Review

Pitfalls and Challenges in Specific Absorption Rate Evaluation for Functionalized and Coated Magnetic Nanoparticles Used in Magnetic Fluid Hyperthermia

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Pitfalls and Challenges in Specific Absorption Rate Evaluation for Functionalized and Coated Magnetic Nanoparticles Used in Magnetic Fluid Hyperthermia

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Abstract: In recent decades, considerable interest has been observed in the field of cancer treatment research in relation to magnetic hyperthermia (MH), due to its ability to target tumors and generate localized effects with a high degree of specificity. Using of biocompatible magnetic nanoparticles, coated with specific organic molecules and functionalized with vectorizing molecules, has enabled the targeting of particular intracellular components of the diseased tissue. The application of localized radiofrequency magnetic fields results in the excitation of magnetic nanoparticles through specific relaxation mechanisms and hysteresis-driven processes, leading to temperatures that exceed physiological thresholds. This triggers a series of apoptosis processes. Additionally, the effects of low-frequency AC fields on high anisotropy magnetic nanoparticles, whether intra- or extracellular, have been shown to be highly effective in disrupting the internal functional structure of cells. A crucial parameter measuring the efficiency of magnetic nanoparticle systems in MFH is the specific absorption rate (SAR), which is experimentally evaluated by different calorimetric and magnetic techniques and methodologies. This review highlights the experimental pitfalls encountered in SAR evaluation and indicates the necessity of standardizing the devices and protocols involved in magnetic hyperthermia SAR evaluation. It also discusses the challenges that arise in magnetic hyperthermia at the cellular level, pointing to a more localized and specialized magnetic hyperthermia perspective.

Keywords: magnetic hyperthermia; magnetic nanoparticles; cellular level

1. Magnetic Hyperthermia as a Hope in Cancer Therapy

Magnetic hyperthermia has emerged as a new approach to cancer therapy, raising hopes of finding an effective solution to this disease, which has spread rapidly throughout the world in the last century. The causes of this disease are not clear, but the hypotheses that the new chemical substances used in agriculture (chemical fertilizers [1] and pesticides [2,3]) or in food processing (chemical additives [4,5] or plastics [6–8]) are responsible are reasonable. On the other hand, the psychological stress felt by all people in this very complex and complicated world could be a source of cancer development. On a microscopic level, all these factors seem to induce changes at the DNA level [9,10]. As a result, the regulatory processes that control cell growth and proliferation in different tissues are altered. It appears that a primary mechanism responsible for inducing mutagenesis is the inflammatory processes in various organs or tissues that precede tumor formation and sustain all stages of tumorigenesis [10,11].

After primary tumor formation, a chain of very sophisticated processes leads to metastasis and ultimately to the death of the living organism [12]: *angiogenesis* - new capillaries with hyperelastic properties and malformed structures are formed around pre-existing blood vessels to support tumor growth. They are highly permeable, facilitating the passage of tumor cells from the primary site into

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the bloodstream; *epithelial-mesenchymal transition (EMT)* - tumor cells gain invasive properties by hijacking the EMT program. This causes epithelial cells to acquire migratory capabilities and drug-resistant properties; *invasion* - primary tumor cells enter the bloodstream by single cell or collective migration mechanisms; *intravasation* - tumor cell invasion is active (cells migrate through blood vessels along nutrient gradients due to the chemotaxis process) and passive; *metabolic reprogramming* - the tumor adapts its metabolic parameters for the purpose of proliferation to the conditions required for invasion; *extravasation* - it is an intermediate step when tumor cells adhere to endothelial cells at the next site; *dormancy* - in micrometastases - cancer cells that extravasate remain in a dormant state as single cells or microclusters (micrometastases) and become non-proliferative; *macrometastasis* - the step when micrometastases come out of the dormant state and start the growth process by forming macrometastases.

Cancer works against life, taking living systems out of their functional equilibrium and forcing them to evolve into a disordered state that ultimately leads to their death. DNA is a very complex and dynamic information system that maintains the proper functioning of living beings in the finest detail, and also has self - error cleaning capabilities through specific repair mechanisms [13]. Damage to DNA information sequences caused by external factors can overcome the informational repair mechanisms and a wave of errors can propagate through the system, contributing to the birth of malignant cells in specific tissues [10,11]. The immune system has a role to play in recognizing and neutralizing this type of threat, but its ability can also be overwhelmed by the amplitude of the malignant cells occurrence. For this reason, cancer treatment is focused in two ways: on the genetic mechanisms that trigger the repair of DNA damage [14, 15], and on the effects of these mechanisms, in particular on the cancer cells, to destroy them by cytostatic drugs or by external factors such as heat in various therapeutic approaches: ultrasonic ablation [16, 17], laser ablation [18, 19] or magnetic hyperthermia [20-24]. The second category of cancer treatment approaches includes immunotherapy [25-27], which aims to boost the immune system to make it more effective in identifying and destroying cancer cells. It is known that cancer cells are less resistant than healthy cells to an increase in temperature above the physiological threshold [28]. Thus, heat can be used to kill cancer cells, but one of the problems is the distribution of heat in the tumor tissue. For successful killing of all cancer cells, homogeneous heat distribution and o[14,15ptimal timing of the heating steps are essential [29]. If some parts of the diseased tissue remain alive, the process of cell proliferation will continue and the tumor will grow again.

The utilization of magnetic nanoparticles in generating heat under the influence of an alternating magnetic field [30] has garnered significant scientific interest due to their capacity to reach deep-seated tumor tissues, if functionalized with appropriate molecules [31,32]. This phenomenon, known as magnetic hyperthermia, has the potential to be employed in cancer hyperthermia treatment due to its ability to target tumors with high precision. Concurrently, magnetic hyperthermia can function as an adjuvant modality in combination with other cancer treatment approaches, including chemotherapy and radiotherapy [33,34]. Furthermore, magnetic nanoparticles have the capacity to function as vectors for diverse drug molecules that target specific diseased tissues, thereby integrating the cytotoxic effects with the heating effects of hyperthermia [35–37]. There are two primary methods for delivering antitumor drugs or diagnostic molecules [38–40]: active targeted drug delivery, which is based on the chemical affinity between ligands (e.g., antibodies, peptides, small molecules) and specific receptors located on the cell surface, and passive targeted drug delivery, which is based on the enhanced permeability and retention (EPR) effect. This effect allows for the movement of large molecular species and fine particles from the bloodstream into the tumor due to the leaky vasculature of this tissue. Passive targeted delivery is a universal mechanism employed in the treatment of numerous cancerous diseases. Magnetic nanoparticles can be used as nanoplatform carriers for both active and passive targeting [41,42]. Drug molecules used in cancer therapy cannot be directly chemically attached to the nanoparticle, and an organic layer with high affinity for specific ligands and drug molecules should cover the nanoparticle surface [43]. A key requirement for materials used in cancer treatment is biocompatibility. All final material products,

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such as nanoparticles or organic layers used for coating, must be compatible with the human body without cytotoxic effects. A wide class of biocompatible organic molecules provide chemical support for drug or marker molecules (chitosan, dextran, different lipids and fatty acids, polyacrylic acids, polydopamine, starch, etc [44,45]). In addition to the conditions of material biocompatibility, magnetic hyperthermia must be applied with some limitations given by the negative effects of alternating magnetic fields in the human body. Radiofrequency magnetic fields induce electric currents in biological tissues, which increase the local temperature. Brezovich, in 1998, established a criterion for the permissible limits in direct application of AC magnetic fields to the human body: the product between frequency and intensity of the applied field ($\boldsymbol{f \cdot H}$) should not exceed the value of $\mathbf{4.85 \times 10^8\ Am^{-1}s^{-1}}$ [46]. Furthermore, more permissive limits have been given [47]: $\cdot \boldsymbol{H} = \mathbf{5 \times 10^9\ Am^{-1}s^{-1}}$.

