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The effect of field parameters, nanoparticle properties and immobilization on the specific heating power in magnetic particle hyperthermia

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Abstract

Magnetic nanoparticles (MNP) are intended for utilization in cancer therapy as they produce damaging heat in the presence of AC magnetic fields. In order to reach the required temperature with minimum particle concentration in tissue the specific heating power (SHP) of MNP should be as high as possible. The aim was to clarify the influence of magnetic field parameters and nanoparticle properties on the SHP. As usual ferrofluids exhibit broad size distributions, a magnetic fractionation of a commercial iron oxide nanoparticle suspension was performed in order to obtain particles with varying properties. The fractions obtained were characterized by means of atomic force microscopy and magnetometry, among other techniques. Frequency spectra of the susceptibility show clear peaks at low frequencies related to the Brown relaxation. This effect vanishes after particle immobilization. Theoretical spectra considering experimentally determined size distributions are in agreement with experimental data. The SHP derived from AC susceptometry is in accordance with that directly determined by calorimetry. A maximum SHP of 160 W g^{-1} (400 kHz, 8 kA m^{-1}) was detected for the largest particles, showing a behaviour in the transitional regime between superparamagnetic and stable ferromagnetic.

1. Introduction

The incidence of cancer diseases in industrial countries is growing with increased life expectancy, yielding incalculable economic risk. Standard treatments are surgery, irradiation and chemotherapy, or combinations of them, dependent on the patient and the specific tumour. Among a multitude of alternative therapy concepts, hyperthermia is proven to have synergistic

effects with standard treatments, but even hyperthermia treatment alone may damage tumour tissue.

Besides whole body hyperthermia (e.g. by means of a heat bath) there are different ways of achieving local intracorporeal heat generation, e.g. by means of microwave radiation, by capacitive or inductive coupling of radiofrequency fields, via implanted electrodes, by ultrasound, or by lasers. As an alternative therapy method, in magnetic particle hyperthermia (MPH) one deposits magnetic media in tumour tissue with subsequent heating by means of an external alternating magnetic field. Former approaches dealt with electroconductive or ferromagnetic implants; recent studies are applying magnetic nanoparticles (MNP) as their heat generation potential appears beneficial and they provide the opportunity of direct tumour targeting through blood circulation. Besides a localized heat generation, the application of MNP offers the possibility of a self-limitation of the temperature enhancement by using a magnetic material with a suitable Curie temperature (e.g. [1]). However, for biomedical applications the vast number of known magnetic materials is strongly restricted by the demand of biocompatibility (e.g. non-toxicity, sufficient chemical stability in the bio-environment, appropriate circulation time in blood and finally harmless biodegradability). Though there are a few suggestions in the literature to use metallic iron particles, several spinel ferrites as well as special magnetic alloys for MPH, the majority of investigations concentrate on magnetic iron oxides Fe_3O_4 (magnetite) and $\gamma\text{-Fe}_2\text{O}_3$ (maghemite) which have been proven to be well tolerated by the human body.

Concerning the therapy temperature one differentiates in the literature *hyperthermia* which aims for a weak temperature enhancement to about 42–45 °C and the more rigorous *thermoablation* above about 50 °C. While in the former case assisting toxic agents (chemotherapeutic or radiotherapeutic agents) are needed the latter is a stand-alone therapy modality. In both cases, the amount of nanoparticles to be applied should be as small as possible. Hence, in order to reach the required temperature with minimum particle concentration in tissue the specific heating power (SHP) of MNP in magnetic AC fields should be as high as feasible. The aim of the present paper is to gain deeper insight into the heat generation mechanisms in MNP and to clarify how the particle properties and the magnetic field parameters may be optimized for future application in a reliable cancer therapy.

2. Theory

2.1. Heat generation mechanisms

The interaction of electromagnetic fields with matter generally is accompanied by the generation of heat. The mechanism accounting depends on the characteristics of the electromagnetic field, mainly frequency and amplitude, as well as the material properties. In the case of magnetic nanoparticles inductive heating does not arise as the material normally exhibits poor electroconductivity and is too small to incorporate a closed eddy current loop. However, the electrical conductivity of biological tissue is high enough that the alternating magnetic field may cause—in addition to the therapeutically wanted selective heating of magnetic particles—also a non-selective heating of both cancerous as well as healthy tissue due to the generation of eddy currents. The latter depend according to the induction law on the square of (HfD) where H is field amplitude, f frequency and D the induced current loop diameter. Accordingly, one expects depending on the diameter of the induced current loops in the patients body a limit for the product of frequency and field amplitude. This was found experimentally by Brezovich to amount to $4.85 \times 10^8 \text{ A m}^{-1} \text{ s}^{-1}$ [2]. Consequently, this limit has to be taken into account for an optimization of the specific heating power of MNP. Hence, ferromagnetic resonance is of

minor relevance as the required frequencies are in the GHz range, where negative side effects on healthy tissue emerge.

Responsible for generation of heat in MNP being useful for MPH are either hysteretic or relaxational losses. In the range of particle diameters above about 100 nm magnetic multi-domain particles react to external magnetic fields by Bloch wall displacement thus spreading a hysteresis loop in the $M-H$ diagram. The area enclosed is a measure of energy dissipated during one re-magnetization cycle and depends strongly on the field amplitude as well as the magnetic prehistory.

