Protein adsorption enhanced radio-frequency heating of silica nanoparticles

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Measurements of specific-absorption-rate (SAR) of silica 30, 50, and 100 nm nanoparticles (NP) suspended in water were carried out at 30 MHz in 7 kV/m radio-frequency (*rf*) electric field. Size dependent, NP-suspension interface related heating of silica NP was observed. To investigate a possible mechanism of heating, bovine serum albumin was adsorbed on the surface of silica NPs in suspension. It resulted in significant enhancement of SAR when compared to bare silica NPs. A calorimetric and *rf* loss model was used to calculate effective conductivity of silica NP with/without adsorbed albumin as a function of silica size and albumin concentration.

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Radio-frequency (*rf*) hyperthermia and *rf* ablation procedures, for which cell temperatures increased to 41–46 °C and above 56 °C, respectively, are well recognized methods for treatment of malignant tumors.^{1,2} However, they suffer from lack of selectivity toward cancer tissue, thus causing unintended thermal necrosis in healthy tissue. In recent years it has been demonstrated that these *rf* procedures can be modified to be non-invasive and cell-selective when functionalized³ nanoparticles (NPs) and an external source of *rf* are used.^{4–6} For both non-magnetic and magnetic nanoparticles working as heating enhancers, *in-vitro* destruction of malignant cells was demonstrated using such technique.^{7–10}

Although these demonstrations are impressive and indicate a potential for developing a new clinical technique, the underlying physics behind the reported *rf* heating of NP is not fully explored. Theoretical simulations based on existing physical models, as well as experimental results, produce, especially for non-magnetic NPs, *significant disparities between reported and expected heating*^{11,12} sometimes leading to major overestimation of heating efficiency of investigated NPs. Furthermore, there are disparities reported for nanoparticles' heating efficiency measured for colloidal suspensions of nano-structures, and for such nanoparticles measured *in-vitro* and *in-vivo*. Besides the well-understood basics of *rf* magnetic field interaction with magnetic NPs¹³ very limited literature is available on other heating mechanisms.^{12,14}

Our measurements of *rf* electromagnetic fields' induced heating of magnetic, dielectric, and metallic nanoparticles indicate that interface losses due to, for example, the double layer between NPs and the host media also play an important role in the overall *rf* heating process.¹⁵ In order to investigate this issue, we have characterized *rf* heating of water-suspended bare silica NPs and also silica with its surface modified by adsorbed bovine serum albumin (BSA). Silica NPs were selected here because they are not expected to produce any noticeable heat by dielectric loss mechanism.¹⁶ The estimated *rf* dielectric nano-sphere loss is a two order of magnitude smaller than the loss needed to explain experimentally observed heating.

When administered to the body, colloidal oxide particles immediately adsorb and become coated with proteins of the biological medium, with albumin being the dominant one. This opsonization process is strongly affected by the surface characteristics of NP. BSA is a soft protein obtained from cow's blood serum, which constitutes about half of the protein in plasma and is one of the most stable and soluble proteins. There are several mechanisms, which explain how a protein can attach to a charged surface.^{17,18} Because of the hydrophilic silica-water interface, the adsorption of BSA here is a simpler case of the protein adsorption process resulting in a smooth and densely packed monolayer of about 5–25 nm thickness.^{19–21} In the case of a hydrophilic surface, there is an electrostatic attraction between the charged surface and an oppositely charged protein molecule.

Our experimental setup, for which a block diagram is sketched in Fig. 1, is based on the inductance-capacitance-resistance (LCR) high Q-factor (300–400) resonator, which can generate, in 12–50 MHz frequency range, E_{rf} and B_{rf} fields up to 100 kV/m and 50 kA/m, respectively.¹² Very efficient water-cooling was applied to all metal parts of the LCR resonator and the capacitor plates are separated by a water-cooled single crystal sapphire ($\epsilon_r = 11$, $\tan \delta = 10^{-5}$). The temperature of the sample was measured by a GaAs Opsens sensor with controlled positioning using a μm resolution x-y-z translation stage. The resonator was driven by a HP synthesizer 83640 and a 10 W *rf* amplifier.

To determine specific-absorption-rate (SAR) of silica NPs with/without BSA, we have used the first 20 s of the linear portion (which is an adiabatic heat exchange case) of the temperature increase, $T(t)$ to calculate slope the $q_c = dT/dt$. The *heat-production-rate* power loss P increases the sample temperature, where P is equal to $C_{ps}\rho_s dT/dt$, and it is equivalent to power absorption per unit volume (W/m^3). Here, C_{ps} and ρ_s are the constant pressure heat capacity ($\text{J/(kg }^\circ\text{C)}$) and the density of the sample (kg/m^3), respectively. For aqueous suspensions, their values are assumed to be close to the values measured for water, which are equal to $4.18 \times 10^3 \text{ J/(kg }^\circ\text{C)}$ and to 10^3 kg/m^3 .