The localization of magnetic nanoparticles in tumor tissue depends on their size, morphology and functionalization [48,49]. Magnetic hyperthermia experiments performed in vitro on DX3 human melanoma cells incubated with iron oxide nanoparticles coated with citric acid and exposed to a variable magnetic field of up to $\mathbf{16.1\ kA/m}$ strength and $\mathbf{950\ kHz}$ for 2 h, showed a high internal uptake of nanoparticles, as demonstrated by transmission electron microscopy (TEM) measurements [50]. Another work [51] studied magnetic hyperthermia in vitro in the glial microtumor phantom incubated with polyacrylic acid-coated and lauric acid-coated Fe_3O_4 nanoparticles. TEM studies showed partial internalization of the nanoparticles into vesicles distributed in the cytoplasm and formation of NP clusters attached to the cell membrane. The effects of magnetic hyperthermia treatment, performed at a frequency of $\mathbf{560\ kHz}$ and a field strength of $\mathbf{24\ kAm^{-1}}$, were compared with those induced in cells by conventional heating in a water bath. Both hyperthermia and classical methods induced the apoptosis process as measured by viability tests, but in the case of magnetic hyperthermia, the local damage at the cellular level was more pronounced than in the classical case, possibly due to the mechanical vibration of the nanoparticles under AC magnetic field excitation. Nanoparticles can be driven to specific locations within the cell, but they can also remain trapped in the extracellular matrix [52,53]. Therefore, the idea arose to study the local efficiency of MH in the intra- or extracellular space. Comparative intracellular and extracellular MH experiments were performed on SK-Hep1 hepatocellular carcinoma cells incubated with polystyrene sulfonic acid-coated magnetic nanoparticles immediately after incubation and after 24 hours. MH results showed that nanoparticle localization in the extracellular matrix was more efficient than internalization in the cytoplasm [54]. In addition, other in vitro experiments have shown that the MH process is more effective at the extracellular level than at the intracellular level [55]. In contrast, in vivo experiments on tumors induced in mice showed that MH was more effective in the intracellular space than in the extracellular matrix, even when the temperature reached in the intracellular space was lower than outside the cell [56]. This was explained by the high temperature reached in the vicinity of the nanoparticles (several tens of degrees), which has a strong impact on the integrity of the organelle membranes by inducing local damage. Ratiometric luminescence thermometers based on Sm^{3+}/Eu^{3+} were developed to detect local temperature increases on the surface of nanoheaters or in specific parts of the cell. Significant temperature differences were found between the nanoparticles and the cellular environment in their immediate vicinity. This suggests that some functional parts of the cell are more sensitive than others, requiring small amounts of heat to trigger the chain of apoptotic processes [57]. Other strategies have used magnetic nanoparticles as immobilizer nanoheaters attached to the cell membrane to induce physical damage (pores) capable of allowing the passage of drug molecules into the cytoplasmic space [58]. The combination of MH with nanoparticle-mediated chemotherapy proved effective in vitro and in vivo studies on colorectal cancer stem cells (CSCs). Thus, iron oxide nanocubes functionalized with doxorubicin killed almost all cancer cells, but a small fraction of cells that survived hyperthermia was neutralized by the effect of doxorubicin, which inhibited the regrowth and implicitly the relapse of tumor cells. In addition, the effects of MH increased the uptake of doxorubicin by tumor cells [59]. Specific assembling behavior was observed in cellular MH experiment in [60] where nanoparticles were aligned in chains. Furthermore, mechanical effects

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induced by vibration or oscillation under AC magnetic field were highlighted by microscopic examination of nuclear debris after MH. Similar works [61–63] showed that low frequency AMF and dynamic magnetic field induce mechanical forces mediated by magnetic nanoparticles of specific shapes (disks of 60 nm thickness and 1μm diameter) inside the cytoplasm, which act on the cell membrane, nuclei or various organelles, leading to cell disruption. The vibration and oscillation effects of nanoparticles are usually exploited in MH under low-frequency fields, where highly anisotropic (rod-shaped) particles of appreciable size (200 nm in length) have been designed for operation in $\mathbf{35}\boldsymbol{kHz}$ magnetic fields [64,65]. Nanoplates of 1 μm diameter have also attracted attention for use in very low and weak fields ($\mathbf{20}\boldsymbol{Hz}$ and $\mathbf{30}\boldsymbol{mT}$), showing lethal effects on cancer cells without significant heat release [66].

In this context, MH may be approached surgically through strategies that identify thermosensitive intra- or extracellular sites of high functionality in the cell life cycle and their targeting pathways. Furthermore, combination of thermal effects and mechanical stress applied directly to the cellular infrastructure may be a successful strategy in MH.

Magnetic particles used as nanoheaters or as mechanical vibration and oscillation sources in MH are prepared by a wide class of chemical and unconventional routes [67,68], depending on the strategy approached in hyperthermia applications; they are designed under different physical or chemical functionalities. Simple or core-shell magnetic particles [69–71], spherical or with different functional shapes: nanocylinders, nanodisks, nanoflowers, nanocubes [72], each of them bringing specific advantages in MH. Magnetosomes are a particular class of magnetic nanoparticles (Fe3O4) with low toxicity and high efficacy in MH, with a size of a few tenths of nm, biologically synthesized by the group of magnetotactic bacteria [73]. Coating magnetic particles with biocompatible layers increases their functionality in terms of targeting diseased tissues and delivering drugs or markers inside cells [74–77]. In addition to its functional role in drug targeting and preventing particle agglomeration, the organic coating directly influences nanoparticle size and morphology during chemical synthesis.