With decreasing particle size a transition to single domain particles occurs. In this case the equilibrium magnetization direction is determined by anisotropy contributions due to the crystal structure, particle shape and surface. In the simplest case we deal with uniaxial anisotropy where only two anti-parallel magnetization directions are allowed. The shape of hysteresis loops relies on the orientation of the particle with respect to the external field direction as elucidated in detail by the well known theoretical treatment of Stoner and Wohlfarth [3]. For aligned particle axes being parallel to the external field a rectangular loop results and the hysteresis loss density per cycle is given by the anisotropy energy density $2 K$. A statistical particle orientation results in a curved hysteresis loop resembling that of multi-domain particles and hysteresis loss is reduced in comparison to the aligned case by a factor $1/4$. Besides this simple model of uniform magnetization reversal there are different theoretical models dealing with more complicated magnetization distributions (e.g. curling, buckling, fanning; see e.g. [4]). The situation is even more complex for cubic anisotropy. For the case of magnetite Butler and Banerjee [5] derived theoretically an upper limit of about 80 nm for the single domain size range which increases considerably with tetragonal particle elongation. Micromagnetic calculations of Fabian *et al* [6] show the existence of more sophisticated magnetization patterns like the so-called flower and vortex states. Experimental results are available only for the diameter range above about 50 nm, where e.g. Heider *et al* [7] found a decrease of coercivity and remanence with increasing particle size which implies a decrease of hysteresis losses in the multi-domain size range. Comparing different types of magnetic iron oxide particles with respect to their suitability for hyperthermia it was shown [8] that hysteresis losses of different types of magnetite particles may differ by orders of magnitude in the range of field amplitudes below 10 kA m^{-1} due to differences of particle size, shape and microstructure. Many magnetite and maghemite powders in a broad size range of about 10 nm up to 100 nm were investigated by Dutz *et al* [9] but in no case was the typical SW behaviour found. Instead, in any case for low field amplitudes generally a third order power law for the amplitude dependence of hysteresis losses is observed which is known as the so-called Rayleigh law for multi-domain particles (e.g. [10]).

The energy barrier KV between different magnetization states can be overcome by either an external magnetic field or by the thermal energy $k_B T$. As the magnitude of the energy barrier depends strongly on particle volume V , decreasing particle size yields superparamagnetic behaviour, i.e. magnetization may relax during observation. As a consequence, in the regime of the transition to superparamagnetic behaviour the loss energy per cycle derived from hysteresis loops becomes smaller than calorimetrically measured loss data if the characteristic time of measurement τ_m is larger than the relaxation time of the particle system. The latter is given for the so-called Néel relaxation by the ratio of the anisotropy energy KV to the thermal energy $k_B T$ [11]:

$$\tau_N = \tau_0 \exp [KV/(k_B T)], \quad (\tau_0 \sim 10^{-9} \text{ s}). \quad (1)$$

A critical particle volume V_c may be defined by $\tau_N(V_c) = \tau_m$. For a measuring frequency of 300 kHz and a magnetic anisotropy energy density of 10^4 J m^{-3} (for magnetite particles of

ellipsoidal shape with an aspect ratio of 1.4) the critical diameter is about 20 nm. If particles are freely movable within a suspension medium of viscosity η , a second relaxation mechanism occurs due to reorientation of the whole particle. The mechanism is also referred to as Brown relaxation with the characteristic relaxation time

$$\tau_B = \frac{4\pi\eta r_h^3}{k_B T} \quad (2)$$

(r_h is the hydrodynamic radius which due to e.g. particle coating may be essentially larger than the radius of the magnetic particle core). The Brown mechanism causes generation of heat as a result of viscous friction between rotating particle and surrounding medium. In the general case, the faster of the relaxation mechanisms is dominant; the effective relaxation time is given by

$$\tau_{\text{eff}} = \tau_N \tau_B / (\tau_N + \tau_B). \quad (3)$$

Experimentally, the frequency dependence of the relaxation of the particle system may be well investigated by measuring spectra of the complex susceptibility. The imaginary part $\chi''(f)$ which is related to magnetic losses may be described by the well known expression (see e.g. [12])

$$\chi''(f) = \chi_0 \phi / (1 + \phi^2), \quad \phi = f \tau_{N,B}, \quad \chi_0 = \mu_0 M_S^2 V / (k_B T) \quad (4)$$

with M_S being the saturation magnetization. Accordingly, spectra show peaks related to Néel or Brown relaxation for $\phi = 1$. Within the validity range of linear response theory the loss power density P is given by (e.g. [13])

$$P(f, H) = \mu_0 \pi \chi''(f) H^2 f. \quad (5)$$

According to these equations at low frequencies ($\phi \ll 1$), i.e. in the superparamagnetic regime, losses increase with the square of frequency while for $\phi \gg 1$ losses approach a frequency independent saturation value $P = \mu_0 \pi H^2 (\chi_0 / \tau)$. The strong size dependence of the relaxation times leads to a very sharp maximum of the loss power density depending on the particle size [8, 14]. Accordingly, a remarkable output of heating power occurs only for particle systems with narrow size (and anisotropy) distribution with the mean diameter being well adjusted in relation to the treatment frequency. The effect of size distribution on loss power density was elucidated theoretically by Rosensweig [15] and will be subject of the present experimental investigations.

2.2. Choice of alternating field frequency and amplitude

In addition to the biological limitation discussed above, also technical–economic reasons determine the choice of the amplitude–frequency pairing for therapy. A simultaneous increase of frequency and amplitude in practice is seriously limited and accordingly a decision has to be made as to which of the factors in the limiting product Hf is favoured. Which combination of AC field amplitude and frequency favourably has to be chosen depends strongly on the type of particles provided for therapy. For particles with mean size in the superparamagnetic regime losses increase non-linearly with frequency according to (5) taking into account the frequency dependence of the imaginary part of the susceptibility (4). In contrast, the dependence of hysteresis losses on frequency is linear for ferromagnetic particles.

