Magnetic Stimulation on Human Blood
Electromotive force analysis

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In this work a comparative theoretical analysis vs. experimental study on human blood under a magnetic field stimulation is presented. Twenty samples of leukoreduced human blood were stimulated with an alternant magnetic field using a Helmholtz coil system; this magnetic field induced an electromotive force in them. Theoretical calculations were performed for the induced electromotive force in a simple model of blood tissue under magnetic stimulation at frequencies: 50 Hz, 100 Hz, 800 Hz, and 1500 Hz. Experimental measurement was performed at the same frequencies for comparison purposes. Results show a high correlation between theoretical and experimental study, as well as effects of agglutination in the stimulated blood cells.

Keywords: Alternating Magnetic field stimulation, Helmholtz coils, Induced electromotive force, Stimulated human blood

Interactions between living systems and magnetic field (MF) on the environment cannot be avoided; therefore, it is important to widely study those interactions in order to understand any potential side effects. Earlier studies have shown the presence of proliferation, agglutination, growth and other effects when some culture cells are stimulated with applied magnetic field (AMF) at low or high frequencies. In particular, H.A. Perez *et al.* 2013 [1] showed the changes on cell motility in Entamoeba invades culture under stimulation with vortex of MF generated with a Rodin coil, they discussed that at low frequencies, the cells showed changes in their motility with rapid response compared to cells stimulated with frequencies greater than 400 Hz. Rodriguez De la Fuente, *et al.* (2008) [2] mentioned the effects of cell proliferation to the same group of cells when it is stimulated at 60 Hz. Nevertheless, they also suggest that the side effects of MF in cells are still considered different, for example, in 1993 Goodman [3] suggested that MF have effects on specific genes responsible for growth and even on certain calcium channels of the cell membrane that might affect cell growth. More recently H.A. Perez *et al.* 2013 [4], while he was working with human lymphocytes showed that, 20 days later, a considerable increase in the number of cells had increased the number of divisions of these.

Blood cells have also been studied, in particular, Higashi *et al* (1997) [5] studied the orientation of erythrocytes under a static MF of 8.0 T where a parallel orientation to the field lines was observed, authors suggested that this is due to magnetic features of the cell membrane. Later, M. Sosa *et al* (2005) [6] studied the changes in the electrical properties of the blood when it was exposed to a magnetic field of 0.5 T finding a resistance increase of 10.4 % and a 1.9 % in capacitance; given the highly non-conductive membrane properties, they were able to explain these changes based on ion currents induced in the interstitial medium and the charge distribution generated in the membrane surface because of ions that remain attached to it. Other authors have studied sedimentation and aggregation behavior when the erythrocytes are in a MF of the order of 6.0 T [7].

Physically, it is known that when a biological system is modeled as a conductor and it is stimulated by a MF, then by Faraday's law, it is induced a electromotive force (*emf)* in the tissue which causes a flow of a current density through the biological system [8].

As part of this work, theoretical calculation for the induced *emf* in a simple model of blood tissue is presented. Also, the experimental evaluation in the blood samples magnetically stimulated at four frequencies with a Helmholtz coils system was also analyzed.

Experimental part
Materials and methods

A Helmholtz coils system was used for the magnetic stimulation, it has two identical coils with 98 windings, a diameter of 20 ± 0.1 cm and a separation form one coil to the other of 10 ± 0.1 cm. Also it has a resistance of 233.28 Ω and an inductance of 5.06 mH.

The coil system is controlled by LabVIEWO software. A signal, with frequency and time well defined, is controlled from the PC's audio output directly to an audio amplifier model MIT-75AZ that increases the signal to 12 v RMS and then to the Helmholtz coil system. Additionally, a multimeter was connected to the circuit to record the current, I_B, through the coil.

In table 1 we show the values of electric current and magnetic field recorded with signals of different frequencies applied to the Helmholtz coil, such frequencies were selected from our previous experience that suggested proliferation changes in the blood cells.

