, 10, 20, 30, 50 and 60 Hz were recorded for three minutes at both 36.7°C and room temperature.

It has been reported that temperatures above 37°C can stress the cells [15]; thus, considering that the Joule effect is not absent in generators that depend essentially on the flow of electric current, it is important to observe the temperature conditions present in long duration tests. Therefore, the temperature inside the incubator was recorded for 60 minutes at frequencies 1 and 10 Hz, at a field magnetic magnitude compensated to its initial value, 25G at room temperature. Before the tests, 10 minutes were waited to allow the initial temperature inside the incubator to stabilize at 36.5°C for both experiments.

Simultaneously, the magnetic field was measured to verify that its magnitude was the same during the experiment.

3. Results

3.1. Simulation

The results of the simulation can be observed both graphically in Fig. 4, and numerically in Table 2.

The flat response of the graph in Fig. 4 allows observing the uniformity of the magnitude of the magnetic field in the region where the filters with cell cultures are intended to be placed.

To corroborate, quantitatively, the uniformity of the field distribution in the region of interest, the mean, standard

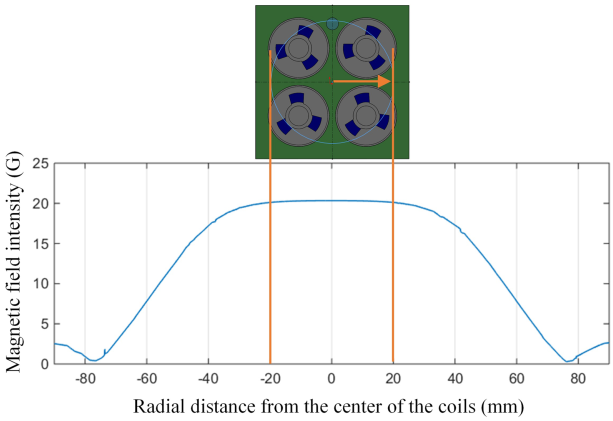

Fig. 4. (Color online) The flat response of the magnetic field generator can be seen in the region delimited by orange lines.

Table 2. Magnetic field values in the region of interest.

Parameter	Well 1	Well 2	Well 3	Well 4
Mean (G)	20.217	20.223	20.210	20.234
SD	0.049	0.053	0.053	0.041
Maximum (G)	20.312	20.307	20.301	20.316
Minimum (G)	20.105	20.080	20.068	20.110

deviation, maximum, and minimum magnetic field magnitude parameters in each well were calculated. The results are shown in Table 2.

3.2. Direct current

The data of the magnetic field measurements made with the criteria defined for the direct current tests are shown in Table 3(a) and Table 3(b). The coefficient of variation determined the degree of dispersion of the magnetic field magnitude for the working volume, which was less than 1 % in all cases. This suggested a uniformity of the magnetic field and a good performance of the Helmholtz configuration when working with direct current.

3.3. Pulse current

The tests with pulsed current were performed at different frequencies, with two temperature conditions, in the environment, and inside the incubator.

Figure 5(a) shows that at ambient temperature, the magnetic field magnitude at low frequencies was conserved practically at 25 G, whereas Fig. 5(b) shows the attenuation of the field as the frequency increased.

Figure 5(c) shows the comparison of the magnetic field magnitude readings under the two proposed temperature conditions. It is observed that there was an attenuation of the magnitude of the magnetic field with the change of temperature, i.e. by placing the magnetic field generator

Table 3. (a) Response of Hall effect sensors of the 10 series of measurements, Mean (SD).

