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Specific heating power of fatty acid and phospholipid stabilized magnetic fluids in an alternating magnetic field

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Abstract
Magnetic fluids (MFs) with a similar narrow size distribution of the iron oxide core were stabilized with lauric acid (MF 1), oleate (MF 2) or, after dialysis in the presence of liposomes, with phospholipid molecules (MF 3 and MF 4, respectively). The hydrodynamic sizes of the MF 1 and MF 3 were half those found for MF 2 and MF 4. The MFs were exposed to inductive heating in an alternating magnetic field at a frequency of 200 kHz and a maximum magnetic field strength of 3.8 kA m^{-1}. Specific absorption rates (SAR) of 294 ± 42 (MF 1), 214 ± 16 (MF 2), 297 ± 13 (MF 3) and 213 ± 6 W g^{-1} Fe (MF 4) were obtained. The data for MF 2 and MF 4 were identical to those found for the commercially available ferucarbotran. The biomedical relevance of the phospholipid-coated MFs is briefly discussed.

1. Introduction

Magnetic nanoparticles possess an increasing relevance for tumour hyperthermia (Wust *et al* 2006). For successful *in vivo* applications, however, the structures should have a diameter that does not exceed 50 nm and demonstrate superparamagnetic behaviour to avoid particle clustering. When brought into an alternating (AC) magnetic field, heat generation occurs as a result of reorientation of the particle's magnetic moment (Néel relaxation) and rotational Brownian motion of the particle as a whole in the carrier liquid. The efficacy of heat dissipation is expressed by the specific heat absorption rate (SAR). At present, most of the magnetic fluids (MFs) applied for hyperthermia contain subdomain iron oxide particles wrapped with a stabilizing coat made of (modified) dextran (Jordan *et al* 1993, Hergt *et al* 2004a, 2004b). However, these polymers are rather weakly bound (Mornet *et al* 2005, Groman and Josephson 1993) and, moreover, may elicit allergic or hypersensitivity reactions after parenteral administration (Runge 2000). Within this context, it remains surprising that magnetoliposomes (MLs), i.e. nanometre-sized Fe_3O_4 cores captured in a phospholipid bilayer, have only been used sporadically in hyperthermia applications (Lacava *et al* 2004, Shinkai *et al* 1994) in spite of the fact that phospholipids are highly biocompatible molecules (Lacava *et al* 2004, Dumitrascu *et al* 2001), which very strongly bind on iron oxide surfaces by chemisorption forces (De Cuyper and Joniau 1988). In the present work we further elaborate on this issue. Four MF types, with identical iron oxide core size but different coating material and hydrodynamic radius (r_h) were synthesized and their SAR values were measured. The SAR of ferucarbotran, an approved magnetic resonance imaging (MRI) contrast agent, was measured and taken as a reference.

[4] Marcel De Cuyper and Michael Hodenius contributed equally to this work.

2. Experimental details

2.1. Materials

Dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) were obtained from Avanti Polar

Lipids Inc. (Birmingham, AL, USA). Ferucarbotran (carboxydextran stabilized $Fe_3O_4/\gamma Fe_2O_3$ particles) was obtained from Schering (Berlin, Germany). All other chemicals were from Sigma-Aldrich (Steinheim, Germany).