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Table 1
MAGNETIC FIELD, FOR DIFFERENT STIMULATION FREQUENCIES

Frequency [Hz]	I_B[A]	B [mT]
50	0.22 ± 0.01	48.482 ± 0.011
100	0.22 ± 0.01	48.482 ± 0.011
800	0.17 ± 0.01	37.463 ± 0.011
1500	0.11 ± 0.01	24.241 ± 0.011

The experimental set includes a couple of copper wires of a coaxial cable as electrodes, which were connected to an amplifier. These electrodes are capable to record the voltage variations in microvolts (μV) order. The amplifier circuit includes the integrated circuit LM324. This amplifier is adjusted such that the gain was 10 times.

For data acquisition, the measurements were recorded using a National Instrument DAQ system, which is implemented in LabVIEW. Twenty measurements were performed with different input voltages to figure out the behavior of the amplifier, this graph is shown in figure 1.
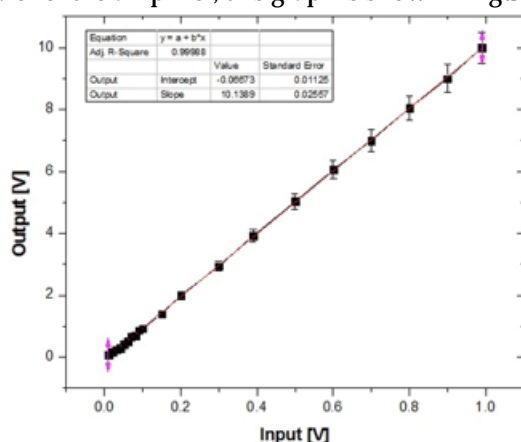

Fig. 1. Output (vertical axis) *vs.* the input signal (horizontal axis)
The linear fit on the graph is linear with slope value around 10.13

Sample preparation and measurements protocol

Twenty leuko-reduced (with no leukocytes) human blood samples from healthy subjects were mixed with antifreeze were magnetically stimulated during four minutes, one minute with each of the four following frequencies of 50 Hz, 100 Hz, 800 Hz and 1500 Hz.

Each sample contained in a testing tube was placed at the center of the coil system, the electrodes were immersed into the sample and then the measurements were performed, see figure 2. For reference purposes or base line, the associated voltage or *emf* was recorded during the first 30 s in each sample, while the MF was still off, then the blood sample started to be stimulated for a minute with a frequency of 50 Hz. Along this stimulation, the voltage or *emf* was simultaneously recorded. Similarly, the same sample was still stimulated for one minute with each of the following frequencies, 100 Hz, 800 Hz, and 1500 Hz.
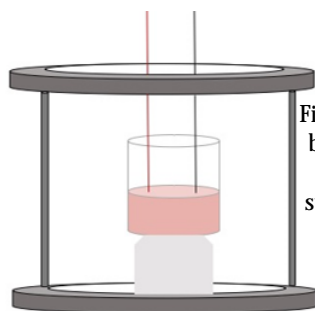

Fig. 2. Setup of stimulation. The blood sample in a test tube is set in the center of the coil supported in a Styrofoam base and the electrodes are immersed in the sample

After the stimulation of each sample, the electrodes were taken out and cleaned with acetic acid in order to eliminate possible oxidation reaction of the electrode caused by ions of blood plasma subjected to electrolysis. In order to visualize the effects in the blood samples before and after the magnetic stimulation, some photographs were taken using an optical microscope. A smear of one of the twenty blood samples was done and Wright's stain was applied to see the stimulated cells under the microscope.

Theoretical analysis

The magnetic field lines in the center of Helmholtz coil are uniform in magnitude and parallel to the coils axis, so that, they cross the blood sample to stimulate it. In order to calculate *emf* induced by the MF in the biological sample, it was considered as a homogeneous fluid formed by concentric spirals which makes concentric disks, the test tube was considered as a cylinder formed by the disks of radius ρ, as it is shown in figure 3.
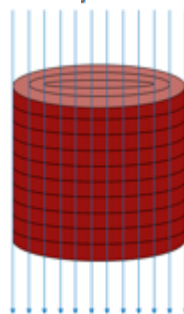

Fig. 3. Magnetic field lines schematization, when the sample was magnetically stimulated in the center of the system coil