2. Physical Mechanisms Involved in Magnetic Hyperthermia. Power Dissipation

The principle behind magnetic hyperthermia is the generation of heat in nanoparticle systems, typically dispersed in fluid phases, under the influence of alternating magnetic excitation. This process is strongly dependent on the magnetic mechanisms involved: hysteresis loss and superparamagnetic relaxation (Neel relaxation and Brownian relaxation). An additional mechanism that can contribute to heat generation is electrical current induction, resulting from magnetic field oscillation within metallic nanoparticles. However, this phenomenon becomes significant only when the particle size is substantial (in the range of micrometers). Two categories of magnetic nanoparticle are distinguished: monodomain nanoparticles and multidomain nanoparticles. Bulk magnetic materials are divided into magnetic monodomains where magnetic spins are all oriented in a specific direction. The magnetic monodomains are separated by walls in which the spins gradually orient from one direction to another, corresponding to the two adjacent monodomains. At a specific size, a particle may experience a single magnetic domain where spins are all aligned in a particular direction, named the magnetic easy axis, defined by the magnetic anisotropy energy. Over a specific temperature (blocking temperature - T_B), spins may fluctuate coherently between the two directions of the easy axis - a phenomenon known as superparamagnetic behavior [78–80]. For magnetic monodomain nanoparticles subjected to AC fields, the mechanisms responsible for heat generation are hysteresis loss, described by the Stoner–Wohlfarth model when the particles are in magnetic frozen regime ($T < T_B$), and superparamagnetic relaxation described by the Rosensweig model ($T > T_B$).

The Stoner-Wohlfarth model is a theoretical framework that elucidates the phenomenon of magnetization in monodomain nanoparticles. In this model, magnetic moments are spontaneously aligned with preferential directions, characterized by effective anisotropy energy. This energy originates from either spin-orbit coupling (magnetocrystalline anisotropy, which is an intrinsic

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property of a material) or is imposed by the particle shape (shape anisotropy). Other sources of magnetic anisotropy include surface anisotropy, which can be attributed to specific phenomena such as symmetry breaking of the crystalline structure, oxidation, coating with organic molecules, uncompensated bonds, surface strain, etc. These phenomena can occur at the particle surface [81]. It is noteworthy that the fraction of atoms at the particle surface is approximately $6/D$ from the total number of atoms contained within the particle, where D is the particle's diameter, [82]. The magnetic anisotropy energy can be defined as the energy required to rotate the magnetic moments from the direction of the easy axis (EA) to a direction that makes 90° to EA, also termed the hard magnetization direction. A general formula for anisotropy energy for a single magnetic domain particle can be written as: $E_A = K_{eff} sin^2\theta$, where K_{eff} is the effective anisotropy constant. The easy axes induced by the magnetocrystalline anisotropy are related to the principal direction of the crystalline lattice (symmetry axes). For example, in the case of iron, the cube edges $< 100 >$ are easy directions and diagonals of the cube $< 111 >$ are hard directions. The anisotropy energy induced by the particle shape may be greater than the magnetocrystalline anisotropy energy, if the ratio of the shape geometry parameters exceeds a certain value. In the case of ellipsoidal particles, if the ratio between the polar axis and, respectively, the equatorial axis is at least 1.4, the shape anisotropy becomes dominant [83]. The total anisotropy of a monodomain particle is, therefore, the sum of these particular contributions:

$$K_{eff} = K_{crystalline} + K_{shape} + K_{surface} \quad (1)$$

Different types of magnetic interactions that can occur in nanoparticle assembly may add supplementary terms to magnetic anisotropy expression [84–86]. In the frame of the Stoner – Wohlfarth model, the total energy of a monodomain particle placed in a magnetic field that makes the angle α with the EA is given by:

$$E_T = K_{eff} sin^2\theta - \mu_0 MHcos(\alpha - \theta) \quad (2)$$

where θ is the angle between magnetization and EA. The second term is the magnetic potential energy. In the particular case of applied magnetic field aligned with EA ($\alpha = 0$), the hysteresis loop is perfectly a square, and the coercive field is identical with anisotropy field: $H_c = 2K_{eff}/\mu_0 M_s$.

The Rosensweig model [30] is an analytical approach to the magnetic relaxation mechanisms induced in monodomain non-interacting nanoparticle assemblies excited by AC magnetic fields. The model quantifies the power loss in such a system dispersed in the liquid phase:

$$P = \mu_0 \chi_0 \pi f H_0^2 \cdot \frac{\omega\tau}{1+(\omega\tau)^2} \quad (3)$$

where $\mu_0 = 1.25 \times 10^{-6}\ \mathrm{N \cdot A^{-2}}$ is the vacuum magnetic permeability and H_0 is the field amplitude, χ_0 – equilibrium susceptibility, f and $\mathrm{H_0}$ - magnetic field frequency and strength and τ – the effective relaxation time integrating contributions from Neel and Brownian relaxation processes:

- Neel relaxation when $T > T_B$ and magnetic moments fluctuate statistically and coherently around the nanoparticle's easy axis between the two energy minima. That happens when thermal energy $k_B T$ becomes higher than the anisotropy energy KV, where K is the effective anisotropy constant, $k_B = 1.380649 \times 10^{-23}\ m^2 kg\ s^{-2} K^{-1}$ is Boltzmann constant and V is the nanoparticle's volume. In this way, the Neel relaxation is defined by a relaxation time: $\tau = \tau_0 \exp(KV/k_B T)$, where τ_0 is a time constant with $\tau_0 = 10^{-10} s$. In association with the relaxation time may be defined a frequency of magnetic moments fluctuation, $\tau = (2\pi f)^{-1}$.
- Brownian relation when the magnetic moments are strongly bound to the nanoparticle (the case of high values of KV) and cannot be driven by the AC field. In this case, the particle rotates as a whole against the fluid viscosity resistance, being characterized by a relaxation time defined as: $\tau_B = 3\eta V_H / k_B T$, related to the fluid viscosity (η) and particle's hydrodynamic volume. Hence, an effective relaxation time can therefore be defined as: $\tau = \tau_B . \tau_N / (\tau_B + \tau_N)$.

The hysteresis loss mechanism in magnetic monodomain nanoparticles in AC regime is observed when the anisotropy energy KV is greater than $k_B T$, so the particles are in frozen magnetic

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state with all spins aligned with the easy axis and if the particle as a whole rigid body doesn't rotate in the fluid dispersion media. This is the case of high viscosity fluids or when the particle's rotation is blocked by strong magnetic or physical interactions (e.g., dipolar interactions or organic molecules chains matrices). In the case of multidomain nanoparticles, the mechanism of heat generation is given by the hysteresis loss, but in contrast with monodomain particles, the coercivity is lower and hence, heat production is not as efficient. Nevertheless, it was reported experimental research studies on multidomain nanoparticles performance in MH [87,88].