The dependence of magnetic losses on the field amplitude obeys a square law for superparamagnetic particles (5) which, however, is theoretically profound only for small field amplitudes. In comparison, for larger ferromagnetic particles in the Rayleigh regime a third order power law is expected. As a consequence, for instance, for particles with hysteresis

losses within the validity range of the Rayleigh regime one should favour the field amplitude against frequency in the limiting product Hf (e.g. when reducing f by a factor one half and simultaneously doubling H one may gain a factor four in specific heating power). In contrast, for superparamagnetic particles the frequency has to be overweighted against the field amplitude. It is one goal of the present investigations to clarify experimentally the relation of specific heating power with particle size distribution on one hand and alternating field parameters on the other in the framework of the above sketched loss mechanisms.

3. Materials and methods

3.1. Magnetic nanoparticles and fractionation

3.1.1. Magnetic nanoparticles. For medical applications, only material with a well characterized fate within the human body can be accepted. We therefore opt for nanoparticles consisting of pure iron oxide as this is found in high amounts within the human body, mainly bound in haemoglobin. The iron homeostasis (cf e.g. [16]) provides for the relatively high tolerance compared to other heavy metals.

The particles we used for our investigation (DDM 128N 1020, Meito Sangyo, Japan) consist of magnetic cores with diameters between 5 and 20 nm. The single cores are partly aggregated and surrounded by a carboxydextran (MW 2600 Da) shell with a thickness of about 4 nm, dependent on the swelling state [17]. The coating is due to reduction of magnetic dipole–dipole interactions, further aggregation and sedimentation. These particles are used as raw material for Resovist[®], a contrast medium for MRI of the liver [18]. We therefore act on the assumption that these particles are non-toxic at rather high doses of 3 mmol Fe kg⁻¹ (cf e.g. [19]).

3.1.2. Fractionation. On account of the particle size distribution and the complex relation of heating power to particle size, a magnetic fractionation was performed. The principle is derived from magnetic cell separation (an early review is given in [20]) where magnetically labelled cells are attracted in a gradient field. The attractive force depends on both the gradient and the particle magnetic moment and counteracts the hydrodynamic force.

The basic procedure of magnetic fractionation as described in [21] had to be modified due to increased demand of material for calorimetry and susceptibility measurements. The scaled-up mode proceeds as follows: a magnetic separation column (MACS[®] XS, Miltenyi Biotech, Germany) is placed between the pole shoes of an electromagnet (Bruker B-E 10v, Germany) and rinsed with distilled water. Then, the column bottom is sealed using a two-way plug and a defined current is switched on. Afterwards, 10 ml of nanoparticle suspension are filled in the column and the plug is opened. Particles are rinsed with additional water drop by drop (1 s⁻¹) until the eluate becomes clear. The eluate is collected. Now the current is switched off and retained particles can be eluted with water in the reverse direction under pressure using a syringe connected to the plug. The obtained fraction is named '0 A' as the particles are eluted at that current. The prior eluate is then refilled in the column, after the current is increased compared to the first run. Washing steps are repeated in the manner described. The fraction obtained after switching off the field is indicated by the primary current as the particles were eluted under that condition during the first run. The last steps are repeated ad lib. The final washing solution containing particles that are not held at any current are indicated by the highest applied current, as the particles are eluted under that condition.

The successive increase of current is due to observed aggregation if high concentrated particles are attracted to high gradients. The circuitous notation is for conformity with prior

fractionations (e.g. [22]), where the current was stepwise reduced. That way altered, the method enables 40 times increased amounts of ferrofluid to be fractionated.

In our case, the measured currents were 0.25, 1 and 6 A. Accordingly, magnetic fields of 14.5, 52 and 320 kA m⁻¹ were generated. The corresponding fractions were named as '0 A', '0.25 A' and '1 A'; the last fraction obtained without being held in the highest magnetic field was named as '6 A'.

3.2. Sample preparation

3.2.1. Ultrafiltration. After fractionation, particles are available very diluted. However, the following characterizations demand highly concentrated suspensions. Therefore, particles were re-concentrated using an ultrafiltration unit (Vivacell 250, Vivascience, Germany) provided with polyethersulfone membrane (PES, cut-off 5000 Da) oscillating with 300 min⁻¹ at 4 bar overpressure. Stored at 4 °C, these concentrated fractions are stable for at least two years.

3.2.2. Particle immobilization. For elimination of Brown relaxation mechanism particles were immobilized in an appropriate container by means of freeze-drying after addition of 5% (m V⁻¹) mannitol to the solution. Therefore, the mixture was frozen at -80 °C and then transferred to the drying chamber of freeze-drying apparatus (Alpha 2-4, Christ, Germany). Water was sublimated at 13 °C and 1 × 10⁻⁵ bar. Alternatively, gelatine was added to the aqueous suspension at elevated temperature allowing for particle immobilization below the gelation temperature of about 30 °C. In some experiments the liquid suspension was simply dried in a thin layer.

3.3. Chemical and structural characterization

3.3.1. Atomic absorption spectrometry. For measurement of iron content portions of obtained fractions were decomposed using hot concentrated nitric acid (Suprapur®, Merck, Germany) until decolorization occurred and then diluted with pure water. The samples were measured by means of flame atomic absorption spectrometry (PU 9200, Pye Unicam, UK) with an air/acetylene flame (acetylene 2.6, Messer Griesheim, Germany) of approximately 2300 °C against the calibration curve. The signal was integrated over 2 s tenfold.

Fractions were adjusted to contain 40 mg γ -Fe₂O₃/ml (according to 0.5 M regarding Fe).