The electromotive force expression [9] is given by

$$|\xi| = \left|\frac{d\Phi_B}{dt}\right|. \tag{1}$$

Here, the magnetic flux is

$$\Phi_B = \int \vec{B} \cdot d\vec{A} = \pi\rho^2 B_z. \tag{2}$$

where ρ is the magnetic field transversal area of interest given for axial component of the Helmholtz coils systems reported by Cordova-Fraga *et al.* [10].

$$B_z(\rho, h) = \frac{8}{5\sqrt{5}}\frac{\mu_0 NI}{a}\left(1 - \frac{54}{125}\frac{\rho^4}{a^4} + \frac{432}{125}\frac{h^2\rho^2}{a^4} - \frac{144}{125}\frac{h^4}{a^4}\right), \tag{3}$$

where N is the number of windings, α is the coils' radius, μ_0 is the magnetic permeability, ρ is the radius for the region where the magnetic field interaction with the sample, I = I(t) the current trough the coil and B_z the axial magnitude of MF.

The electromotive force is then

$$\xi_z = \pi\rho^2 \frac{dB_Z}{dt} = c\frac{dI(t)}{dt}, \tag{4}$$

The Helmholtz coils system was fed with an alternating current

$$I(t) = I_B \sin \omega t, \tag{5}$$

where I_B is the amplitude and $\omega = 2\pi f$ is the frequency of the electric current; such the above expression for the magnetic field contains the time dependence and the time derivative only affects $I(t)$ in the induced *emf* due to the area being constant.

$$\frac{dI}{dt} = I_B \omega \cos \omega t,$$

$$\xi_z = \frac{d\Phi_B}{dt} = \frac{8}{5\sqrt{5}}\frac{\pi\rho^2\mu_0 N}{a}\left(1 - \frac{54}{125}\frac{\rho^4}{a^4} + \frac{432}{125}\frac{h^2\rho^2}{a^4} - \frac{144}{125}\frac{h^4}{a^4}\right)I_B \omega \cos \omega t. \tag{6}$$

Substituting the correspondent values of the stimulated sample, the induced *emf* is:

$$\xi(t,\omega) = 6.921 \times 10^{-8} I_B \omega \cos \omega t. \quad (7)$$

Considering the data in table 2, where it is shown the whole features of the coils system, the four frequencies and the four I_B electric current values used, so that, the *emf* is from 1.05μV to 16.23μV, this is: $\xi(t,\omega) \in (1.05\mu V, 16.23\mu V)\cos\omega t$.

Table 2
SPECIFIC VALUES OF THE PARAMETER IN THE EXPRESSION OF THE INDUCED *EMF*. NOTE THAT THERE ARE FOUR DIFFERENT VALUES FOR , THESE CORRESPONDS AT EACH STIMULATION FREQUENCY

ρ	0.5 ± 0.1 cm
a	10.0 ± 0.1 cm
h	2.0 ± 0.1 cm
μ_0	$4\pi \times 10^{-7}$ N/A^2
N	98
ω [Hz]	I_B [A]
50	0.31 ± 0.01
100	0.31 ± 0.01
800	0.25 ± 0.01
1500	0.16 ± 0.01

Results and discussions

A typical behavior of the whole measurements of the induced *emf* in blood samples is shown in figure 4. The first 30 s there is no magnetic stimulation, then follows the measurement for the first minute in a frequency of 50 Hz and is succeeded by a minute for each of the following other frequencies. In the figure 5 is shown a decrease in the voltage when there is no stimulation, however, when stimulation starts, the behavior of the signal changes dramatically to increase.

For each sample, the Wilcoxon rank sum test was done in order to know whether there were significant differences between the average voltage measured in 50 Hz and 1500 Hz and between 800 Hz and 1500 Hz. Furthermore, 95 % of cases showed significant differences between the measured voltage at 50 Hz and 1500 Hz. 90 % of cases showed significant differences between the voltages measured at 800 Hz and 1500 Hz.

On the other hand, additional information of the side effects of the magnetic stimulated sample is summarized in the images of cells taken by microscope. They reveal changes in stimulated samples compared to non-stimulated samples, see figure 6. While stimulation of 100 Hz for one minute caused the agglutination of erythrocytes, see figure 7, a longer exposure caused side effects in cells.

