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(Article begins on next page)

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Why does thermomagnetic resonance affect cancer growth? A non-equilibrium thermophysical approach

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Abstract
Recently, the low frequency thermomagnetic effects on cancer cells have been analysed, both theoretically and experimentally. They have been explained by introducing an equilibrium thermodynamic approach. But, in this context, two related open problems have been highlighted: (1) Does there exist a magnetic interaction or do there exist any other processes? (2) Do there exist also thermal effects? Here, we introduce a non-equilibrium thermodynamic approach in order to address an answer to these questions. The results obtained point out that: (a) the effect produced by the electromagnetic wave is just a consequence of the interaction of the magnetic component of the electromagnetic wave with the biological matter; (b) the interaction of the electromagnetic wave causes also thermal effects, but related to heat transfer, even if there have been applied low frequency electromagnetic waves; (c) the presence of the magnetic field generates a symmetry breaking in the Onsager's coefficients, with a related perturbation of the cancer stationary state.

Keywords Biothermodynamics · Bio-thermomagnetism · Cancer · Symmetry breaking

List of symbols

Latin letters

A	Surface (m^2)
B	Magnetic field (T)
c	Specific heat ($J\,kg^{-1}K^{-1}$)
ELF	Extremely low frequency
IF	Intermediate frequency
F	Heat power surface density ($W\,m^{-2}$)
$\mathbf{J}_e$	Current density ($A\,m^{-2}$)
$\mathbf{J}_Q$	Heat flux ($W\,m^{-2}$)
K_J	Thomson coefficient
L_{ij}	Phenomenological coefficients
ℓ	Length of the cell membrane (m)
$\dot{Q}$	Heat power (W)
$\langle R \rangle$	Cell characteristic length (radius) (m)
Re	Reynolds number
RF	Radio frequency
Pr	Prandtl number
s	Specific entropy ($J\,m^{-3}\,K^{-1}$)
t	Time (s)

T	Temperature (K)
u	Internal energy density ($J\,m^{-3}$)
$\mathbf{v}$	Velocity ($m\,s^{-1}$)
V	Volume (m^3)

Greek letters

α	Coefficient of convection ($W\,m^{-2}\,K^{-1}$)
ϕ	Membrane potential (V)
λ	Conductivity ($W\,m^{-1}\,K^{-1}$)
μ	Chemical potential ($J\,mol^{-1}$)
μ_e	Electrochemical potential ($J\,mol^{-1}$)
ν	Frequency (Hz)
σ	Entropy production ($J\,K^{-1}m^{-3}$)
τ	Finite time of the process (s)

Constants

c	Speed of light in vacuum $= 3\times10^3\,m\,s^{-1}$
F	Faraday constant $= 96485\,A\,s\,mol^{-1}$
R	Ideal gas constant $= 8.314\,J\,mol^{-1}\,K^{-1}$
μ_0	Permeability $= 4\pi \times 10^{-7}\,H\,m^{-1}$

Subscripts

0	Environment
surf	Surface

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Introduction

During the 20th century, the electromagnetic fields (EMF) have increased their relevance in any application, biomedical applications included, in relation both to safety and direct use. So, over the last decades, the analysis and the study of the effects of electromagnetic fields, on human health, has gained increased attentions [1]. Indeed, electromagnetic-based treatments have achieved therapeutic potentials and effects, in a large number of medical fields [2].

The electromagnetic frequency spectrum is usually classified in [3]:

- Static, with a frequency $\nu = 0$ Hz;
- ELF (Extremely Low Frequency), with a frequency in the range $0 < \nu \leq 300$ Hz;
- IF (Intermediate Frequency), with a frequency in the range $300 < \nu \leq 1 \times 10^4$ Hz;
- RF (Radio Frequency), with a frequency in the range $1 \times 10^4 < \nu \leq 3 \times 10^{11}$ Hz;
- IR (Infrared), Visible and UV (Ultraviolet), with a frequency in the range $3 \times 10^{11} < \nu \leq 3 \times 10^{15}$ Hz;
- IR (Ionizing Radiation) for higher frequencies.