3. Evaluation Methods in Magnetic Hyperthermia

3.1. Specific Absorption Rate (SAR). Bioheat Equation

Almost all the attention in the MH research field was focused on monodomain magnetic nanoparticles mainly due to the heat efficiency of superparamagnetic relaxation phenomena and secondly for avoiding particle agglomeration and possible issues related to cellular uptake. Even if the MH may have high specific localized effects as it was experimental proved, the standard approach of this technique works in the approximation of homogeneous heat distribution in the tumor tissue, able to rise internal temperature over the physiological threshold with few degrees (~45°C), required for triggering the apoptosis process. In this way, a physical quantity, called specific absorption rate (SAR) was introduced to quantify the amount of power release by MH mechanisms in the tissue mass unit:

$$SAR = P_{abs}/m = [J \cdot kG^{-1}] \quad (4)$$

SAR must have enough high values to compensate the heat loss driven by the physiological thermoregulation processes which try to reestablish the temperature at normal limits. MH may be numerically modelled in vivo by the so-called bio-heat transfer equation (BHTE) [89]:

$$\nabla \cdot k\nabla T + q_p + q_m - Wc_b(T - T_a) = \rho c_p(\partial T/\partial t) \quad (5)$$

where $T(℃)$ and $T_a(℃)$ – local temperature recorded inside the diseased tissue and respectively, the arterial temperature, $c_b\ (J/kg/℃)$ and $c_p(J/kg/℃)$ - specific heats of the blood and respectively, of the tumor, $k(w/m/℃)$ - thermal conductivity of the tumor tissue and $W(kg/m^3/s)$ - blood flow rate. The terms $q_p(w/m^3)$ and $q_m(w/m^3)$ quantify the power generated in the MH process and respectively, by the metabolism processes. MH can be optimized in relation with q_p parameter for biological accepted limits. The term q_p is directly related to SAR parameter by relation:

$$SAR = P_{abs}/m = c \times \Delta T/\Delta t = q_p/\rho \quad (6)$$

with ρ is the tumor tissue's density, c - specific heat of its density and $\Delta T/\Delta t$ - temperature increase rate. Another formulation of SAR can be related to the mass of magnetic material spread into the tumor volume and is known under name of specific loss power (SLP) [90]:

$$SLP = P_{abs}/m_{NP} = c \cdot m/m_{NP} \times \Delta T/\Delta t \quad (7)$$

were m is the mass of tissue and m_{NP} is the mass of the magnetic nanoparticles contained by the investigated tissue.

The SAR factor can be evaluated in vivo experiments, monitoring the temperature increments by specific methods such as ultrasound echo measurement [91] or thermosensitive light emission effect [92], in vitro experiments and directly in ferrofluid samples where temperature may be recorded with a simple optical fiber thermometer. Most of the SAR evaluation measurements are performed by calorimetry techniques in ferrofluid samples containing "fresh" synthesized nanoheater systems dispersed in liquids (water, physiological serum or different oily phases) which are subjected to oscillating magnetic fields (usually in the radiofrequency range (RF): 50-1000 kHz) in order to quantify their heat efficiency. As a standard method, the temperature increase in ferrofluid samples is measured with optical fiber thermometers (metallic sensors are not allowed in RF fields),

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but also, IR imaging is used [93]. The ferrofluid samples are placed inside circular coils connected to RF generators that may be commercial (most of them) or home-made. Depending on the coil geometry and setting of the inductor capacitors, the working frequency can be adjusted. The SAR evaluation methods relied on calorimetric measurements require recording time-temperature heating curves during the MH experiment on the temperature range that include the physiological point. Considering that the heat dissipated in MH process is strongly dependent on the field parameters (f, H_0), a more specific loss power term, called intrinsic loss power (ILP), can be expressed independently of these parameters [94] as:

$$ILP = SLP/f \cdot H^2 [H \cdot m^2/kg \quad (8)$$

This is very useful in evaluation of heat performance in the case of superparamagnetic nanoparticles where dissipated power depends on the square of field intensity according to Rosensweig model. In the case of ferrofluids, SLP may be expressed as [95]:

$$SLP = (\rho_{FF}/\eta \times \rho_{NP}) \times SAR \quad (9)$$

where η represent the ferrofluid volume fraction, ρ_{FF} and ρ_{NP} are ferrofluid and nanoparticles densities.

Along with calorimetric methods, magnetic methods may also provide information about heat efficiency of nanoparticles in MH application. SAR may be seen as the product between frequency of the applied magnetic field and the energy released in dispersion media during a field oscillation cycle. This energy can be evaluated through dynamical hysteresis measurements integrating the area of the magnetization loop over a complete field oscillation. Hence, SAR may be written as [96]:

$$SAR = f \cdot A = f \cdot \int_{-H_0}^{+H_0} \mu_0 M(H) dH \quad (10)$$

Another SAR evaluation magnetic method uses susceptibility measurements [97]. The volume of experimental SAR evaluation data has increased tremendously in the last decades and a huge scientific effort for evaluation of nanoparticles performance in MH was done. In the following, calorimetric and magnetic techniques and methodologies for evaluating the SAR parameter in magnetic fluid sample will be mentioned by few concrete examples, highlighting their main advantages and disadvantages.

3.2. Calorimetric Methods in SAR Evaluation

As it was mentioned above, RF induction devices are commercially available and they are usually equipped with a set of coils of different geometry parameters (diameter, length, pitch) allowing working with multiple frequencies and sample volumes and shapes. These coils are cooled with water or other special cooling liquids. Typical SAR measurements involve small volumes of ferrofluid enclosed in vials of a maximum of a few ml placed in the inner space of the RF coil. Depending on the morpho-structural characteristics of nanoparticles and dispersion liquid, the oscillating magnetic field generated by the coil activates relaxation or hysteresis mechanisms in nanoparticles, leading to a temperature increase in the sample volume that is time-measured with optical - fiber thermometers. The shape of the heating curve acquired during a MH experiment is dictated by the competition between heating rates and loss rates (induced by conduction, natural convection and radiative processes).

Most of the RF commercial induction heating setups don't provide adiabatic conditions during the measurements and that may induce imprecisions in SAR evaluation. Adiabatic environments around the ferrofluid samples are not trivial to build, in particular when the sample volume is very small (0.5-2ml). Even sample holder walls may store consistent amounts of heat generated during the MH experiment. Another important issue that arise in MH experiments is related to large temperature gradients especially generated when the induction coils are cooled by water at low temperatures (under 15°C), usually from a standard water tap. In this situation, water vapor from the