3.3.2. X-ray diffraction X-ray diffraction was applied for phase identification as well as for determination of the mean core size of the nanoparticles. Identification of the two magnetic iron oxide phases magnetite (Fe₃O₄) and maghemite (γ -Fe₂O₃) is complicated by the fact that these materials exhibit very similar diffraction patterns. The similarity of the crystal lattices of both iron oxides—spinel lattices in either case—allows for the existence of solid solutions, the so-called Bertholloid phases. In general, iron oxide ferrofluids may vary considerably between magnetite and maghemite. While the oxidation of magnetite in macroscopic samples at room temperature is kinetically inhibited, one observes significant ageing effects in small particles which show that under access of oxygen (e.g. storing of aqueous fluids under air). MNP transform into the stable phase which is maghemite instead of magnetite as often claimed in the literature. Although there are some isolated reflections in XRD patterns which are typical for either one or the other of these phases they are of minor intensity not allowing for a sufficient signal-to-noise ratio within reasonable time of measurement. Therefore, the (440) reflections of magnetite and maghemite positioned at diffraction angles of 62.515° and 62.925 °C (for Cu K α radiation), respectively, were used to identify the present iron oxide phase. Of course,

the diffraction equipment (in the present case the X'pert-Twin diffractometer, Philips) must allow sufficient angular resolution.

From a line profile analysis the diffraction linewidth B may be determined which is a measure for the mean particle size d . They are correlated by the so-called Scherrer equation, $d = K\lambda/[(B-b)\cos\theta]$, with the wavelength λ , the peak width B , the instrumental broadening b , the Bragg angle θ , and the shape factor $K \approx 0.9$.

3.3.3. Photon correlation spectroscopy. Measurements were performed in water of 298 K using a ZetaSizer 3000HS (Malvern, Germany). The instrument is operating with a 10 mW laser at 633 nm and at a fixed observation angle of 90°.

For analysis, the obtained diffusion constants and polydispersity indices were converted into size distributions using an internal procedure based on the Contin algorithm [23].

3.3.4. Atomic force microscopy. For sample preparation, a piece of mica ($1 \times 2 \text{ cm}^2$) was freshly cleaved, yielding a clean and atomically flat surface. As the surface exhibits negative charge the pieces were incubated for 20 min in 0.003 monomol/l poly(ethylene imine) (PEI, MW 50 kDa, Aldrich) for charge reversal. Subsequently, pieces were soaked three times with pure water for 1 min and then incubated with nanoparticle solutions (1×10^{-4} M regarding Fe, fraction 6 A 1×10^{-5} M) for 5 min. Afterwards, the slices were dried in laminar flow.

Pictures were obtained in tapping mode in air using a Multimode AFM (Digital Instruments, CA, USA). For analysing the particle size distribution height images were evaluated by measuring about 100 particles and approximating the distribution using a log-normal fit function

$$f(d) = \frac{1}{\sqrt{2\pi}\sigma_L d} \exp\left[\frac{-(\ln d - \mu_L)^2}{2\sigma_L^2}\right] \quad (6)$$

where σ_L and μ_L are the parameters of the probability density function $f(d)$. Results are indicated by the expectation according to $\exp(\mu_L + \sigma_L^2/2)$.

3.4. Magnetic analyses

3.4.1. Magnetization measurements (VSM). Quasi-static magnetic measurements were performed with a vibrating sample magnetometer (VSM). According to the Langevin theory the magnetization of superparamagnetic particles close to saturation is described by

$$M = M_S \left(1 - \frac{k_B T}{\mu_0 M_S \text{Bulk} \frac{\pi}{6} d_{\text{core}}^3} \frac{1}{H}\right). \quad (7)$$

Accordingly, saturation magnetization data M_S were extrapolated from magnetization loops measured with high field amplitude (1114 kA m^{-1}) via a $1/H$ plot. Comparing the experimental data with the saturation magnetization of bulk material ($M_{S \text{ Bulk}} = 400 \text{ kA m}^{-1}$ according to [24]) the volume fraction of magnetic material of the ferrofluid samples was determined from the ratio $M_S/M_{S \text{ Bulk}}$.

Measured magnetization data were approximated by a Langevin function and assuming a log-normal size distribution the distribution parameters were determined according to the method described by Chantrell *et al* [25].

DC susceptibility data were obtained from magnetization loops with a field amplitude of 80 kA m^{-1} and with high resolution of data points close to the origin of the $M-H$ diagram. In the same way the small residual coercivity of the ferrofluids was determined.

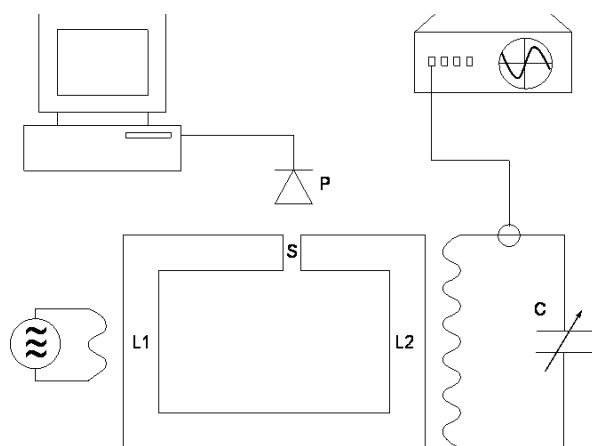


Figure 1. Scheme of the calorimetric device: power supplied by HF generator via coil L1, frequency tuned by adjustment of resonance circuit consisting of variable capacitor C and coil L2, sample S placed between the pole shoes of ferrite core, sample temperature measured by pyrometer P.