In this context, in particular, the ELF range has attracted the interest of the researchers, because it characterizes some physical phenomena, such as the geomagnetic fluctuations [4], the Schumann resonance [5], the cell pulsations [6], etc. In particular, the vibration of a cell membrane has been pointed out to play a key role in the regulation of cell shape, and in the behaviour of the cells. Moreover, new directions, based on bio-inspired approaches, have been highlighted to develop possible future therapies, based on the use of Electromagnetic Fields, for various diseases, including cancer, diabetes, neural diseases, immune diseases, etc. [6].

In recent papers [7–9], the thermomagnetic effects on cancer cells have been shown and explained from an equilibrium thermodynamic point of view. The experiments have been carried out by comparing the growth of some cancer cell lines, under the exposure of an ELF-EMF, at their proper characteristic resonant frequencies, with the same lines of untreated cells. The characteristic resonant frequency has been evaluated for each cell line, by considering the cells average geometric parameters as required by the theoretical results [10]. The ELF-EMF exposure system is constituted by two independent couples of coaxial coils, wound into a cylindric frame, with external radius of 8 cm, and a distance between the couples of coils of 8 cm. The outer casing of the exposure system is constituted by a box, that shields it from the background magnetic field,

in order to expose the cells at their resonant frequencies. The treated cells plate was set in the centre of the shielded exposure system, inside an incubator, while the untreated cells (the control ones) of the same cell line were placed inside the incubator, without any shield.

Some results obtained are summarised in Table 1, where the resonant frequency has been calculated for each cancer cell line. The cells have been exposed to their proper characteristic resonant frequency, as derived by our previous thermodynamic approach [10]. The effect on the cancer cells growth has been compared with that of the untreated cells. Always in Table 1, it is possible to highlight that the cancer growth reduction depends on the frequency, which is a function of the cell lines shapes [10].

In particular, during the experiments, the frequencies of the magnetic field were in the range of the order of $1 - 30$ Hz, with the maximum amplitude of the wave was always $B = 100 \, \mu$T.

Consequently, the power applied was of the order of $B^2 cA/\mu_0 \sim 10^{-3} - 10^{-2}$ W, where $c \approx 3 \times 10^8$ m s^{-1}, $A \sim 10^{-10}$ m^2 is the area of cell membrane external surface, and $\mu_0 \approx 4\pi \times 10^{-7}$ H m^{-1}.

The fundamental results obtained can be summarised as follows:

- the key role of the volume-area ratio has been highlighted in relation to the cells heat exchange;
- the thermomagnetic resonance effect has been characterised by its resonant frequency, obtained by a thermodynamic analysis of the cell system, based on the heat outflow from the cell towards its environment;
- the related consequences on the behaviour of cancer cell have theoretically been shown, and experimentally confirmed, too;

Table 1 Growth variation of some cancer cell lines after the exposure to the calculated resonant frequencies [8, 9]

Cell line	Frequency/Hz	Growth variation/%
A375P	31	−15
HT-29	24	−19
GTL16	14	−24
MCF7	5	−22
SKBR3	8	−18
MDA-MB-231	6	−18

In particular, A375P is a human melanoma cell line, HT29 is a human colorectal adenocarcinoma cell line, GTL16 is a human gastric cancer cell line, MCF7, MDA-MB-231 and SKBR3 are three different cell lines of human breast cancer

- a device, based on the use of the electromagnetic field, has been designed, in order to trigger the behaviour of the cancer cell.

These experimental results open a theoretical problem in biophysics: which is the biophysical process involved in the thermomagnetic effect? In order to address an answer to this open problem, other two questions must first be analysed:

- is the effect produced by the electromagnetic wave a consequence of the magnetic interaction?
- Does the interaction of the electromagnetic wave cause also a thermal effect?

Since 1956, cancer cells were proven to be electrically different from normal cells [11]. Indeed, the start of the M phase of the cell life cycle has been highlighted to be characterised by a hyperpolarized state; consequently, a hypothesis has been introduced on the correlation between the cell cycle advancement and the modifications of the membrane electric potential [12]. Moreover, membrane hyperpolarization was shown to stop in a reversible way the DNA synthesis and the mitosis [13]. So, the membrane potential has been found to be one of the causes of the increase of the cancer cells proliferation [14–18], with relation also to migration and differentiation [19–25]. In Table 2, the membrane potential - both for some healthy and cancerous tissues - has been summarised by considering the data in literature [25].