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surrounding air may reach the condensation temperature point (dew point) around the coil, and water drops on the coil surface may appear, complicating the surrounding thermal transfer conditions. This can happen particularly in the summer when air humidity is high. For example, if the air temperature is 25 ^{o}C and indoor humidity is about 45%, the dew point will be 13.8 ^{o}C [98]. Considering that MH experiments are performed in a huge number of laboratories around the world, air temperature and humidity can vary considerably. Artificial environment conditions may be established using air conditioners, but in this case, they produce air currents that may influence the thermal regime around MH experimental setup. Technical solutions for improving the environmental thermodynamic parameters require enclosing the MH experimental setup in specially sealed walls where the inside air may be removed or dry before the experiments. However, even with these special experimental arrangements, in the case of using cooled water at low temperatures, high thermal gradients remain a major issue in evaluating the SAR, especially in the case of slow MH heat rates. Some of the heat generated in the sample's volume will still leak out, resulting in measurement inaccuracy. Cooling the RF coils with water or other cooling agents provided by chillers at a precise temperature may solve the issue, but additional costs are involved. However, the cooling water temperature should have optimal values depending on the values of RF currents through the coil. High RF currents induce heat in the coil body (made by copper) by resistive mechanisms and the cooling process should be optimal in order to not allow temperature to increase inside the coil metal and not to generate high thermal gradients around the sample. The MH measurements should be done in constant and low thermal gradients, adjusting the cooling liquid temperature according to RF current intensity and time measurements, avoiding coil temperature increases and possible instabilities in RF field delivery. Large temperature distributions inside the sample led to high inaccuracy in SAR evaluation. The optical-fiber thermometers, acquire data from a single point (most likely from the middle of the sample's volume), even if the temperature at the sample extremities could be a few degrees lower than in the recording point in the case of high thermal gradients. On the other hand, the coil geometry may induce inhomogeneities in the field distribution inside the sample, which, in turn, may induce heat nonuniformities. In this respect, RF coils with diameters fitting the sample's geometry, optimal length, and small pitches between turns should be used to ensure relatively uniform field distributions within the sample. However, another issue is related to the working frequency used in the MH experiment. RF coils are designed in specific geometries depending on the frequency at they work. Adjusting the capacitors of the RF work head, also, allows the frequency to be adapted to a new value. For different frequencies, along with a change in coil geometry, a change in copper tube thickness is another important aspect. Reporting SAR from MH experiments where various coils of different geometries are used may contain inaccuracies given by the changes in the field distributions inside the sample volume that induce changes in the thermal gradients. Solving this issue involves using a single RF coil for different ferrofluid samples placed in the same type of vials, which should be identically positioned related to the coil geometry. The optical fiber sensor should also be placed carefully in the same position inside the ferrofluid volume for all samples. Therefore, SAR evaluation by calorimetric techniques encounters a series of specific pitfalls related to the MH experimental, becoming a challenge in these technical circumstances. Special experimental setups and innovative methodologies capable of mitigating their effects are required in order to compensate heat loss induced by thermal gradients and to allow reporting comparable results without errors induced by technical artefacts. There are different experimental approaches that have been implemented in SAR evaluation procedure to avoid or to compensate the heat loss effects.

The most used technique that try to avoid the effects of heat loss is to record the temperature increase just for a small period of time with the assumption that the sample "has not enough time" to lose heat in the surrounding environment. This technique considers only the initial part of the heating curve which is properly fitted to extract the time-temperature initial slope of the heating process. Therefore, the SLP factor can be expressed as [99]:

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$$SAR = \frac{C}{m_{np}} p \tag{11}$$

where C – heat capacity [J/K] of the ferrofluid sample, m_{np} - mass of magnetic material contained by the sample and p - initial slope of the acquired heating curve. The crucial condition involved here is related to the thermodynamic equilibrium between sample and the external environment during the measurement. In the case of high heating rates, the initial slope method may work very well, but in the case of low heating rates a consistent amount of heat in respect with that generated through MH could leaks in the surrounding environment, inducing substantial inaccuracies in heating slope determination. Using of highly sensitive and fast thermometers adequate for operation in RF magnetic fields may bring advantages in this case, but probable with high costs. The versatility of the initial slope methods makes it very attractive for SAR measurements [100–102], but other methods have been developed in order to record the heat loss and relied on them, to find methodologies for compensating the real heating behavior and therefore, reconstructing the adiabatic experimental heating curve. In this way, Iacob, et al [89,95] propose two simple methodologies involving recording of time-temperature behavior in both, MH heating regime and, cooling regime where sample is subjected to the natural convection after the RF magnetic field is turned off. This can be done continuously, where the temperature is recorded on the entire ranges of heating and cooling processes, or in steps, where the temperature is recorded in successive short intervals in both heating and cooling regimes in correspondence to the intervals of applying the magnetic fields. In the case of temperature step profiles [95] the experimental points are fitted with linear functions, in order to extract the heating and cooling velocities ($v_H(T)$ and $v_C(T)$) in correspondence to the temperature points. The continuous dependence of $v_H(T)$ and $v_C(T)$ is further obtained by proper fitting of the experimental points. The next step is to numerically compute the adiabatic heating velocity as: $v_A(T_n) = v_H(T_n) + |v_C(T_n)|$ in all temperature points equally separated in arbitrary mode ($T_{n+1} - T_n = \Delta T = constant$) on the experimental range ($[T_0, T_1, \dots, T_n]$). The $\Delta T / v_A(T_n) = \Delta t_{n,n+1}$ ratio gives the time required for the temperature to increase between two consecutive points. The summation of all these time intervals $\sum_{n=0}^{n} \Delta t_{n,n+1}$ provide the time during which the temperature increases between T_0 to T_n. The generation of the adiabatic heating curve $T_n(t_n)$ is therefore possible by the inverse representation of the $t_n(T_n)$ values. In the case of continuously MH mode [89] a more versatile way for calculating the adiabatic heating behavior based on experimental data was developed. The heating and cooling temperature profiles are acquired continuously in the MH process and during the natural convection after the magnetic RF field was turn off. The cooling temperature curve that containes information about heat loss due to thermal gradients around the ferrofluid sample can always be fitted by an exponential function: $T_C(t) = a_C e^{-t/b_C} + c_C$, with a_C, b_C, c_C as fitting parameters. The heating curve may be fitted in the general case by polynomial functions: $T_H(t) = a_m t^m + a_{m-1} t^{m-1} + \cdots a_0$ with a_m coefficients, but for low heating rates, it can be fitted by exponential functions: $T_H(t) = a_H e^{-t/b_H} + c_H$ with a_H, b_H c_H as fitting parameters ($b_H < 0$). In the case of low heating rates, the derivative of the exponential heating and cooling profiles give the temporal heating and cooling rates ($v_H(t)$ and $v_C(t)$). Further on, using a simple mathematical trick of eliminating the time variable either numerical or analytical, between $v_H(t)$ and $T_H(t)$ and $v_C(t)$ and $T_C(t)$, the correspondence between heating and cooling rates (($v_H(T)$ and $v_C(T)$ respectively) and temperature is therefore obtained: $v_H(T) = (c_H - T)/b_H$ and $v_C(T) = (c_C - T)/b_C$. Following the same procedure as in the temperature steps approach, the experimental adiabatic heating curve $T_H{}^*(t)$ is constructed. In the case of high heating rates, where the heating curves are fitted by polynomial functions, the correspondence $t_n(T_n)$ can be simply found by solving the equation $a_4 {t_n}^4 + a_3 {t_n}^3 + \cdots a_0 = T_n$ where ($a_0, .., a_4$) are polynomial coefficients. The heating velocity $v_H(t_n)$ is obtained by numerical evaluating of the derivative of the polynomial function at each t_n. But, t_n corresponds to T_n and the $v_H(T_n)$ dependence is found. Further on, the adiabatic heating rate $v_A(T_n)$ and $T_H{}^*(t)$ are easily computed. In this case it was evidenced the less linear profile of the adiabatic heating curve. If the Rosensweig model (eq. 3) is computed with the physical parameters of the real nanoparticle system as input values, the dissipated power P_n can be calculated

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in each consecutive point T_n of the experimental temperature range. Therefore, the heat dissipated become $Q = P_n \cdot \Delta t_{n,n+1} = m \cdot c \cdot \Delta T_{n,n+1}$ and $t_n(T_n)$, the time needed for temperature to increase between T_0 to T_n is obtain by summation: $t_n(T_n) = \sum_0^n \Delta(t_n, t_{n+1})$. In this way, a theoretical heating curve can be generated by the inverse representation of $t_n(T_n)$. These two methodologies relied on the continuously and steps MH approaches are strongly validated by the overlapping of both heating profiles: the experimental adiabatic and the theoretical one in the case of SPM nanoparticle systems. Nevertheless, a poorer overlapping is observed in the case of high heating rates (the samples of high nanoparticle concentration) possible induced by changes in SPM nanoparticles behavior given by magnetic dipolar interactions or due to thermal inertia that induce deviation from the linear heat transfer behavior. Both experimental approaches used oleic acid coated Fe_3O_4 superparamagnetic nanoparticles dispersed in a polar fluid (transformer oil [103]) in low and high-volume fractions (0.004 and 0.15). The samples vials were positioned in a PVC tube with vacuum walls centered in a commercial 235kHz RF coil setup. The main advantage of these methods is given by the simple construction of the experimental setup. The mathematical approaches also, are not complicated.

