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Ferrofluids of magnetic multicore nanoparticles for biomedical applications

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ABSTRACT

For a variety of magnetically based biomedical applications, it is advantageous to use sedimentation stable suspensions of relatively large ($d > 20$ nm) magnetic core–shell nanoparticles. Water-based suspensions of multicore nanoparticles were prepared by coating of the particles (synthesized by means of a modified alkaline precipitation method) with a carboxymethyldextran shell. The resulting ferrofluids were structurally and magnetically characterized. It was found that these fluids show a specific heating power of about 60 W/g ($f = 400$ kHz, $H = 10$ kA/m). This value was increased up to 330 W/g by a simple fractionation method based on centrifugation. Finally, the cellular uptake of the multicore nanoparticles was demonstrated.

0. Introduction

Magnetic nanoparticles (MNP) are a promising tool for a wide spectrum of magnetically based biomedical applications. For the majority of applications (e.g. hyperthermia, cell separation, and drug targeting), it is advantageous to use aqueous suspensions of relatively large ($d > 20$ nm) magnetic nanoparticles. Unfortunately, MNP of this size show a tendency to form aggregates which results in the sedimentation of the ferrofluid. To solve this problem, so-called multicore nanoparticles (MCNP) were synthesized by a modified alkaline precipitation method. Water-based suspensions of these particles were prepared by coating of the MCNP with a carboxymethyldextran (CMD) shell. In a previous work [1] only a weak influence of the CMD shell on the magnetic properties of the coated particles was found. For reasons of biocompatibility only iron-oxide-based MCNP were prepared.

For the medical heating applications of MNP (hyperthermia), the applicable amplitude of the alternating magnetic field is limited to a value of about 15 kA/m due to medical and technical restrictions [2,3]. Therefore, MNP with a comparatively high specific heating power (SHP) at a relatively low field amplitude are needed. It is well known that the SHP is a function of the mean particle diameter of the MNP as well as of the size-distribution width [4,5]. Fractions of different particle diameters were extracted from the prepared fluid by a separation method based on centrifugation. By this way, for some fractions the SHP could be increased to a higher value than the SHP of the original fluid.

Magnetic core–shell nanoparticles are of growing importance in labelling and separation of cells. Nanoparticles attached to antibodies which are directed against cell surface markers are widely used for magnetic cell separation and allow a specific and high enrichment of the target cells [6,7]. On the other hand, the nanoparticle shell itself could be used for cell-specific interaction [8–10]. Depending on the size of the particles as well as the nature of the shell MNP are attached to the cells or incorporated [11,12]. Small-sized MNP interact with human cells in a cell-type and time-dependant manner, allowing the precise separation of tumor cells from leukocytes [9].

In the present paper, the preparation route of the MCNP is described and structural and magnetic properties of the ferrofluid and the single particles are shown. A fractionation method based on centrifugation as a tool for the increasing of the SHP is investigated. First results for the cellular uptake of the CMD-coated MCNP in tumor cells are given.

1. Methods

1.1. Preparation and fractionation of the ferrofluids

The particles introduced in this paper were prepared similar to the well-known wet chemical precipitation methods [13,14], but using another alkaline medium and a slower reaction velocity.

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In detail, a 1 M $NaHCO_3$ solution was slowly added under permanent stirring to a $FeCl_2/FeCl_3$ solution (total Fe-concentration: 0.625 M; Fe^{2+}/Fe^{3+} ratio = 1/1.3) with a rate of 0.75 ml/min. This procedure was stopped when the pH value reached 8. During this routine, a brownish precipitate was formed. This precipitate was heated to 100 °C for 5 min and iron oxides with a spinel structure were formed under the release of CO_2. To remove excess reaction products from the prepared particles, they were washed with de-ionized water three times.

For the production of sedimentation stable suspensions of the MCNP, the particles were coated with a CMD shell. After washing of the particles the pH value of the dispersed particles was adjusted to pH 2–3 by the addition of diluted HCl. Then the suspension was homogenized by ultrasonic treatment for a few seconds (Sonopuls GM200, Bandelin electronic) and then temperated at 45 °C. An aqueous solution of CMD (initial material: CMD sodium salt, Fluka) with an CMD/MCNP ratio of about $\frac{1}{3}$ was added to the suspension and stirred for 60 min at 45 °C. Finally, the coated particles were washed with de-ionized water to remove the remaining salts.

For the fractionation of the initial MCNP fluid into fractions of different mean particle sizes, 30 ml of this fluid were filled in a cylindrically shaped centrifugation vessel made of glass (sample height ≈70 mm and diameter ≈20 mm). The sample was centrifuged in a laboratory centrifuge (Cryofuge 6000, Heraeus Sepatech) at 1000*g* with a temperature of 20 °C. The sediment was stored and the supernatant was recovered. A portion of the supernatant was centrifuged again with 1500*g*. This procedure was repeated twice with increasing centrifugal accelerations (2500*g*, 3000*g*). In consequence, 4 sediments and 4 supernatants were obtained representing 8 fractions of the MCNP.