3.4.2. AC susceptibility measurements. In addition to characterization of static magnetic properties of MNP for the present high frequency application of magnetic colloids the behaviour in alternating magnetic fields is of interest. A valuable method for investigation of the dynamic response of a magnetic particle system on RF excitation in a wide frequency range of 20 Hz up to 1 MHz is AC susceptometry. The method is based, in principle, on a lock-in balance technique using two identical pick-up coils one of which contains the sample. Both pick-up coils are fixed in an external solenoid that is driven by the oscillating current of a RF generator. From the in-phase and the 90° phase shifted components of the induced differential voltage signal which is measured by means of a lock-in amplifier the real and the imaginary parts of the complex susceptibility are calculated.

3.4.3. Calorimetric measurements. An alternating magnetic field is generated in an air gap ($d = 10$ mm) of a ferrite core (N87, Epos, Germany) using an oscillating circuit; dissipated power is replenished by a 1000 W RF generator (LF10A, RF Power Products, NJ, USA). Adjustment of the oscillating circuit by means of a variable air capacitor (M73, WVS-Technology, Germany) allows continuous variation of frequency in the range of 50–460 kHz; field amplitudes of 6.5 kA m^{-1} over the whole frequency range are achievable (figure 1).

Field strength can be measured using a pick-up coil applying the linear $U_{\text{ind}}-B$ relation. The probe has been calibrated within a Helmholtz coil, where B can be calculated exactly. The dependence is proven to be linear in the operating frequency range. A vessel of volumetric capacity of $300 \mu\text{l}$ is placed in the gap between the two pole shoes. The temperature of the sample is measured continuously by a pyrometer (Inspacto 900, Infrapoint, Germany) in a non-contacting mode. The recorded temperature charts were approximated according to

$$T(t) = \frac{P}{kC} [1 - \exp(-kt)] \quad (8)$$

with T being the sample temperature and k a correction factor accounting for the heat dissipation. The initial temperature rise ($t \rightarrow 0$) equals P/C . If the sample's heat capacity C is known, one obtains values for the sample heating power P .

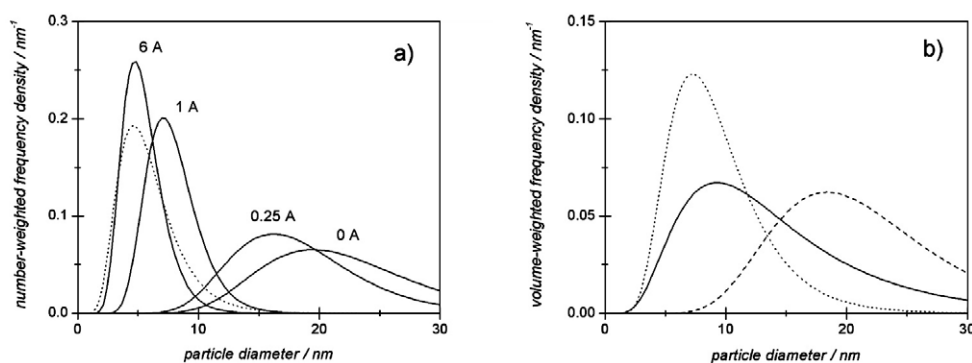


Figure 2. (a) Particle size distribution of fractionated samples from AFM measurements according to a log-normal fit (number weighted), the dotted line representing the original ferrofluid. (b) Comparison of reconstructed volume-weighted size distributions of the original ferrofluid by means of magnetometry (full line), AFM (dotted line) and PCS (dashed line).

3.4.4. Magneto-optical analyses. It has been proven that *static* magnetic fields induce a state of order into a ferrofluid yielding an optical birefringence of the solution [26]. For exploring the ferrofluid behaviour within *alternating* magnetic fields an optical bench has been arranged consisting of a diode laser (635 nm, 0.9 mW), two crossed polarizers, a quarter-wave plate and a photodiode detector (Femto, Germany). The signal is intensified by a current amplifier (DLPCA-100, Femto, Germany) and recorded by an oscilloscope (TDS 2002, Tektronix, Oregon, USA). The $\lambda/4$ plate is adjusted to minimize passing light intensity at zero field. Between the two polarizers the ferrite core of the calorimetric device is arranged, in order that the laser beam passes the air gap. The field direction is oriented at 45° to both polarizers.

4. Results and discussion

4.1. Particle size analyses

Phase identification analysis via x-ray diffraction revealed that the investigated MNP completely consist of maghemite. The particle size distribution parameters of the original ferrofluid and fractionated samples obtained by different methods are compiled in table 1. The deviation of particle size from values from earlier investigations of the same ferrofluid [22], especially for the fraction of the largest particles, is due to the scale-up of the fractionation method (see section 3.1). The expectation values represent the barycentre of the log-normal distribution. The distributions of particle sizes determined by AFM are displayed in figure 2(a).

The charts reveal an effective splitting into differing particle size ranges. The larger the electromagnet's current during separation, i.e. the larger the magnetic gradient, the smaller the particles eluted. The dotted line representing the original unfractionated particles denotes an overestimation of small particles due to faster diffusion to the positively charged surface. Thus, the amount of smallest particles appears unrepresentative for the whole solution. For comparison of particle sizes derived from different methods, the different weighting of large particles had to be taken into account. This was accomplished by conversion into volume-weighted size distributions as displayed in figure 2(b).

The expected order $d_{\text{VSM}} < d_{\text{AFM}} \ll d_{\text{PCS}}$ could not completely be recovered. AFM diameters should exceed those of VSM measurements due to considering the whole core plus dried particle shell, whereas VSM detects only the magnetic core less a potential dead

Table 1. Particle sizes (nm) of magnetically fractionated samples and original ferrofluid. PCS: photon correlation spectroscopy using Contin analysis, distribution weighted by d^6 , mean \pm SD; AFM: atomic force microscopy, distribution number weighted by d^0 , expectation of probability density function according to $\exp(\mu_L + \sigma_L^2/2)$; XRD: mean values obtained from x-ray diffraction linewidth; VSM: particle size reconstructed from magnetization curve according to [25], distribution volume weighted by d^3 , expectation values. (Note: n.d.—not determined.)