*G	Sensor 1		Sensor 2		Sensor 3		Sensor 4	
1.50	1.57	(0.05)	1.59	(0.05)	1.57	(0.07)	1.55	(0.06)
3.20	3.37	(0.08)	3.38	(0.09)	3.37	(0.09)	3.34	(0.08)
5.40	5.44	(0.08)	5.47	(0.09)	5.46	(0.09)	5.43	(0.06)
7.60	7.57	(0.06)	7.58	(0.06)	7.59	(0.07)	7.57	(0.07)
9.70	9.81	(0.07)	9.85	(0.08)	9.85	(0.08)	9.82	(0.08)
11.90	12.08	(0.10)	12.12	(0.11)	12.11	(0.09)	12.10	(0.09)
14.30	14.37	(0.09)	14.43	(0.10)	14.44	(0.09)	14.40	(0.08)
16.70	16.67	(0.11)	16.73	(0.10)	16.74	(0.10)	16.69	(0.09)
19.00	19.01	(0.09)	19.08	(0.09)	19.04	(0.09)	19.03	(0.07)
21.10	21.36	(0.09)	21.42	(0.09)	21.37	(0.11)	21.39	(0.08)
23.30	23.72	(0.17)	23.81	(0.17)	23.74	(0.19)	23.75	(0.15)
25.70	26.05	(0.12)	26.11	(0.12)	26.06	(0.13)	26.07	(0.10)
28.30	28.39	(0.12)	28.46	(0.12)	28.38	(0.09)	28.40	(0.10)
30.50	30.76	(0.11)	30.79	(0.12)	30.74	(0.12)	30.75	(0.11)
32.80	33.08	(0.06)	33.12	(0.07)	33.09	(0.08)	33.06	(0.06)
35.20	35.41	(0.11)	35.45	(0.12)	35.43	(0.13)	35.35	(0.12)
37.50	37.78	(0.11)	37.75	(0.10)	37.80	(0.12)	37.72	(0.11)
40.00	40.11	(0.10)	40.08	(0.10)	40.12	(0.10)	40.03	(0.09)

*Magnitude of Magnetic Field (G)

(b) Results of the sensors dispersion

Mean	(SD)	CV (%)
1.57	(0.02)	0.99
3.37	(0.02)	0.52
5.45	(0.02)	0.37
7.58	(0.01)	0.18
9.83	(0.02)	0.20
12.10	(0.02)	0.14
14.41	(0.03)	0.22
16.71	(0.03)	0.20
19.04	(0.03)	0.17
21.39	(0.03)	0.12
23.75	(0.04)	0.16
26.07	(0.03)	0.10
28.41	(0.03)	0.12
30.76	(0.02)	0.07
33.09	(0.03)	0.08
35.41	(0.04)	0.12
37.76	(0.04)	0.10
40.09	(0.04)	0.10

*CV, Coefficient of Variation

inside the incubator at 36.7°C. Also, the attenuation tendency was maintained by increasing the frequency in both temperature conditions.

Figure 6 shows the temperature changes within the incubator during magnetic field exposures for 60 minutes with the pulsed magnetic field at two frequencies, 1 and 10 Hz. A temperature increase about 0.4°C was observed

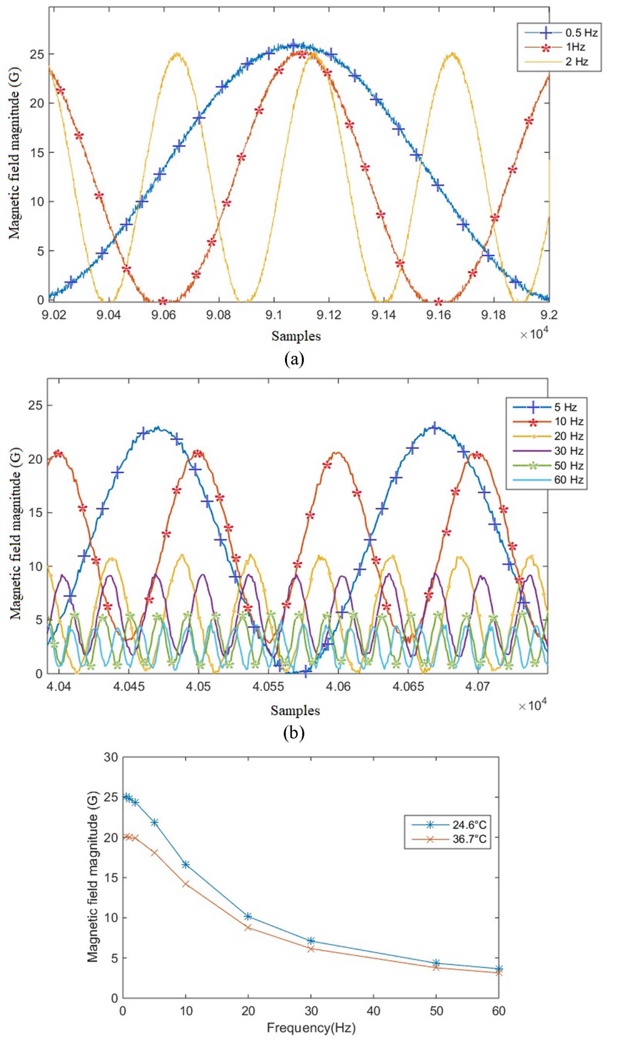

Fig. 5. (Color online) Magnetic field magnitude at different frequencies, (a) f < 5 Hz, (b) 60 ≥ f ≥ 5 Hz, (c) response of the magnetic field generator through the frequency and temperature.