FIG. 1. A sketch of the experimental setup is shown. RLC resonator consisting of a parallel plate capacitor and multi-turn solenoid allows for placing the sample in uniform electric or magnetic rf fields.

First, we have measured the $T(t)$ dependencies for silica NPs of diameter 30, 50, and 100 nm (Kisker-Bio) suspended in deionized (DI) water (50 mg/ml). Next, in order to investigate the influence of albumin on the heating of silica NPs, we have merged silica NPs in DI water solution with four different concentrations of BSA: 0.5%, 1%, 2%, and 4% (weight/volume). All measurements were carried out for a constant volume of sample (2 ml), while the volume fraction for each NP size was also kept constant. It has been demonstrated previously that the amounts of BSA adsorbed on silica NPs surface are highly correlated with the zeta potential²² and of hydrodynamic radius values. Measurements of these two parameters as a function of silica NP size confirmed BSA adsorption.

Experimental plots of temperature, $T(t)$ for 30 nm SiO_2 NPs with/without an added 2% BSA, are shown in Fig. 2. The reference temperature $T(t)$ plots for 2% albumin and DI water are also included. The measured slope of $T(t)$ for DI water is very close to the slope obtained from rf absorption calculations. It can be observed that albumin addition increased the heating efficiency of NPs. We have also observed that the heating efficiency of silica NPs depends on the particle diameter as it can be seen from the inset in

FIG. 2. Temperature vs. time dependence of 30 nm silica particles in water suspension and in 2% w/v albumin solution is shown. Respective dependencies of control samples such as 2% albumin only solution and DI water are also presented. Inset illustrates size dependent heating of 30, 100 nm silica NPs (the same volume fraction).

Fig. 2, where comparison of the $q_c = dT/dt$ slopes calculated from temperature, $T(t)$ plots for 30 and 100 nm particles indicate that smaller particles are experiencing more efficient heating. Similar observation was reported for gold nanoparticles.²³ This observation confirms that mechanism of heating is correlated with the overall surface area of the particles in the suspension.

The power loss, P , is directly related to the electromagnetic material properties such as conductivity σ , complex permittivity ϵ , and/or complex permeability μ . Because of $\sigma = \epsilon_0 \epsilon'' \omega$ (ω is the angular frequency of the rf field, ϵ_0 is the free space permittivity and ϵ'' is the imaginary part of the dielectric constant), the same sample can be treated either as a lossy dielectric material or as a conducting material with an effective conductivity σ_{eff} . The latter term is used to describe components of rf losses related to the sample. When a material is exposed to an rf local electric field E_L , the power loss per unit volume can be expressed as

$$P = \frac{1}{2} \sigma_{\text{eff}} |E_L|^2 = \frac{1}{2} \epsilon_0 \epsilon''_{\text{eff}} \omega |E_L|^2. \quad (1)$$

Since σ_{eff} represents power loss per unit volume and per “unit” of electric field strength ($|E_L|^2 = 1$), it can be used to express SAR of a measured material, which represents power loss per unit mass

$$\text{SAR} = \frac{\sigma_{\text{eff}}(f) |E_L|^2}{\rho}. \quad (2)$$

It needs to be noted that in order to compare two different SAR measurements of investigated material, the local E_L field and frequency values should always be specified. Furthermore, SAR was introduced in literature as a dosimetric parameter, not a material parameter as it is used in nanotechnology.

In literature, for SAR measurements of NPs, polarization effects in sample containers and their influence on the local rf field around NPs are usually neglected, resulting in an overestimation of the local electric field component. In our system the local E_L field is expressed as

$$E_L = \beta \gamma_c (\sigma_{\text{sol}}) E_y, \quad (3)$$

where σ_{sol} represents effective conductivity of a solution, γ_c is the depolarization factor due to free ion charges in this solution (in our case for $\sigma_{\text{sol}} < 0.01$ S/m, $\gamma_c \sim 1$ and $\sigma_{\text{sol}} > 0.1$ $\gamma_c \sim 0$), and E_L is the local electric field in the sample region, equal to βE_y , where E_y is the external electric field measured between the two metal plates of the capacitor and β is the dielectric polarization-screening factor calculated to be ~ 0.1 for the experimental configuration used here. Such factor depends on dielectric constants of sapphire, quartz tube, and the geometry of the holder.