In this study, a response to the previous open problems is suggested, by introducing the role of the membrane electric potential into the previous thermodynamic approach. To this purpose, an approach, based on the non-equilibrium thermodynamics, recently introduced in biophysics [10], is considered. The fundamental result of this research consists in the analysis of the resonant approach in heat transfer, when low frequency electromagnetic fields are applied to cancer cells.

Table 2 Some cell membrane potential in different tissues [25]

Tissue	Membrane potential	
	Normal/mV	Cancer/mV
Liver	−51	−44
Thyroid	−47	−25
Muscle	−89	−16
Gastric	−34	−24

Theory

Here, a non-equilibrium thermodynamic analysis of the membrane heat and mass transfer is introduced [10, 26–34], in order to improve the analysis of the thermomagnetic effects on cancer, in agreement with the experimental results reported in literature [11–21, 35–59].

In non-equilibrium thermodynamics, it is accepted to follow the Onsager's approach [60], for which every flow $\mathbf{J}_i$ is linearly dependent on all the forces $\mathbf{X}_j$ operative in the system, such that:

$$\mathbf{J}_i = \sum_j L_{ij} \mathbf{X}_j \tag{1}$$

where the coefficients L_{ij} are always positive, independent of the forces and satisfy the reciprocal relations $L_{ij} = L_{ji}$. So, in relation to the analysis of heat and mass transport through the cell membrane, the Onsager relations can be written in relation to the phenomena which cause the fluxes themselves. They are the temperature gradient in relation to heat transfer and the gradient of chemical potential in relation to ion transport (mass transport). Consequently, the relation (1) results [26, 27, 31, 61–63]:

$$\begin{cases} \mathbf{J}_e = -L_{11} \frac{\nabla \mu_e}{T} - L_{12} \frac{\nabla T}{T^2} \\ \mathbf{J}_Q = -L_{21} \frac{\nabla \mu_e}{T} - L_{22} \frac{\nabla T}{T^2} \end{cases} \tag{2}$$

where $\mathbf{J}_e$ represents the current density per surface area, $\mathbf{J}_Q$ is the heat flux per surface area, $\mu_e = \mu + Ze\phi$ denotes the electrochemical potential, with μ the chemical potential, ze the electric charge per mole, and ϕ the membrane potential, T is the living cell temperature, and L_{ij} are the Onsager coefficients, with [64] $L_{12}(\mathbf{B}) = L_{21}(-\mathbf{B})$ (Onsager-Casimir relation [65]), and $L_{11} \geq 0$ and $L_{22} \geq 0$, and [64] $L_{11}L_{22} - L_{12}L_{21} > 0$. Now, when there are ion and metabolites fluxes, $\mathbf{J}_e \neq \mathbf{0}$ and $\mathbf{J}_Q = \mathbf{0}$, it follows [26, 61, 62]:

$$\frac{d\mu_e}{dT} = -\frac{L_{21}}{L_{11}} \frac{1}{T} \tag{3}$$

with [61, 62]:

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_Q \tag{4}$$

where u is the internal energy density.

Living cells outflow heat power to the environment by convection, thus, we can write [10]

$$\frac{du}{dt} dV = \delta \dot{Q} = -\alpha (T - T_0) dA \tag{5}$$

where $\alpha \approx 0.023 Re^{0.8} Pr^{0.35} \lambda / \langle R \rangle$ is the coefficient of convection, with $\lambda \approx 0.6$ W m^{-1}K^{-1} conductivity, $Re \approx 0.2$ the Reynolds number and $Pr \approx 0.7$ the Prandtl number [66], A is the area of the external surface of the cell membrane, V is the cell volume, T depicts the mean temperature of the external surface of the cell's membrane, and T_0 is the temperature of the cell environment.