1.2. Investigation of cellular uptake

The interaction of the MCNP with human cells was studied with the breast cancer cell line MCF-7. The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) +10% fetal calf serum. For incubation experiments, adherent cell culture cells were detached with a trypsin/EDTA solution and inoculated with MCNP in PBS/EDTA (2 mmol) for the times indicated. After the treatment, the magnetically labelled cells were separated by MACS (SuperMACS and MS-columns, Miltenyi-Biotec). The efflux was designed as 'negative fraction' and the cells retained in the column as 'positive fraction'. Total cell numbers were estimated by cell counting (Coulter Z2, Beckman-Coulter).

1.3. Structural and magnetic characterization

Hydrodynamic diameters (d_h) of the CMD-coated particles in the ferrofluid were determined by using photon correlation spectroscopy (PCS; HPPS-ET, Malvern Instruments). Dry samples of the fluids were structurally characterized by field emission scanning electron microscopy (FE-SEM; JSM 6300-F, JEOL), transmission electron microscopy (TEM; JEM 2010FEF, JEOL) and X-ray diffraction (XRD; X'pert-Twin diffractometer, Philips). The mean sizes (d) of the magnetic cores were calculated from measurements of the XRD line width by using the Scherrer method.

For the investigation of the quasistatic parameters, the MCNP in the fluid were immobilized by drying. Minor loops of the dry particles were measured by vibrating sample magnetometry (VSM; Micromag 3900, Princeton Measurement Corporation). By integrating of the area of the measured minor loops, the specific hysteresis losses (SHL) per cycle depending on the field amplitude were calculated. The results were compared with a commercial sample (iron(III)oxide, gamma, 99+%, AlfaAesar) with high SHL determined in earlier investigations and magnetosomes from magnetotactic bacteria—up till now the particles with the highest SHL at relatively low field amplitudes [15]. The specific heating power was measured by means of magnetic field calorimetry (MFC) at a field amplitude of 10 kA/m and a frequency of 400 kHz. This parameter combination is suitable for hyperthermia treatment [16].

The Brownian relaxation behavior of the MCNP in the fluid was investigated by magneto relaxometry (MRX) [17]. For these measurements, the samples were diluted by de-ionized water (dilution factors from 1:9 to 1:900). A magnetizing field of $H = 1300$ A/m is applied for $t = 1$ s. A total of 450 μs after switching off the field, a highly sensitive low-T_C-SQUID sensor measures the magnetic induction $B(t)$ at a distance of 10 mm above the sample. The measurement time window is $450\,\mu s \leqslant t \leqslant 0.45$ s. From these relaxation curves, the size distribution of hydrodynamic diameters of the CMD-coated particles was calculated by fitting the so-called cluster moment superposition model (CMSM) to the relaxation data [18]. The CMSM describes the relaxation of the magnetic moment of an ensemble of particulate magnetic entities, the magnetic moment of which can relax via the Brownian and Néel mechanism. The distribution $f(d_h, \mu, \sigma)$ of the hydrodynamic diameters d_h with the median diameter μ and the distribution parameter σ is assumed to be a lognormal one. In previous investigations, it was empirically found that the hydrodynamic diameter of the entities with the mean volume is in good agreement with hydrodynamic diameter obtained by PCS [17].

Furthermore, the MCNP were investigated by AC susceptometry (DynoMag, IMEGO) in the frequency range from 1 Hz to 200 kHz at a field amplitude of 0.4 kA/m. The hydrodynamic diameters distribution was determined by fitting the complex susceptibility data to a model that assumes Brownian relaxation of the MCNP.

2. Results

It was found that during the precipitation primary particles with a mean diameter of 14 nm (XRD) were formed. Phase identification by XRD demonstrates that the particles consist of solid solutions (or of a mix) of maghemite and magnetite. These particles form clusters (Fig. 1a) of 40–80 nm (TEM) surrounded by a CMD shell. Clearly, a size distribution of the cluster diameters could be observed (Fig. 1b). The dynamics of the cluster formation as well as their magnetization configuration are yet unknown. For the hydrodynamic diameters of these clusters values of 158 nm (PCS) and 160 nm (AC susceptometry) were determined. These values are affected by a hydrate shell on the CMD layer.