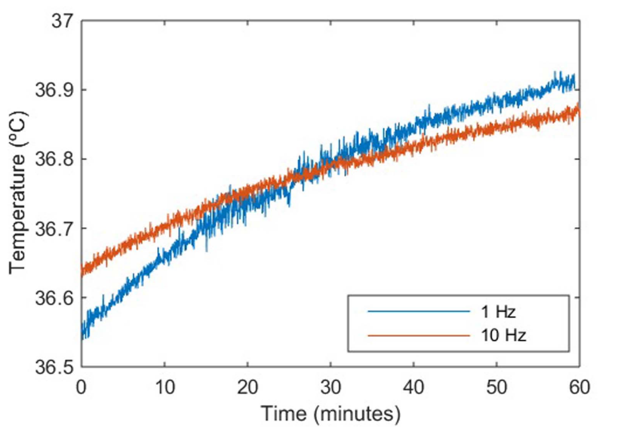

Fig. 6. (Color online) Temperature measurement for 1 hour at 1 and 10 Hz.

throughout the experiment for both frequencies.

In relation to the magnetic field magnitude records during the 60-minute tests, the mean and standard deviation at 1 Hz were 20.21 ± 0.13 G meanwhile at 10 Hz were 20.05 ± 0.05 G. Data showed a minimum standard deviation, which suggested that the magnitude of the field was conserved throughout the experiment.

4. Discussion

The simulation and modeling allowed for a good approximation to the developed physical model since it offered a relatively simple way to establish the design parameters of the coil system specifically, the dimensions of the coils and wire gauge to meet the requirements for the desired working volume. The simulation showed the distribution of the magnetic-field magnitude inside and outside of the region of interest. Moreover, because of the uniformity of the magnetic field in the working volume, it was not necessary to carry out point-to-point measurements throughout the area.

The results of the physical prototype with direct current agreed with those obtained by the simulation: that is, there was no variation in the magnitude recordings made with the four sensors in the area of interest. The coefficient of variation was less than 1 % over the entire frequency range wherein work with cell cultures typically is carried out.

As expected, there were attenuations in the magnitude of the magnetic field initially established for the tests, as shown in Fig. 5. It should be emphasized that temperature was a critical factor, because when the room temperature was changed from 24.6°C to 36.5°C inside the incubator, the magnetic field underwent an attenuation; this is shown in Fig. 6. This is why the developed system was designed to compensate for such attenuations by adjusting the voltage and current parameters that define the desired magnetic-field magnitude. The compensation at 10 Hz and 1 Hz in the 60-minute tests showed that, despite the frequency, the field magnitude remained constant.

It should be noted that the tests inside the incubator were performed under temperature conditions similar to those reported for optimal cell development [10, 11]. The data of the working volume temperature for the long-term tests showed only increments of 0.4°C. These increments are according to cell growth and survival under usual culture conditions.

It is noteworthy that the equipment developed in this manuscript has a total volume suitable to be introduced in commercial incubators to keep the standard cell culture conditions to guarantee that magnetic field variable in frequency and magnitude will be the only stimulation factor when experiments are carried out with *in situ* cell cultures.

5. Conclusions

The simulation in COMSOL Multiphysics® allowed for the physical-model design and development according to the parameters specified for studies with cell cultures inside an incubator. Both the physical size, and the working volume (i.e., the region wherein crops will be exposed to the same magnetic field magnitude) were considered. This uniformity of field in the physical model was confirmed by the coefficient of variation obtained from the data on the arrangement of the four Hall effect sensors, which was less than 1 %.

In this work the inherent performance of the coils was observed in relation to the working frequency and temperature; as expected, there were attenuations in the magnetic field magnitude as the frequency and temperature increased.

Considering that during tests with cell cultures the same magnetic field magnitude must be guaranteed independently of the working frequency, a control and compensation procedure was implemented through the developed graphical interface. In long-term tests, it was demonstrated that the magnitude of field was maintained for one hour and that the changes in temperature did not interfere with cellular activity. These results served to demonstrate that the magnetic field was the only stimulating factor.

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