So, considering Equations (4) and (5), and the Divergence Theorem [67], the heat flux can be written as:

$$J_Q = \alpha (T - T_0) \tag{6}$$

and the related power flux yields:

$$\dot{Q} = \int_A \mathbf{J}_Q \cdot \hat{\mathbf{n}} dA = \alpha A (T - T_0) \tag{7}$$

Furthermore, considering Equation (2), together with $\mathbf{J}_e = \mathbf{0}$ and $\mathbf{J}_Q \neq \mathbf{0}$, it follows [26]:

$$\frac{d\mu_e}{d\ell} = \frac{T J_Q}{\left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right)} = -\frac{\alpha T(T - T_0)}{\left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right)} \tag{8}$$

with ℓ the length of a cell membrane and $|\nabla \mu_e| \approx d\mu_e / d\ell$. This relation is the link between the cell membrane electric potential and the temperature of the cell itself.

From Equations (6) and (8) follows:

$$J_Q = \alpha (T - T_0) = -\frac{1}{T} \left(L_{22} \frac{L_{11}}{L_{12}} - L_{21} \right) \frac{d\mu_e}{d\ell} \tag{9}$$

where:

$$\left(L_{22} - L_{21} \frac{L_{12}}{L_{11}} \right) = K_J T^2 \tag{10}$$

with $K_J = \lambda$ being the Thomson coefficient. Consequently, it follows:

$$\frac{\partial \mu_e}{\partial \ell} = \frac{\partial \mu_e}{\partial T} \frac{\alpha}{K_J} \left(T_{surf} - T_0 \right) \tag{11}$$

from which, taking into account that $\mu_e = \mu + ze\phi$, becomes:

$$\frac{\partial \mu}{\partial \ell} = -ze \frac{d\phi}{d\ell} + \frac{\partial \mu_e}{\partial T} \frac{\alpha}{K_J} \left(T_{surf} - T_0 \right) \tag{12}$$

So, we can obtain:

$$\frac{\partial \mu_e}{\partial T} = \frac{K_J}{\alpha} \frac{F + ze}{T_{surf} - T_0} \frac{d\phi}{d\ell} - \frac{K_J}{\alpha} \frac{2.3 R T_0}{T_{surf} - T_0} \frac{dpH}{d\ell} \tag{13}$$

which links the electrochemical potential to the pH.

The effect of the application of an electromagnetic wave with the thermal resonant frequency is to force the heat transfer, with a related thermal and electric perturbation.

Thus, cancer cell must activate ions fluxes in order to restore its initial condition, so $\mathbf{J}_e \neq \mathbf{0}$, and the concentration of ions varies in time [61, 62]:

$$\frac{dc_i}{dt} = -\nabla \cdot \mathbf{J}_i \tag{14}$$

where c_i is the concentration of the i-th ion (Na$^+$, K$^+$, Ca^{2+}, Cl$^-$, etc.), t is the time, and $\mathbf{J}_i$ is the current density of the i-th ion. In this condition, considering the Eq. (2), it follows the Thomson's Second Relation [61, 62]:

$$\frac{d\phi}{dT} = -\frac{L_{21}}{L_{11}} \frac{1}{T} \tag{15}$$

Therefore, the ion fluxes generate a variation in the electric potential and, consequently, a temperature variation and a heat flux occurs, too [61, 62, 68]:

$$\frac{du}{dt} = -\nabla \cdot \mathbf{J}_u \tag{16}$$

where u is the specific internal energy. As a consequence of this temperature change, the specific entropy rate occurs [69]:

$$T \frac{ds}{dt} = \nabla \cdot \left(\mathbf{J}_u - \sum_{i=1}^{N} \mu_i \mathbf{J}_i \right) - \sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla \mu_i \tag{17}$$

where T is the temperature, s is the specific entropy, and μ is the chemical potential, $\mathbf{J}_S = \mathbf{J}_u - \sum_{i=1}^{N} \mu_i \mathbf{J}_i$ represents the contribution of the inflows and outflows, and $T\sigma = -\sum_{i=1}^{N} \mathbf{J}_i \cdot \nabla \mu_i$ is the dissipation function [61].