Fraction	PCS	AFM	XRD	VSM
Original DDM	56.3 \pm 0.9	6.0	11.0	12.5
6 A	11.2 \pm 1.9	5.5	8.6	7.0
1 A	20.9 \pm 0.7	7.9	n.d.	n.d.
0.25 A	43.8 \pm 0.6	18.4	n.d.	n.d.
0 A	69.0 \pm 1.1	22.3	12.6	17.5

layer. The observed deviation should be due to the above discussed overestimation of small particles. The strongly exceeding particle size of PCS is due to measuring the hydrodynamic particle diameter considering the hydrated shell. In the case of concentration dependent particle agglomeration, which is frequently observed for ferrofluids, this must reflect in both AFM and PCS measurements as they operate in the same concentration range. This is obviously not the case.

The core size by VSM measurement quoted for fraction 0 A may be doubtful as that sample exhibits small coercivity; thus the model of Chantrell [25] overestimates the particle size which may explain the observed difference of the XRD and VSM sizes of that sample.

4.2. Magnetic properties

4.2.1. Quasi-static magnetization. Though measured magnetization data may be well approximated by the Langevin function, high resolution measurements at low field strength reveal that there is a small coercivity, at least for the 0 A sample. Experimental coercivity data are extremely small in comparison to the anisotropy field of ellipsoidal single domain maghemite particles. With increasing mean diameter differences of coercivity between fluid samples and immobilized ones are observed as reported previously [22]. The coercivity data show the general tendency to increase with increasing mean size of magnetic cores which is qualitatively reasonable. However, it is difficult to estimate the small fraction of particles showing hysteresis losses and quantitative conclusions considering different contributions to the coercivity of the particle ensemble are not drawn.

The concentration data derived from magnetization measurements are in acceptable agreement with data derived from iron analysis by atomic absorption spectrometry.

4.2.2. Frequency dependence of susceptibility. Frequency spectra of the complex susceptibility were determined as described above for the fractionated ferrofluids as well as for fractions immobilized in mannitol or gel. While frequency spectra of common ferrofluids due to a broad size distribution are rather flat, in the present case clear peaks are observed which could be shown to be related to Brown relaxation. As an example, figure 3 shows the real (figure 3(a)) and the imaginary part (figure 3(b)) of the specific susceptibility depending on the frequency for fraction 0 A as liquid aqueous suspension (full points) as well as for immobilized particles (open circles). By comparing the experimental results for the fluid suspension with the data for the same particles being immobilized the effect of Brown relaxation became obvious. The low frequency peak of χ'' found for the liquid suspension vanishes after immobilization of the particles. From the position of the Brown peak at 1 kHz one may estimate the hydrodynamic

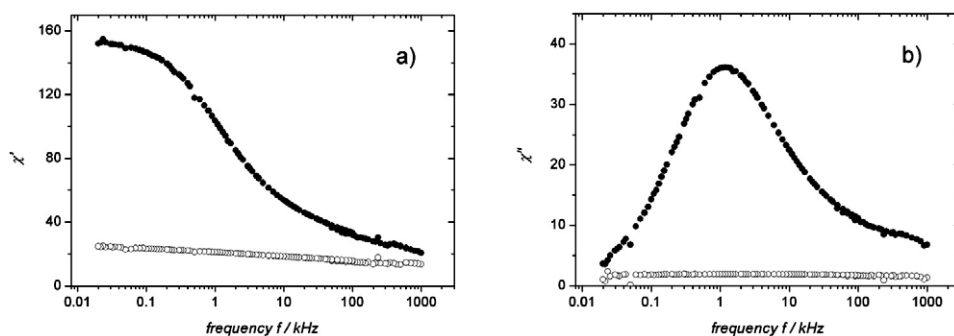


Figure 3. Real (a) and imaginary parts (b) of the specific susceptibility depending on the frequency for fraction 0 A; full points—particles suspended in water; open circles—particles immobilized.

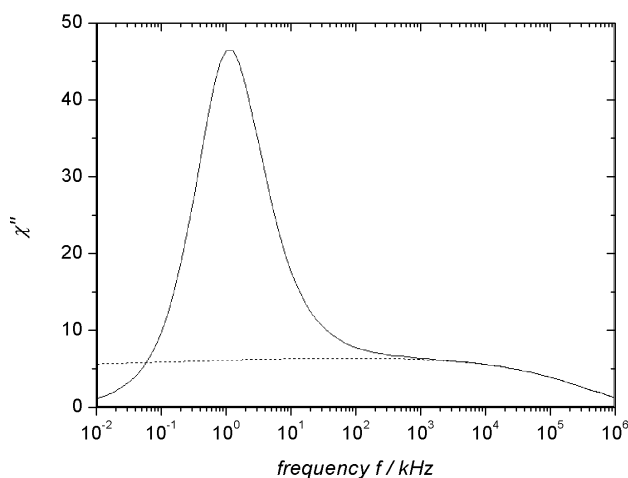


Figure 4. Theoretical spectra of the imaginary part of the complex susceptibility of the 0 A fraction with consideration of the particle size distribution (derived from magnetization measurements): full line—suspended particles; dashed line—particles immobilized.

particle diameter using (2). The resulting value of 73 nm is in good accordance with the value of 69 nm determined by means of PCS (cf table 1).