2.2. Methods

2.2.1. Synthesis of aqueous magnetic fluids. The magnetic fluids stabilized with laurate (MF 1) and oleate (MF 2) were prepared essentially as described by Khalafalla and Reimers (1980). In a typical synthesis 4.2 ml of 25% NH_4OH solution was slowly added to 8.25 ml of a solution of 2 g $FeCl_3{\cdot}6H_2O$ (7.5 mmol) and 1 g $FeCl_2{\cdot}4H_2O$ (5.0 mmol) in water under vigorous stirring at 25 °C. The iron oxide thus formed was a black precipitate with a strong response to a toric permanent magnet (NdBFe). To remove residual chloride ions, the iron oxide slurry was then washed with a 1.25% ammonia solution and subsequently peptized with 0.375 g lauric acid (1.9 mmol; MF 1) and 0.75 g sodium oleate (2.5 mmol; MF 2). In the case of MF 1 a colloidal stable 'fluid' was obtained. The oleate treated mixture (MF 2) appeared as a viscous suspension and required a twofold dilution with water, followed by ultrasonic disintegration of particle clusters with an UP 200S probe-tip sonicator (Hielscher, Teltow, Germany). The suspensions of both MF types produced were centrifuged at 4200 g to remove a small number of larger particle clusters (<5% with respect to iron content). Phospholipid stabilized magnetic fluids MF 3 and MF 4 (derived from MF 1 and MF 2, respectively—see below) were prepared following a procedure described earlier (De Cuyper *et al* 2003, Hodenius *et al* 2002). In short, small unilamellar phospholipid vesicles, composed of a mixture of DMPC and DMPG in a molar ratio of 9/1 and suspended in 5 mM *N*-Tris[hydroxymethyl] methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.0) were mixed with an aliquot of MF 1 or MF 2, at a phospholipid/iron weight ratio of 6.9. The mixtures were then dialyzed (Spectra/Por membrane, molecular weight cut-off 12 000–14 000 Da) against TES buffer for 96 h at 37 °C. During this step, the fatty acids were (partly) removed and replaced by phospholipids. In case we started with laurate coated nanocolloids (MF 1), the final phospholipid to Fe ratio (mmol g^{-1}) equalled 0.82; with the oleate coated structures (MF 2) this ratio was 0.33.

2.2.2. Iron and phospholipid content. The iron concentrations of the MFs were determined by complexing Fe^{3+} with Tiron according to Yoe and Jones (1944). The following Fe contents for the various MF stock solutions were found: 115±6 (MF 1), 40 ± 2 (MF 2), 1.7 ± 0.09 (MF 3) and 2.7 ± 0.14 mg Fe/ml (MF 4). Phospholipid contents were measured according to the method of Vaskovsky *et al* (1975) and equalled 1.4 ± 0.07 (MF 3) and 0.9 ± 0.04 μmol phospholipid/ml (MF 4).

2.2.3. In vitro inductive heating of magnetic fluids. The SAR values (W g^{-1} Fe) were determined for all magnetic fluids at various Fe concentrations (see section 3.2) by the method of Jordan *et al* (1993). The experimental set-up consisted of a test tube with 2 ml of MF placed in the centre of a copper coil (external ⌀ 3 cm, eight turns and 7 mm distance between each turn), which was connected to an induction heating generator TIG 5/300 (Hüttinger GmbH, Freiburg, Germany). The generator was operated at maximum output: power 5.2 kW; current 26.5 A and frequency (f) 200 kHz. In these conditions a maximum magnetic field amplitude at the coil centre ($H(0)$) of about 3.8 kA m^{-1} was calculated using the following equation (Jordan *et al* 1993):

$$H(0) = \left(\sum_{n=1}^{N} 2 \frac{R^2}{\left(R^2 + n^2 a^2\right)^{\frac{3}{2}}} + \frac{1}{R} \right) \frac{I}{2} \qquad (1)$$

where R is the coil radius, n the number of turns, a the turn-to-turn distance and I is the current through the coil. The SAR values were determined by:

$$\mathrm{SAR} = c \frac{\mathrm{d}T}{\mathrm{d}t} \frac{1}{[\mathrm{Fe}]} \qquad (2)$$

where c = specific heat capacity of the sample, which can be approximated by the value of water (4.187 J g^{-1} K^{-1}), $\mathrm{d}T/\mathrm{d}t$ = the temperature increment expressed in °C s^{-1} and which is derived from linear regression of the initial data points, and [Fe] = the iron concentration expressed in g ml^{-1}.