Another SAR evaluation method in non-adiabatic conditions that computes heat loss based on recording both heating and cooling curves were developed in [104]. Here, the MH setup is completed with a water shell surrounding the magnetic fluid sample based on Fe_3O_4 nanoparticles in order to protect the sample against possible short temperature variations from the environment. The entire holder is placed in a home-made RF device working at $100kHz$. SAR is evaluated trough a developed methodology relied on solving a set of coupled differential equations describing the heat exchange between MH setup components:

$$P - \frac{c_s m_s}{\tau_s}[T_s(t) - T_w(t)] = c_s m_s \frac{dT_s(t)}{dt} \tag{12}$$

$$\frac{c_s m_s}{\tau_s}[T_s(t) - T_w(t)] - \frac{(\tau_{w1} + \tau_{w2}) c_w m_w}{\tau_{w1}\tau_{w2}}[T_w(t) - T_a] = c_w m_w \frac{dT_w(t)}{dt} \tag{13}$$

were P is the power term, c_s and m_s - specific heat and mass of the heat source (nanoparticles), c_w and m_w – specific heat and mass of the water τ_s - the time constant of the heat exchange between the nanoparticles and the water, T_s is the nanoparticles temperature and T_w is the temperature of the water, τ_{w1} and τ_{w1} – time constants of exponential functions that fitted the cooling curve of the magnetic fluid sample in the natural convection conditions and $T_a = 23.5^oC$ - equilibrium temperature of the environment. The method brings some complexity regarding to experimental setup and mathematical methodology, also involving a calibration procedure, but has as a main advantage, possibility for estimating the temperature of the magnetic nanoheaters dispersed in the fluid sample. The methodology exploits the thermal equilibrium condition attained during the MH process when the heat released by the nanoparticles is completely lost in the environment. In this situation the system of equations is reduced to:

$$P = \frac{(\tau_{w1} + \tau_{w2}) c_w m_w}{\tau_{w1}\tau_{w2}}[T_M - T_a] \tag{14}$$

where T_M – maximum temperature reached in the heating process when the sample enter in the thermal equilibrium. Similar MH experiments in non-adiabatic conditions counting heat loss from the recording of natural cooling temperature may be found in [105]

A general equation relied on a developed thermodynamic approach for determining SLP factor containing terms corresponding to different thermodynamic regimes: adiabatic approximation, the non-adiabatic and non-radiating conditions, and the isothermal case may be found in [106]:

$$SLP = \frac{1}{m_{np}} C_{susp} \frac{dT}{dt} + \frac{1}{m_{np}} \in (T - T_{air}) + \frac{1}{m_{np}} \sigma \eta A_t (T^4 - T_{air}^4) \tag{15}$$

where m_{np} - nanoparticle mass, C_{susp} - heat capacity of nanoparticles suspension, $\in$ - effective thermal conductance of the sample's surrounding environment, σ – Stefan Boltzmann constant, η - emissivity and A_t – total sample surface. The equation was validated with high accuracy by HM

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experiments performed on magnetite and magnesium ferrite dispersed in water in non-adiabatic and radiating conditions. Heating in RF field conditions of 70.5kHz and 70Oe and cooling curves were recorded and by their proper fitting, $\in$ parameter and finally SLP was obtained.

In [107] can be found an alternative method for SAR evaluation in MH experiments that claim high precision due to non-transient measurements. The experimental setup is complicated providing the almost adiabatic conditions accomplished by maintained a controlled thermal equilibrium between the environment and fluid sample. The method is based on applying consecutive AC magnetic pulses that generate heating ramps in ferrofluid sample. It is assumed that the entire the heat generated during a heating pulse remains in the sample allowing measuring adiabatic temperature increment ΔT. The method has, along with precision, the advantages of measuring low SAR values.

MH experiments performed in [108] using different dextran and Citrate coated magnetic water-based colloidal systems, a range of frequencies (150-375kHz) and field intensities (4-44kA/m) generated by a single coil cooled with a water close circuit demonstrated a consistent error in SLP measurements even the filed parameters and sample's volume and shape were identical for each experiment. Coupled effects between the magnetic field inhomogeneities generated by the coil's geometry and particle distributions in the sample's volume are identified as main sources of errors. SLP values were estimated considering the heating slope of the linear part of the heating curve and the first derivative of it in order to confirm the quasi adiabatic regime. The magnetic fluid samples were placed in a thermal insulated holder.

Thermographic approach of MH was investigated in experiments were glucose coated iron-oxide nanoparticles dispersed into different polyacrylamide gels, for emulating the intracellular viscosity, were subjected to RF magnetic fields of $H = 32\,kA/m$ and $f = 350\,kHz$ [93]. The temperature behavior of the sample was monitored through a commercial thermographic camera before, during and after MH experiment. Sample of discoidal shape of 13mm diameter was inserted inside a holder adjusted in the inner space of a RF coil. The method shown spatial inhomogeneities in the particle concentration proved by 2D temperature mapping of the sample's surface also, evidenced by difference seen in the recorded temperature profiles by an optical fiber thermometer. Radial temperature distribution also offers information about lateral thermal gradients. The method is quite simple having specific advantages, but required quasi adiabatic protection of the sample and high camera resolution in order to analyses more efficient thermal distributions inside the sample. A low thickness sample would have the advantage of eliminated the volume thermal gradients.

Based on a heat diffusion equation, a device-independent approach was developed in [109] where SLP was calculated by a new method called Peak Analysis Method (PAM) using 1D temperature diffusion model and a zigzag protocol of intermittent heating and cooling steps (similar with [95]). Experimental validation of this approach was performed using three devices with the same field parameters (~165kHz and 35mT) where 1ml magnetite nanoparticles suspension was heating. The temperature was acquired with an optical fiber thermometer. The SLP values were compared with those obtained from single heating-cooling cycle experiment recorded on the same devices). The SLP results were consistent for all three devices in the zigzag protocol proving drastically reduction of the errors between the devices and the measuring time of the SLP evaluation.