In order to check in more detail the validity of the relaxation theory sketched in section 2, theoretical spectra were derived by introducing the experimentally determined size distribution after Chantrell into (1)–(4). For details of the method see e.g. [27]. As an example, theoretical spectra of the imaginary part of susceptibility for the 0 A fraction are shown in figure 4 for the liquid suspension (full line) in comparison with the immobilized particles (dashed line). In the latter case a very large value of 10^3 Pa s was assumed for the viscosity instead of 10^{-3} Pa s of water used for the liquid suspension. Other parameters entering the model calculations are: magnetization 410 kA m^{-1} and the hydrodynamic diameter following from the experimentally found Brown frequency as well as PCS measurements. As a reasonable value of the anisotropy energy density 15 kJ m^{-3} was chosen.

While experimental spectra are limited to a frequency range below 1 MHz for technical reasons, calculations were done up to 1 GHz in order to cover the large range of the Néel relaxation spectrum. The experimental data are in remarkable accordance with the relaxation

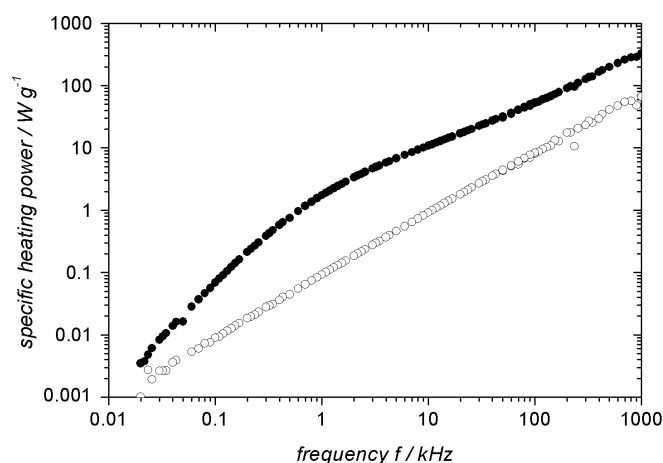


Figure 5. Specific heating power [$\text{W}/\text{g}_{\text{magnetite}}$] for a field amplitude of 8 kA m^{-1} calculated from the χ'' data measured with the 0 A sample displayed in figure 3(b); full points—particles suspended in water; open circles—particles immobilized.

theory. The too large contribution of Néel relaxation in the low frequency range may be caused by the inadequacy of the Néel model in this range.

4.2.3. Specific loss power. The relaxation theory discussed above allows deriving the specific heating power from the imaginary part of susceptibility according to (5). The spectra of specific heating power calculated from the χ'' data measured with the 0 A sample (figure 3(b)) are shown for a field amplitude of 8 kA m^{-1} in figure 5. According to relaxation theory the frequency spectrum of the specific heating power is predicted to be a square law below the Brown frequency and to become linear between the latter and the Néel peak where it reaches saturation. Just that behaviour is shown by the curves of figure 5. The predicted Néel maximum, however, is above the frequency range of practical interest. The vanishing of the Brown relaxation peak after immobilization implies a considerable reduction of specific heating power in hyperthermia, e.g. when particles circulating in blood get sticking on tumour cells or enter into the cell plasma. However, the results of figure 5 show that this detrimental effect may be reduced by choosing a suitable frequency of the exciting magnetic AC field well above the Brown peak.

Since (5) is defined for small values of the magnetic field the validity of the extrapolation to higher fields being done with the data of figure 5 was checked by measuring specific heating power directly by a calorimetric method. Results are compiled for different samples in figure 6(a) for liquid suspensions and figure 6(b) for immobilized particles. Comparison of these data with the extrapolation from AC susceptibility shows a fair accordance for both the aqueous suspensions and the immobilized particles.

In order to get a more detailed check of the validity of the relaxation theory the dependence of SHP on the field amplitude was measured; experimental results are shown in figure 7. The predicted square law is found for the majority of samples investigated with the exception of the large diameter 0 A fraction. Reasonably, this fraction contains a portion of large particles showing hysteresis losses. As established for a great number of different particle types [9], in the so-called single domain size range (25–70 nm predicted theoretically for magnetite, e.g. [5]) a third order power law is found for the dependence of SHP in the field amplitude range shown in figure 7.

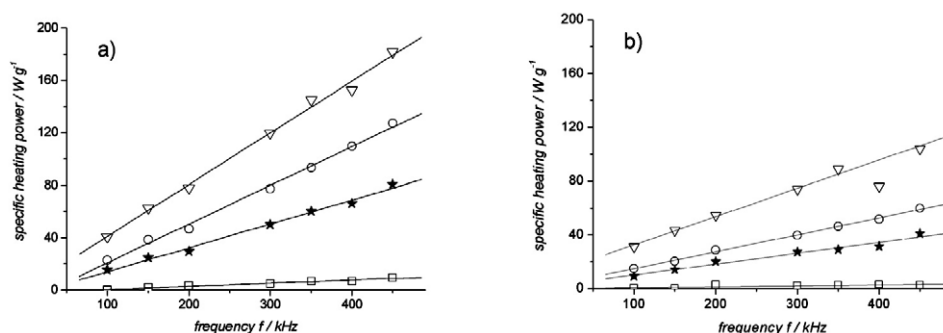


Figure 6. Specific heating power [$\text{W}/\text{g}_{\text{maghemite}}$] of fractionated nanoparticles determined calorimetrically at 8 kA m^{-1} over frequency: open triangles—fraction 0 A; open circles—fraction 0.25 A; open squares—fraction 1 A; asterisks—original unfractionated nanoparticles; fraction 6 A not shown as values are not distinguishable from the x -axis; straight lines representing linear fit; (a) particles suspended in water; (b) particles immobilized in mannitol by means of freeze-drying.