In general, when the rf electromagnetic wave is applied, the heat loss equation can be written in the following manner:

$$\begin{aligned} Gq_c &= \sigma_{\text{sol}} V_{\text{fsol}} + \sigma_{\text{NP}} V_{\text{fNP}} + \sigma_{\text{Alb}} V_{\text{fAlb}} \\ &= \sigma_{\text{sol}} (1 - V_{\text{fNP}} - V_{\text{fAlb}}) + \sigma_{\text{NP}} V_{\text{fNP}} + \sigma_{\text{Alb}} V_{\text{fAlb}}, \end{aligned} \quad (4)$$

where $G = 2C_{ps}\rho_s/|EL|^2$ is a measure of power loss normalized to unit volume and square of the electric field. Here, σ_{Alb} is the conductivity of an albumin; V_{sol} , V_{fAlb} , and V_{fNP} are volume fractions of solution, albumin, and silica, respectively. Since we are interested in σ_{NP} , which is a function of both NP diameter “a” and albumin concentration “c,” we have modified Eq. (4) to the following form:

$$\sigma_{NP}(a, c) = \frac{1}{V_{fNP}} \left(Gq_c(a, c) - \sigma_{sol}(1 - V_{fNP} - V_{fAlb}(c)) - \sigma_{Alb}V_{fAlb}(c) \right). \quad (5)$$

In order to calculate the rf loss associated with NPs only (σ_{NP}), it is necessary to subtract the heating of the dispersion medium (solution) and albumin. From measured heating slope q_{sol} of the solution, Eq. (4) is used to calculate the effective conductivity of the solution, σ_{sol} by setting V_{fNP} and V_{fAlb} to zero. For the case of solution with a known concentration of albumin added (known V_{fAlb}), we obtain σ_{Alb} by solving Eq. (5) with V_{fNP} set to zero. The solution slope, q_{sol} is assumed to be an average of the zero concentration albumin and filtered supernatant produced by centrifugation of silica/water suspensions.²⁴

When DI water is the suspending medium, $\sigma_{sol} = \sigma_{DI}$. However, determination of σ_{sol} values turned out not to be so straightforward. From our other measurements, carried out using both magnetic and electric rf fields, we found that σ_{sol} is higher than σ_{DI} .²⁵ It can be explained by the presence of contamination groups coming from silica powder that are most likely partially dissociated in water. In addition, we should also expect the presence of metal salt residue from the production process.²⁶ As a result, the silica powder introduction is increasing conductivity of the solution in which silica particles are suspended.

The heating slopes q_c with respect to albumin concentration “c” and silica NP diameter “a” as parameters are shown in Fig. 3. It can be seen that the measured slopes q_c are linear with albumin concentration “c” as expected from Eq. (5), where q_c is linear with volume fraction which in turn is proportional to albumin concentration. Extrapolation of albumin

FIG. 3. Slopes for temperature rise vs. albumin concentrations adsorbed on 30, 50, and 100 nm silica NPs dispersed in DI water. Extrapolation of σ_{sol}/G value for $c = 0$ is marked on q_c axis.

concentration “c” to 0%, i.e., no-albumin heating slope q_c is also shown in this graph.

In the plot of heating slope q_c vs. NP diameter “a,” we also see that q_c can be assumed to be linear with “a” as well in the range of 30 nm to 100 nm. In has to be noted that in general heating rate q_c would not be linear with NP diameter, specifically where all samples have the same NP volume fraction ($V_{fNP} \sim 0.0036 \pm 0.0003$). In such a case, decrease in diameter is compensated by increase in NP number to maintain constant volume fraction resulting in a change in the dominating loss mechanism. It can be seen from measurements of heating efficiency of NPs with diameters exceeding 100 nm (not shown here), that q_c dependence on size becomes very weak approaching a constant value. However, we can interpolate values within the size range used in our experiments: 30 nm to 100 nm, as we can assume that the curvature of the size dependent function in this range is large enough to be approximated by a straight line.

Furthermore, measured heat slopes q_c for different “a” and “c” values together with albumin-only and silica NPs-only data are listed in Table I which can be represented by a bilinear regression of the “a” and “c” parameters equation

$$q_{cNP}(a, c) = D + Aa + Bc + Cac. \quad (6)$$

By using the least square regression method on the output variables q_{cNP} and the two input variables “a” and “c,” we can determine the four regression coefficients: {A,B,C,D} leading to the following form of bilinear regression equation:

$$q_{cNP}(a, c) = 0.11 - 4.17 \cdot 10^{-4}a + 3.297c + 0.039ac, \quad (7)$$

with $R = 0.996$ and standard deviation $s = 0.009$. If we assume uniform variation between experimental data the measurement error of the heating slope is determined to be $q_c \pm 0.01$. Inversely proportional dependence of q_c on NPs diameter is reflected by negative regression coefficient A in Eq. (7). It indicates that the heating slope vs. size dependence will have a negative slope. Linear regression for the albumin-only data is as follows:

$$q_c^{Alb}(c) = 0.77 + 2.77c. \quad (8)$$

The SAR of silica NPs with/without albumin as a function of diameter “a” and albumin concentration “c” is calculated using Eq. (2), where σ_{eff} is derived by inserting Eq. (7) in Eq. (5) and E_L from Eq. (3). The results of both conductivity and SAR calculations are shown in Table II. Surface function of the SAR of silica nanoparticles vs. the size of silica NPs “a” and the concentration of albumin “c” is shown in