Even if many and complex calorimetric methods and methodologies were developed for achieving high accuracy in SAR evaluation, utilizing a wide class of experimental equipment undoubtedly generates pitfalls regarding to the comparative analyzing of results reported from different parts of the world. Therefore, the necessity for standardization of calorimetric methods for SAR evaluation has arisen. In this sense, there have been proposed recommendations for SAR evaluation under non-adiabatic conditions, regarding the linear loss regime, the configuration of the experimental setup and the mode of operation [110]. However, it is challenging to develop a standard calorimetric method for SAR evaluation that can be used to compare results obtained by different research groups, due to the variety of experimental setups, environmental conditions, magnetic fluid concentrations, and evaluation methodologies employed. In the case of well-established

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experimental techniques, such as X-ray diffraction, electron microscopy, and magnetometry, standardized commercial devices are utilized. The question therefore arises as to why standardized devices for SAR measurements are not employed in the context of magnetic hyperthermia. While there are indeed such devices on the market, they are limited in terms of the range of models and options available. In addition to the standardization of working methods in the laboratory, therefore is a necessity to utilize standardized commercial equipment that offers a wide range of measurement options with regard to magnetic field parameters and their modulation. The holder in which the magnetic fluid sample is placed should be standardized and integrated into each commercial device where the ambient temperature around the sample should be controlled. Developing fully approved working protocols allowing the most realistic estimation of SAR values under different magnetic field and fluid samples (viscosity, concentration) conditions is also imperatively required.

3.3. Magnetic Methods in SAR Evaluation

Dynamic magnetic hysteresis measurements performed in MH frequency regime bring high accuracy in SAR evaluation [111–117], but commercial devices which deliver proper field intensities are not available. Instead diverse locally implemented solutions have been built with good results [111,112,115]. Comparative analyses with calorimetric methods have been done indicating a good agreement [111,112]. The advantages of this technique are mainly given by the speed and accuracy of measurements. Drawbacks is given by the impossibility of measure hysteresis loops in the case of superparamagnetic nanoparticles. For that AC susceptometers working at the MH frequencies were built in different laboratories because commercially were not available [97,118,119]. The most commercial devices (e.g. PPMS from Quantum Design) for magnetic measurements allow χ'' measurements as a function of temperature and field parameters ($f \leq 10kHz$ and $H_0 \leq 1.2$) which are not useful in the range MH. Each magnetometer technique has advantages and disadvantages regarding to the type of nanoparticles that can be investigated. These techniques also are not standardized, allowing errors between different experimental setups that may arise from the quality of electronic components, calibration procedures and working protocols. Hence, building standardized MH AC magnetometers / susceptometers on large scale is clearly a necessity. The calorimetric technique allows SAR measurements for any type of nanoparticles. In the case of standardization and marketing of both calorimetric and magnetic techniques, probable the price will define any choice made. However well-defined working protocols in both cases are required because of a wide class of magnetic fluids are synthesized and part of them have issues regarding the time-stabilization of the suspension.

4. SAR Optimization

SAR optimization involves improving the thermal response of magnetic nanoparticles with respect to the applied radiofrequency field parameters and allowed biological limits. Depending on the mechanism by which the nanoparticles release heat when they are magnetically excited in radiofrequency fields (superparamagnetic relaxations or hysteresis losses), the saturation magnetization (M_S) and the magnetic anisotropy barrier (KV) are parameters that play a crucial role in the efficiency of heat generation. The enhancement of the magnetic anisotropy barrier (KV) is contingent upon the improvement of the intrinsic properties of the magnetic material, particularly in the case of spherical or cubic particles, where K is independent of the particle shape. However, the optimization of the shape and size of these particles becomes imperative when KV is predominantly induced by the particle morphology. In many instances, the nanoparticles utilized in HM are superparamagnetic and possess ellipsoidal shape. A theoretical study on the dimensional optimization of superparamagnetic ellipsoidal particles in relation to the maximum power dissipated under the imposed biological limits can be found in [83]. In this study, the dissipated power of a mono-dimensional superparamagnetic magnetite nanoparticle system without magnetic interactions and dispersed in fluid media with low and high viscosities was evaluated based on the Rosensweig model [30]. The particle's equatorial axis size (a [nm]) ranged from 5 to 15 nm, and the aspect ratio

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($r = c/a$) varied from 1. 2 to 10, where c [nm] denotes the polar axis size. Subsequent to the implementation of the maximum condition for the dependence of the power dissipated on the frequency $f \times \omega\tau/(1 + (\omega\tau)^2)$ attained for $\omega\tau = 1$ [30], the analytical calculations identified the optimal pairs (a, r) of the shape parameters. For instance, it was estimated that, in the case of applying a magnetic field with a frequency of 250 kHz and an amplitude of 20 kA/m, the optimized geometrical parameters of ellipsoidal magnetite nanoparticles dispersed in a high viscosity fluid have an equatorial dimension of 1. 0 nm and aspect ratio of 2. Theoretical (120, 80) as well as experimental [121–125] studies have shown that dipolar magnetic interactions between superparamagnetic nanoparticles can inhibit their relaxation processes and, therefore, influence their heat generation capacity in the hyperthermal application. The influence of magnetic dipolar interactions on magnetic anisotropy energy and transferred power is perturbative, according to [126], and only becomes relevant at volume fractions greater than 0.01. This is considerably higher than the values typically employed in biomedical applications. Theoretical studies have identified dipolar magnetic interactions occurring in concentrated ferrofluids as a factor that modifies the superparamagnetic behavior of the nanoparticle assembly [80]. An experimental study of the dependence of the SAR decrease on the volume fraction of a magnetic fluid based on the dispersion of a system of superparamagnetic magnetite nanoparticles coated with oleic acid in transformer oil can be found in [127]. In this study the experimental SAR values in overlapped theoretical values generated by the Rosensweig model only when the relaxation time constant τ_0 (usually considered material-dependent) was modified. The changes in the relaxation time constant value is also theoretically proved by [120]. The experimental work was conducted for volume fractions ranging from 0.005 to 0.16 and different values of the applied magnetic field strength and demonstrated that SAR decreased with the volume fraction, decreasing amplified by the magnetic field strength. For the maximum volume fraction of 0.16, SAR decreased by 43% for an applied magnetic field of 14KA/m and by 69% for an applied field strength of 35kA/m, when compared to the reference value of 0.005 (interactions between particles are negligible). The generation of high concentrations of nanoparticles in clusters has been experimentally observed in tumor cells, particularly when the particles are functionalized to target specific cellular components [51]. In this regard, the optimization of injected magnetic fluid doses emerges as a pivotal task to ensure the preservation of the heat dispersion efficiency of the nanoparticles utilized in HM. Conversely, a specific geometrical configuration of a nanoparticle cluster might prove advantageous in facilitating optimal heat transfer to the targeted cellular component to hinder its functionality. As previously mentioned, the cellular effects of magnetic hyperthermia are predominantly influenced by the heat transfer in the proximity of the nanoparticle. A cluster consisting of nanoparticles measuring a few nanometers in size is comparable in dimensions to subcellular components. From this perspective, the size and shape of the cluster may be optimized with respect to the size of the cellular component to ensure efficient heat transfer. Additionally, large particles (hundreds of nm) that can be internalized by cells and come into physical contact with the thermosensitive part of the cell are of particular interest in cellular hyperthermia [24,128]. These particles dissipate thermal energy through hysteresis loss, exhibiting, in general, a lower SLP factor compared to superparamagnetic nanoparticles. However, the total caloric energy transferred to a vital component of the cell could exceed the minimum necessary for it to lose its functionality. Another type of nanostructure that is of interest in the context of cellular hyperthermia is given by nanoflowers, which possess particular shapes and complex geometries, allowing them to attach to cellular components in specific ways that properly to amplifying the heat transfer. In this regard, the significance of SAR/SLP/ILP diminishes when assessing the consequences of hyperthermia at the subcellular level. Therefore, in this context, SAR optimization assumes novel dimensions, particularly at the level of particle or cluster design, and their functionalization to target the cell's most thermosensitive and functionally significant components.