2.2.4. Determination of iron oxide core size and hydrodynamic particle radius r_h by dynamic light scattering (DLS). Size analysis of the Fe_3O_4 cores was done by transmission electron microscopy (Zeiss EM10C). The diameters of about 500 individual crystals were measured. From the size distribution data, the average diameter was deduced (De Cuyper *et al* 2003). The r_h of the magnetic particles was determined before and after the heating process by DLS measurements. A 3D cross-correlated light scattering device was used (Schätzel 1991, Overbeek and Sinn 1999). The set-up consists of an LS Instruments apparatus with two avalanche photodiodes (Perkin Elmer, Type SPCM-AQR-13-FC) and an ALV 5000 correlator. A laser diode module (KOHERAS GmbH, wavelength = 681.3 nm, power = 16 mW, Type LGTC 685-35) was used as light source. Temperature was kept constant at 25 °C with an external heating bath and controlled with a Pt-100 temperature sensor. Further details of the set-up are described in the work of Urban and Schurtenberger (1998).

3. Results

3.1. Fe_3O_4 core size and hydrodynamic radii of the MFs

The magnetic crystals in the MFs we synthesized had an arithmetic mean value of the core diameter of 9.4 ± 2.8 nm (±SD). The distribution of the core diameter fulfilled a log–normal function distribution, since the Gaussian fit of the distribution of the core diameter's logarithm was a normal function distribution:

$$y = y_0 + \frac{A}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2} \right) \qquad (3)$$

where y = percentage by number, y_0 = off-set (−0.4 ± 0.7%) (±error), A = Area of the Gauss curve (5.3 ± 0.5%) (±error),

Table 1. Core size, hydrodynamic radius r_h and SAR of MF 1–MF 4 and ferucarbotran.

MF No	Stabilizing agent	Core diameter (nm)	Hydrodynamic radius r_h[a] (nm)	$\overline{SAR}$ (W g^{-1} Fe)
1	Lauric acid	9.4 ± 2.8[b]	51 ± 3	294 ± 42
2	Oleate		91 ± 3	214 ± 16
3	Phospholipid		46 ± 3	297 ± 13
4	Phospholipid		98 ± 3	213 ± 6
Ferucarbotran	Carboxydextran	4.2[c]	31[c]	215 ± 23

[a] Measured at a scattering angle of 90°.
[b] The same core size as measured for the four MF types.
[c] From Reimer and Balzer (2003).

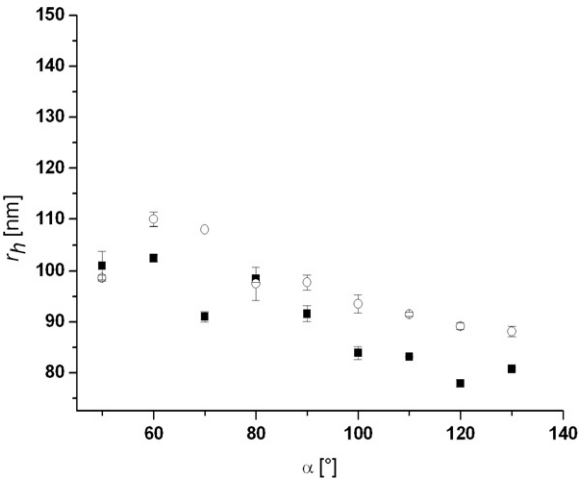

Figure 1. Comparison of the r_h measured at scattering angles between 50° and 130° of oleate stabilized particles before (MF 2—closed symbols) and after exchanging the fatty acids for phospholipids (MF 4—open symbols). The correlation functions of each DLS measurement were analysed with cumulative analysis of second and third order. Both results are averaged to obtain each individual r_h.