The contribution of the dissipation function is to generate a continuous entropy outflow, which generates order from disorder inside the cancer cell, as Schrödinger himself pointed out [70]. We must highlight that all these equations can be analytically solved; indeed, any term presents coefficients that are constant, as a consequence of the lamped model, introduced for the temperature values, and any gradient is constant, if we consider any particular cell line, because of their defined membrane electric potential, ion concentrations and the length of the membrane.

Discussion and conclusions

In this paper, we have analysed the thermomagnetic effect on cancer, by introducing the non-equilibrium thermodynamics.

We have pointed out the emerging of two thermoelectric effects on the cell membrane: a Seebeck- and a Peltier-like effects, related to the heat and the ion fluxes. Both these effects are related to the membrane electric potential, that results modified.

In relation to our previous experimental results [8], the findings, here obtained, allow us to suggest that:

- the effect produced by the electromagnetic wave is just a consequence of the magnetic interaction, due to the low frequencies used, as the optical properties of the biological matter suggest [71];
- the interaction of the electromagnetic wave cause also a thermal effect, which are obtained as Seebeck- and Peltier-like effects;
- the presence of the magnetic field generates a symmetry breaking in the Onsager's coefficients ($L_{12} \neq L_{21}$), which perturbs the stationary state of the cancer.

Now, some considerations can be added, in order to highlight a possible use of the low frequency electromagnetic waves in medicine, as a possible support therapy to the actual anticancer treatments. Indeed, we wish to highlight that, in our experiments, only a decrease in growth has been shown [8, 9]. This is due to the interaction of the electromagnetic fields with the cancer cell membrane. Indeed, following Blank et al. [72], membrane Na/K-ATPase activity is affected in opposite ways by electric and magnetic fields. Under optimal conditions, enzyme activity is inhibited by electric fields and stimulated by magnetic fields. Magnetic fields (in the range 0-70 Hz) were shown to increase Na/K-ATPase activity of around 5-10%, with little dependence on field intensity. The effect of an electric field is similar to an increase in binding of activating ions on the enzyme surface. Ion activation depends on the electric field effect, but also on the frequencies. Magnetic fields influence charge flow within the enzyme during the reaction.

Our results point out how the electromagnetic wave generates changes in the membrane electric potential at the resonant frequency, with the consequence of inducing a hyperpolarization of the membrane. Therefore, some related fluxes could be activated. When an active flux occurs against the membrane electrochemical potential, the required energy is obtained by hydrolysis of ATP, or by the movement of a co-transported, or coupled ion, along its electrochemical gradient. Within this framework, the H^+-ATPase plays a central role, because it transfers positive charges into the cell, generating a membrane voltage (negative inside the cell), and pH gradients [73–76]. Protein phosphorylation is a fundamental cellular regulatory mechanism; indeed, it activates or deactivates many enzymes and receptors [77, 78], by involving kinases and phosphatases, both involved in the cellular transduction signalling [79].

In the analysis of the mitotic activities in sarcoma cells, the membrane potential was found to undergo hyperpolarization before entering M phase. It suggests that the level of membrane potential is correlated with cell cycle progression.

Moreover, membrane hyperpolarization was shown to block reversibly DNA synthesis and mitosis and to be correlated with the level of differentiation [12–14]. Consequently, the membrane electric potential represents a fundamental quantity to control critical cell functions, with particular regards to proliferation, migration and differentiation. Lastly, cell migration is controlled by the movement of ions and water [35], because an acidic environment furthers this phenomenon. This environmental pH is regulated by the H^+ concentration, which is related to the H^+-ATPase functions. In addition, the membrane potential is considered an indirect factor of cell migration, strictly related to the electrical driving force for Ca^{2+} whereas a hyperpolarized membrane potential increases intracellular Ca^{2+} through the Transient Receptor Potential (TRP) channels; in contrast, membrane depolarization activates the Ca^{2+} channels [21]. Notably, migrating cells have a high intracellular Ca^{2+} concentration gradient [80].

In this context, the results, here obtained, introduce a non-equilibrium viewpoint, by pointing out the fundamental role of the thermoelectric phenomena in the comprehension of the thermomagnetic effects on cancer cells, by considering the key role of the membrane electric potential, of the heat and ion fluxes, and of the pH changes.

Declarations

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