TABLE I. dT/dt slopes (in $^{\circ}C/s$) for 30, 50, and 100 nm silica NPs for different concentration of albumin.

c (w/v%)	q_c (30 nm)	q_c (50 nm)	q_c (100 nm)
0.0	0.98	0.09	0.06
0.5	0.116	0.11	0.11
1.0	0.129	0.12	0.12
2.0	0.182	0.22	0.197
4.0	0.269	0.29	0.35

TABLE II. Effective conductivities (in S/m) and SAR (in kW/kg) for different c and a .

c (%)	SiO ₂ , a = 30 nm		SiO ₂ , a = 50 nm		SiO ₂ , a = 100 nm		Albumin	
	σ_{eff}	SAR	σ_{eff}	SAR	σ_{eff}	SAR	σ_{eff}	SAR
0.0	4.10	1.17	1.66	0.47	-4.47	-1.27	0.56	0.11
0.5	6.64	1.89	5.34	1.52	2.07	0.59	0.69	0.13
1.0	9.19	2.62	9.04	2.57	8.61	2.45	0.83	0.16
2.0	14.3	4.06	16.40	4.67	21.70	6.18	1.09	0.21
4.0	24.4	6.96	31.13	8.86	47.86	13.62	1.62	0.31

Fig. 4. Two reference planes are shown here. Zero-SAR-plane describes a case where the same volume silica NP produces the same amount of heat as the solution does. SAR(a,c) points placed below the SAR = zero-plane value represent a case where silica NPs produce less heat than their equivalent volume of the solution. The second SAR = 6 kW/kg plane illustrates for what size of NP and for what concentration of albumin the SAR will exceed such SAR value.

In the SAR(a,c) vs. silica diameter cross section lines (marked as white lines), for $c=0\%$, $c=0.5\%$, $c=1\%$, $c=2\%$, and $c=4\%$, it can be noticed that above $\sim 1.5\%$ of albumin concentration $\Delta\text{SAR}_{\text{NP}}/\Delta a$ slope is positive. It indicates that above certain concentrations of albumin, effective silica rf loss will be increased with the increase of both silica size and albumin concentration. From the SAR(a,c) vs. albumin concentration cross-section lines, for silica 30 nm, 50 nm, and 100 nm diameter, it can be seen that SAR increases for any silica diameter when albumin is added. It can be interpreted by using a simple silica in water and albumin suspension model. A sketch depicting all components of rf electric field induced loss is shown in Fig. 5.

The effective conductivity vs. size dependence includes all three components listed in Eq. (3). Dielectric loss of silica itself can be neglected, and as a result we should consider only two rf loss components related to NPs to be present, the first one due to the double-layer and the second to albumin adsorption. Size dependence of these two components is different. Below $\sim 1.5\%$ of albumin the double layer related

FIG. 4. Surface plot of SAR (which is proportional to effective conductivity) of silica NPs is shown as a function of silica size and albumin concentration.

FIG. 5. Components of overall rf loss which is converted into heat in the case of SiO₂/albumin water suspension.

component has the main influence on SAR, which results in overall inversely proportional dependence on size.

Here it is assumed that each nanoparticle of silica is surrounded by an inner shell, the electrical double layer created at the particle electrolyte interface due to the redistribution of ions in the presence of the surface charge/potential on the surface of the particles. Such charge appears because of the electrical polarization by the applied alternating electric field and consists of the ions and/or polar water molecules. When albumin is added, we assume that outside of the shell there is another layer consisting of adsorbed albumins. Silica NP in this model is enclosed by the two shells and is suspended in the water solution host with free albumin molecules.

The methodology and results shown in this paper should help to better understand the effects of rf fields not only on protein “corona” formation but also on NP surface-bound molecules or other complex structures. Observed rf heating does not come from silica particles, what makes the dielectric NPs case similar to that of gold NPs,²⁷ but from rf losses in the double layer interface and from adsorbed proteins on the NP surface. Rf field induced polarization of NP enhances rf loss in the interface near the particle surface.

In order to further investigate this issue, a combination of local temperature measurements²⁸ with the systematic rf characterization of complex dielectric permittivity, local heat capacity, and conductivity for NP with different complex bio-NP architectures and surrounding media is needed.

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