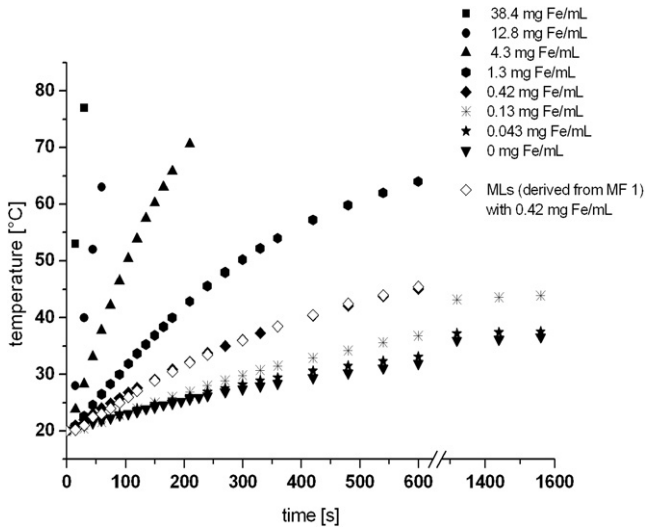

Figure 2. Temperature rise of laurate stabilized Fe_3O_4 (MF 1) with varying iron concentrations (closed symbols), demineralized water (closed symbols) and MLs, derived from MF 1 (open diamonds) in AC magnetic field.

μ = the mean value of the core diameter's logarithm (0.917 ± 0.006) (±error) and σ = standard deviation (0.14 ± 0.01) (±error). The values of the key parameters μ and σ correspond to a mean value of the core diameter of 8.3 ± 2.7 nm (±SD). The r_h deduced from DLS at a scattering angle (α) of 90° was about 50 nm for MF 1 and MF 3 and 98 nm for MF 2 and MF 4 (table 1). Angle dependent r_h measurements at $50° \leqslant \alpha \leqslant 130°$ were conducted to check for polydispersity and the presence of large aggregates (see figure 1 for the oleate coated MF and the MLs derived thereof). Replacing the fatty acid coat by phospholipids has only a minor influence on the r_h of the MF particles. The polydispersity of MF 2 and MF 4 was approximately 15% and no significant aggregation was observed.

3.2. Temperature rise and SAR of the magnetic fluids in an AC magnetic field

To determine the inductive heating characteristics of the MFs, samples at different iron concentrations were subjected to an AC magnetic field as described in the methods. As an example, the profiles of temperature increase of laurate stabilized magnetic particles (MF 1) at different iron concentrations are shown in figure 2 (closed symbols). At iron concentrations $\geqslant$0.42 mg Fe ml^{-1}, a significantly steeper slope of the heating curves, compared to that of a sample with demineralized water containing no magnetic particles (figure 2), was observed. Substitution of the laurate ions (MF 1) for phospholipids (MF 3) did not influence the inductive heating trajectory (compare the values at an iron concentration of 0.42 mg ml^{-1} for the laurate stabilized particles and for the derived MLs in figure 2). Roughly speaking, similar observations (not shown) were made upon substitution of the oleate acid coating for phospholipids.

To determine the SAR values for the MFs, dT/dt was obtained by linear regression of the data points in the initial heating phase. The dT/dt values were then corrected for heating increments of a control sample without magnetic particles, and SAR values were calculated thereof by applying equation (2). To gather reliable dT/dt slopes, $dT/dt < 0.05$ K s^{-1} (at [Fe] < 1.3 mg Fe/ml) or >0.75 K s^{-1} (at [Fe] > 13 mg Fe/ml) were omitted. For each ferrofluid, mean values $\overline{SAR}$ were then calculated by averaging the individual SAR values. The results in table 1 show that changing the fatty acid coating by phospholipids did not significantly alter

the $\overline{\text{SAR}}$ values. For instance MF 1 (laurate coating) and MF 3 (phospholipid coating) gave similar high values of 294 ± 42 W g^{-1} Fe and 297 ± 13 W g^{-1} Fe. Somewhat lower values (about 215 W g^{-1} Fe, see table 1), were obtained for oleate (MF 2) and the corresponding phospholipid-coated MF 4. The $\overline{\text{SAR}}$ value of ferucarbotran was 215 ± 23 W g^{-1} Fe.