The average induced *emf* for the 20 samples is shown in figure 8. nine of them were under 10 mV, five of them were from 10 to 15 mV, and three of them were from 15 to 20 mV and one for each of the next segments of five units of mV. Additional information is observed in the signal, as we can see in table 3.
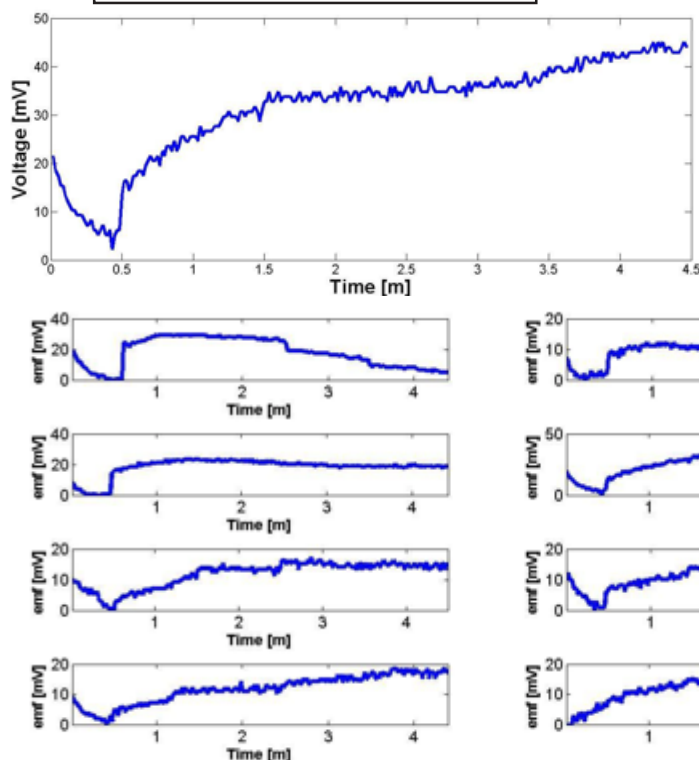

Fig. 4. Voltage behavior recorded in a sample. The voltage decrease when it is not stimulated, the first 30 s and it is increased when stimulation starts

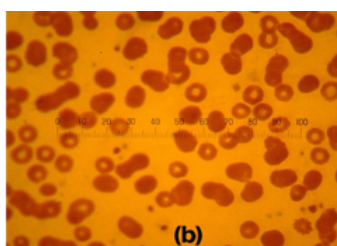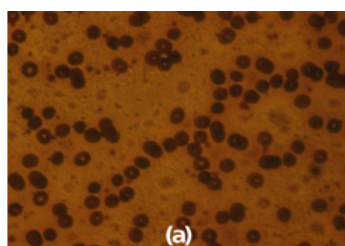Fig 5. Induced *emf* in 8 different samples

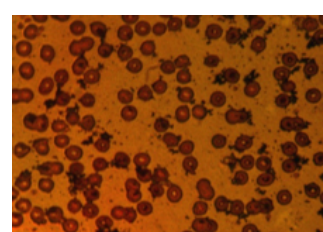Fig 6. Microscopic pictures at 40x after a magnetic stimulation for a minute (a) at 0 Hz, (b) at 100 Hz.

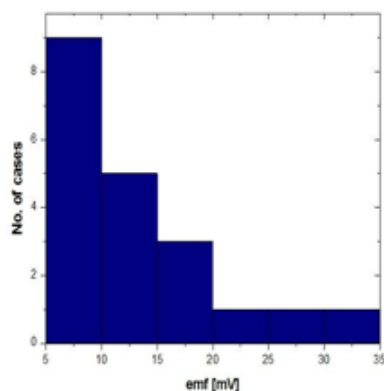Fig 7. A microscopic picture at 40x, after a agnetic stimulation for 4 min erytrocites present agglutination

Fig 8.Histogram of frequencies for the induced *emf*.

Fig. 9.Comparison of induced *emf* between an Entamoeba invadens culture (up) and human blood (down). The up image was taken from [4]

The *emf* variation is accorded to the cosine function, it was calculated and its magnitude is proportional to the frequency of the magnetic field (table 3 for the specific values of the *emf*). The predicted *emf* ranges varies from 1.05 μV to 16.23 μV depending on the operating frequency of the coil and the current flowing through it, the *emf* experimentally recorded averaged 12.81 μV.