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5. Final Conclusions and Remarks

Magnetic hyperthermia constitutes an alternative therapeutic approach for the treatment of cancer, with its principal mechanism of action involving the generation of heat in tumor tissues subsequent to the application of a radiofrequency field on a system of magnetic nanoparticles inoculated into the tissue. The magnetic nanoparticles release heat by specific magnetic relaxation processes, Neel and Brownian [30], or by hysteresis losses [111–117]. It is well-established that cancer cells exhibit heightened sensitivity to temperature elevations that surpass physiological thresholds, leading to a process known as apoptosis.

In the classical MH approached, the implementation at the clinical level necessitates prior knowledge of the magnetic material distribution within the tumor tissue and the magnetic field parameters that can be utilized in safe patient conditions. This can be achieved through theoretical and experimental modeling of magnetic hyperthermia. From a theoretical standpoint, the modeling of magnetic hyperthermia can be achieved through the utilization of the BHTE equation [95], contingent upon the availability of data pertaining to the power dissipated in the tissue (q_P) by the mechanisms of magnetic hyperthermia and the bio-thermodynamic parameters of the tissue under normal physiological metabolism. To determine the q_P term, it is necessary to employ laboratory experimental methods involving calorimetric and magnetic measurements.

Calorimetric methods, however, face limitations in directly evaluating the q_P term due to their inability to maintain adiabatic conditions within the measurement system, which results in inaccuracies in heat loss measurements. In a real, non-adiabatic system, the most straightforward and accessible method to circumvent the impact of heat dissipation is to consider solely the initial heating slope [100–102] of the temperature curve recorded during magnetic hyperthermia. Despite its versatility, the method has drawbacks, particularly for slow magnetic hyperthermia processes, where the ferrofluid sample heats up at a slow rate and the system under test has time to lose heat. Another strategy is not to avoid the heat losses, but to record them and use them to reconstruct the adiabatic heating curve that would have been recorded if the system under calorimetric measurement had been completely adiabatically isolated. For this purpose, various simple or advanced heat loss calorimetric methods providing high accuracy results [104–109] have been developed based on the considering the heat loos induced by the thermal conduction between the experimental setup components, natural convective cooling and radiative loss. If the initial slope method gives good results in the case of high heating rates, the heat loss compensation methods could be complementary used in the case of low heating rates.

Magnetic methods (dynamic hysteresis and susceptibility measurements) are versatile, straightforward to utilize, and provide accurate and rapid information on the magnetic response of nanoparticles in alternating magnetic fields [111–117]. The principal disadvantage of these techniques is that magnetometer devices operating in the range of magnetic hyperthermia field parameters are not commercially available, only locally constructed in the laboratory, but with remarkable results.

However, it's hard to believe that the calorimetric and magnetic methods or methodologies will be able to allow comparison with high accuracy SAR values obtained from different laboratories around the world without involving standardized measurement equipment and protocols. It's the unique way to avoid pitfalls given by a wide class of experimental factors such as: various geometries used in coil building, their cooling systems, ambient thermal conditions, volume and shape of the sample's holder and its material composition, ferrofluid volume fraction and suspension stability, etc, in the case of calorimetric approach and different electronic parts, constructive design and calibration protocols in the case of magnetic approach.

At the cellular level, the dissipation of heat during hyperthermia exerts a specific influence on the growth and multiplication capacity of tumor cells. While the underlying mechanisms remain to be fully elucidated, ongoing research endeavors seek to identify the heat-sensitive cellular components that could expedite the process of apoptosis following magnetic hyperthermia treatment. In this regard, a novel trend in the study of magnetic hyperthermia entails the targeting of specific subcellular structures within the cytoplasmic environment or extracellular matrix [48–56].

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This approach aims to localize the effect of magnetic hyperthermia and optimize the utilization of magnetic nanoparticles and applied fields. This objective can be achieved by binding molecular vectors with chemical affinity for specific cell receptors to the nanoparticle surface. In addition to the effects of cellular magnetic hyperthermia, scientific interest has shifted to the study of the kinetic effect [61–66] of nanoparticles on subcellular components by applying low-frequency magnetic fields to nanostructures with high magnetic anisotropy (nanodisks, nanorods, nanoflowers, etc.). The oscillations and vibrations of these particles have the potential to induce local cellular damage, thereby triggering apoptosis processes.

Considering the classical aspects of magnetic hyperthermia, but also the new trend to focus the effect of magnetic nanoparticles on cell growth and proliferation mechanisms at the cellular level, questions inevitably arise such as: What is the relationship between SAR/SLP/ILP and the local effect of magnetic hyperthermia on the cellular infrastructure? Does it still make sense to consider the power dissipated by magnetic nanoparticles in tissue as a continuous macroscopic variable? Or should we focus our scientific interest on local thermal and mechanical effects at the subcellular level, looking for the optimal way to release the heat and kinetic energy of a single nanoparticle or a cluster of nanoparticles? In this case we would need to find ways of expressing SLP/ILP for a single particle, incorporating the kinetic term. However, it is difficult to establish a link between the power (caloric or kinetic) dissipated by a single particle and its effect on cellular metabolic mechanisms. In this way, the new approach of magnetic hyperthermia becomes highly specialized, adding destructive mechanical effects to the local thermal effects. Combinations of these effects, depending on the targeted cell part, could be the safest way to approach magnetic hyperthermia. Most of the research in MH field are done by chemists and physicists, but strong implication of biologists and biochemists is crucial in understanding fundamental cellular mechanisms, interactions between magnetic particles and cells and therefore, designing optimal ways for MH approaching with high precision and efficiency and less side effects. It's like in a modern war: instead of using a lot of low precision projectiles, you can use few projectiles with high targeting precision. High local heat waves and kinetic disrupting effects targeting the most thermo and structural - sensitive parts of the cell will probably be the magic bullet in MH. But until there is a challenging long way and material and design optimization steps in nanoparticle production should be carried out continuously. On the other hand, a systematic and complex review of all clinical and preclinical experimental results, including the vast amount of in vitro and in vivo experimental results, is needed to provide focused research directions. This may be possible with the new AI algorithms.

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