3.3. Colloidal stability of the magnetic fluids in an AC magnetic field

To check the colloidal stability during the heating process, r_h values were determined by angle dependent DLS. MF samples with an iron concentration of 1.3 mg Fe/ml were exposed to AC magnetic heating until a temperature of 60 °C was achieved. No change of r_h occurred after heating of MF 4; the r_h value measured at 90° remained 98 nm. Identical behaviour was observed for MF preparation No 3. With this preparation the r_h measured at 90° remained about 45 ± 3 nm.

4. Discussion

The size and size distribution of magnetic iron oxide cores tremendously influence the heat dissipation generated in an AC magnetic field (Wang *et al* 2005, Mornet *et al* 2004). In earlier work (results not published), too, we found that citrate and tartrate-stabilized MFs with a mean iron oxide core diameter of only 2–3 nm exhibit SAR values which are about 10 times lower than those observed with the present MFs. To avoid the problem of variations in core sizes, we purposely worked with one batch of iron cores, which was divided into two parts after which the particles were enveloped with either laurate or oleate anions.

In the case of the laurate-stabilized particles, Khalafalla and Reimers (1980) claimed that a water stable dilution-insensitive MF is formed. Our observations were in agreement with this statement. The observed water compatibility resulted from the close packing of the fatty acids, adsorbed on the iron oxide surface via their carboxylic group, restricting the water contact to a limited number of $–CH_2–/–CH_3$ groups exposed towards the external medium. In contrast, the oleate coated particles are known to be organic solvent-adapted MFs and, thus, in an aqueous medium they tended to aggregate, thereby reducing the surface free energy. A consequence of these different MF features is that phospholipid exchange is more difficult with the oleate in the larger particle clusters than with the laurate surfactant in the smaller nanocolloids. This behaviour is supported by the larger r_h and the lower phospholipid-to-Fe ratio observed for the oleate covered structures as compared to the laurate coated ones. Furthermore, we found that—at a fixed core size—the lower the hydrodynamic particle size was, the higher the SAR values were. This is reasonable since, besides the Néel mechanism, which mainly relies on the core size and iron oxide composition, Brownian motion also contributes to the SAR value. For reasons of the different experimental parameters (core size and composition, AC magnetic field amplitude and frequency, etc) it is difficult to compare in an unambiguous way the heat production capacity of the MFs examined in the present work with those reported in the literature for other MF types. Since common iron oxide-based MRI contrast agents are frequently applied in hyperthermia experiments, we included ferucarbotran (Resovist) as a reference. The observed SAR value, however, was distinctly lower than those obtained for the laurate-based MF and the ML derived thereof, and thus the latter nanocolloids may be successfully used in future hyperthermia applications. Within this context, it is worth mentioning that very recently Hergt *et al* (2006) found superior heating properties with bacterial magnetosomes having a mean diameter of the magnetite crystals of about 35 nm, but concerns about the biocompatibility of the bacterial protein coating were raised. In contrast, the coating of ML types we have developed solely consists of phospholipids and, consequently, the colloids are completely biocompatible (Lacava *et al* 2004). Considering the superior heating properties of the above-mentioned magnetosomes, it should be possible to improve the SAR values of our MLs just by using 35 nm diameter magnetite crystals and covering them with phospholipids. However, the development of an adequate protocol to produce such large particles, which still demonstrate superparamagnetic behaviour, is still a real challenge and will require fine-tuned experimental conditions during iron oxide precipitation (e.g. type and concentration of iron salts, temperature) (Massart 1981). Apart from these considerations and in contrast to common dextran coated MFs, the phospholipid bilayer coat of MLs allows the incorporation of lipophilic drugs (Cocquyt *et al*). As a result, the ML particulates we presented in this work show up as unique bionanocolloids which may combine therapeutic (drug delivery, hyperthermia) as well as diagnostic features (MRI—Bulte *et al* 1999).

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