Table 3
VALEUS FOR THE ELECTRIC CURRENT IN THE COILS SYSTEM AND INDUCED EMF FOR EACH FREMNQUENCY

ω[Hz]	I_B [A]	ξ[mV]
50	0.31 ± 0.01	2.29 ± 0.01
100	0.31 ± 0.01	4.59 ± 0.01
800	0.25 ± 0.01	29.62 ± 0.01
1500	0.16 ± 0.01	35.54 ± 0.01

In all experimentally measured cases the effect of the emf was presented as an increase in the measured voltage as soon as the coil began to stimulate at 50 Hz frequency. The increase of the *emf* indicates an increase in the resistance of the sample; this behavior suggests that the *emf* induced in human blood results in increased internal resistance. The possible explanation of this phenomenon may be induced eddy currents, currents induced in a piece of conductive material in much smaller paths that physically modeled, causing an increase in internal energy and hence an increase in the material resistance.

The literature has reported an increased blood resistance when it is exposed to static MF [6], in this work similar effect is shown using AMF.

In the microscope image, figure 6b, only when the sample is exposed to the AMF for one minute at 100Hz agglutination of erythrocytes can be observed, which could also be associated with the currents induced in the medium and interstitial charge distributions generated in the membranes of erythrocytes *pushing* each other. In the case of the image figure 7, erythrocytes are exploited which may be due to excessive heating of the sample exposing the erythrocytes to abnormal conditions according to work described in [4], shows a graph similar at the graph show in figure 9.

Where an Entamoeba invadens sample is placed to be magnetically stimulated with a Rodin coil. In ths graph, the voltage closer to 0 V means that there is a higher conductivity of the sample. This is supposed to be caused by the induced *emf*. This graph compared to the behavior found in this work may suggest that the direction of the field lines play a role in magnetic stimulation since the Helmholtz coil field lines are parallel and the Rodin coil field lines generates a vortex in the center of the coil. Since the register of the *e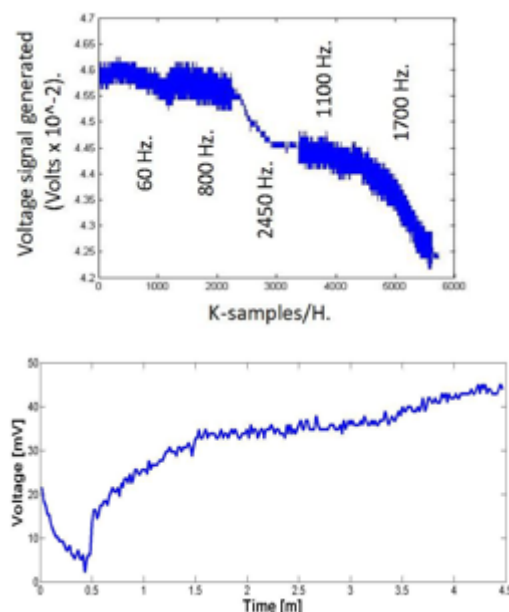mf* signal of the blood plasma was opposite to those of the blood samples, then it has an electrolyte behavior, and it follows that there is an interaction between the plasma ions and the membrane currents induced by the blood. The comparison with median test revealed that the changes of the *emf* measured at different frequencies were significant.

An advantage of making the magnetic stimulation with leuko-reduced human blood is that they do not contain nucleated cells (leukocytes), and this is how it was possible to study the effects of MF on cells only with the induced currents in the interstitial medium and possible transportation of ions in the cell membranes, discarding the biological effects that the magnetic field may have on the cell nucleus containing DNA.

Conclusions

It was found that when an harmonic magnetic field is applied into blood samples an *emf* is induced with a range of 1.05 to 12.23 μV, and this *emf* is directly proportional to the amplitude of the magnetic field. The experimental average for the *emf* was 12.8 μV. Although the theoretical model gives the order of the magnitude of the electromotive force, it does not predict the behavior of blood found experimentally.

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