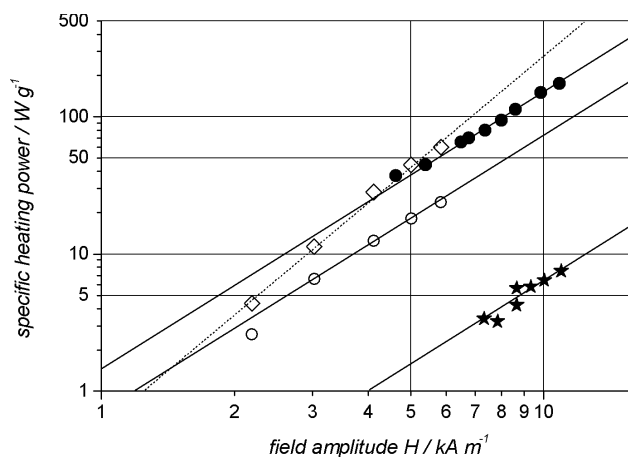


Figure 7. Specific heating power depending on external magnetic field strength: filled symbols representing suspended particles (fixed frequency 410 kHz), open symbols representing particles immobilized in mannitol (fixed frequency 400 kHz); circles—original unfractionated MNP, asterisks—fraction 6 A, rhombi—fraction 0 A; full lines corresponding to square law dependence, dotted line to an $H^{2.7}$ fit for fraction 0 A.

Figures 6 and 7 reveal clearly a negative displacement of curves by a factor of ≈ 2 after particle immobilization. This reduction of specific heating power occurs for frequencies well above the Brown relaxation peak. Relaxation theory predicts only a small reduction at frequencies above 100 kHz (cf figure 4). One explanation of the unexpected large difference of heating power between fluid and immobilized particles may be due to texturing effects in the fluid. Earlier investigations on texture effects [14] resulted in increased area of the magnetization loop anticipating enhanced heating power after alignment of particles and subsequent immobilization. Similarly, Jordan *et al* [28] found reduced specific absorption rates after applying a strong permanent magnetic field perpendicular to the AC magnetic field direction. The present results may indicate that particles persist in orientation forced by the magnetization direction of the AC field. This assumption is supported by magneto-optical analyses. The birefringence for different investigated samples is given in table 2. For comparison, the birefringence intensity is given for quasi-static fields, too.

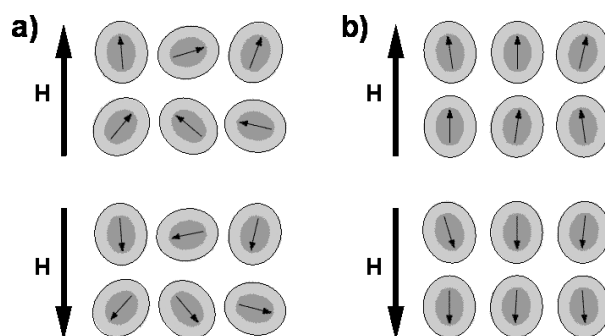


Figure 8. Illustration of hypothesized magnetic nanoparticle orientation after applying HF magnetic fields: (a) immobilized particles with statistical orientation; (b) suspended particles with induced texture due to particle inertia in a viscous environment.

Table 2. Magneto-optical birefringence of fractionated ferrofluid samples in a quasi-static field (4 kA m^{-1} , approx. 1 mM Fe) and in an AC magnetic field (8 kA m^{-1} , 400 kHz , 60 mM Fe). (Note: BLD—below limit of detection.)

Fraction	Quasi-static field	AC field (400 kHz)
Original DDM	120 mV	10 mV
6 A	BLD	BLD
1 A	20 mV	BLD
0.25 A	470 mV	15 mV
0 A	1040 mV	170 mV

The values of quasi-static and high frequency fields differ as the optical path length (cuvette) and concentration (light absorption by dark brown ferrofluid) vary. The largest particles (0 A) possess a remarkable birefringence without a static component of the magnetizing field. Smaller particles and the original solution show only minor effects as they do also in the quasi-static case. Birefringence as a result of chain formation is implausible due to the relatively low volume concentration ($<1\%$) of the investigated ferrofluid samples. This observation is a clear evidence for a preferential particle orientation, though the amount of it is hardly determinable. The idea of particle orientation is illustrated in figure 8.

If particles are at least partly oriented, the hysteresis loop will spread over a larger area compared to the statistical orientation, by a factor between 1 and 4. This would be in good agreement with the observed doubled heating power of freely movable particles compared to immobilized ones (cf figures 5–7).

5. Conclusions

The results presented show that a typical commercial ferrofluid may be improved by magnetic size fractionation for application in magnetic particle hyperthermia. Magnetic properties could be well described by the classical relaxation theory comprising Néel as well as Brown relaxation. In particular, the dependence of specific heating power of the superparamagnetic fractions on the frequency and amplitude of the external alternating magnetic field obeys the predictions of relaxation theory. Only the largest fraction shows hysteresis effects due to a portion of stable single domain particles. Specific heating power increases strongly with increasing mean particle size. While for the fraction of smallest mean size (about 6 nm)

the heating capability is negligibly small the largest fraction delivers about 160 W g^{-1} at a frequency of 400 kHz and a field amplitude of 8 kA m^{-1} . These are very satisfying data for magnetic particle hyperthermia as long as the particle application is performed by direct tumour injection. However, for antibody targeting of the magnetic particles the expected low target concentrations need even (much) higher heating potential. The investigations have also shown that the large heating power of the fluids may essentially decrease in the immobilized state, i.e. for sticking of particles onto cell membranes. In summary, the present results have demonstrated one way towards optimization of nanoparticles for magnetic particle hyperthermia and may help for choosing suitable parameters of the alternating magnetic field for a future therapy.

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