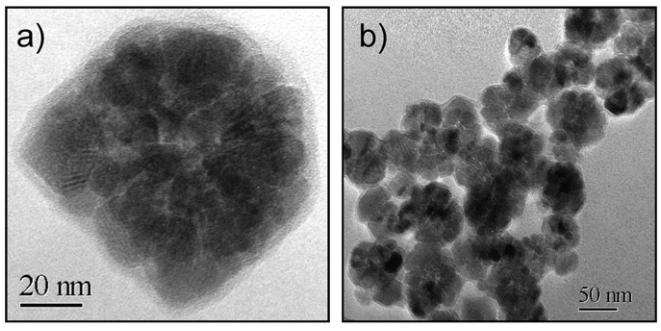

Fig. 1. Typical TEM images of a single cluster (a) and an ensemble of clusters (b). The aggregation of the clusters in Fig. 1b occurs during the sample preparation (drying of the fluid) for TEM imaging.

Immediately after the preparation of the fluid, a sediment consisting of large aggregates is formed. After removal of this sediment, the supernatant shows a high stability against sedimentation over a period of several months.

Due to the size distribution of the clusters, the fractionation of the original fluid based on centrifugation appeared to be useful. Starting from a mean hydrodynamic diameter of 158 nm (PCS) for the original fluid eight fractions with a size range from 50 to 164 nm (PCS) were obtained (Table 1). Because of the very low particle concentration in the fraction with the smallest particles, it was not possible to determine its magnetic parameters. This fraction was excluded from further investigations.

VSM measurements with immobilized particles reveal a thermally blocked behavior of the particles. The clusters of different mean diameters (consisting of primary cores with a nearly constant mean diameter of 14 nm, measured by XRD) show a coercivity depending on the size of the clusters (Table 1). This implies that in this case the magnetic behavior is mainly determined by the cluster size and not by the constant size of the primary cores. As demonstrated earlier [19], the coercivity (H_C) correlates linear with the relative remanence (M_R/M_S). Hence, the applied fractionation method allows the adaption of the relative remanence of the ferrofluid to optimal values for applications based on magnetic attraction, e.g. cell separation or drug targeting.

Table 1
Hydrodynamic diameter (d_h, measured by PCS), fractionation parameters, coercivity (H_C, immobilized particles) and SHP (measured at liquid samples) of the original fluid and of the prepared fractions.

	d_h (nm)	Fractionation parameter	H_c (kA/m)	SHP (W/g)
Original fluid	158	–	3.10	60
Fractions	50	2500 g, supernatant	0.86	–
	61	3000 g, sediment	1.99	274
	82	1500 g, supernatant	2.21	332
	95	2500 g, sediment	2.55	145
	120	1000 g, supernatant	2.60	152
	142	1500 g, sediment	2.68	81
	164	1000 g, sediment	3.33	53

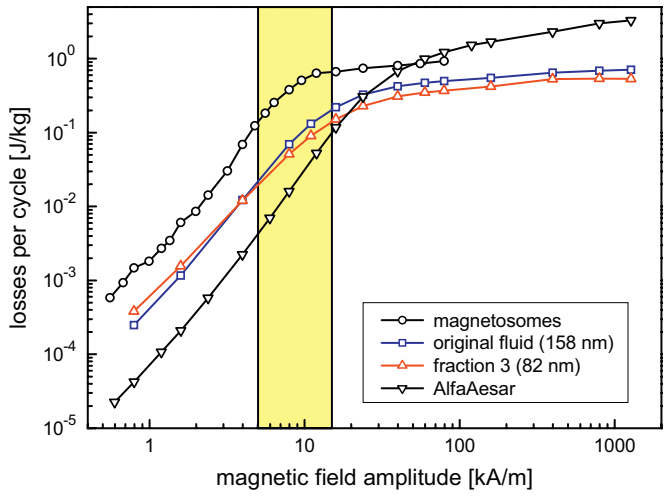

Fig. 2. Dependence of specific hysteresis losses (SHL) of immobilized particles on the field amplitude of minor loops for the original fluid ($d_h = 158$ nm) and the fraction with the highest SHP ($d_h = 82$ nm). For comparison, the curves of magnetosomes and of a commercial sample with high SHL (Iron(III)oxide, gamma, 99+%, AlfaAesar) are shown. The marked frame indicates the interesting field range for biomedical heating applications.

In Fig. 2, the field amplitude dependence of SHL per cycle measured by VSM is shown for immobilized particles of the original fluid and the fraction with the highest calorimetrically measured SHP in the interesting field amplitude range from 5 to 20 kA/m. Additionally the curves of magnetosomes [15] and a commercial sample (Alfa Aesar) with high SHL are presented.

The SHP of the fluid particles was determined by the magnetic field calorimetry measurements. The original fluid shows a moderate SHP of about 60 W/g (at 400 kHz and 11 kA/m). This value was increased up to 330 W/g for the fraction with $d_h = 82$ nm (PCS, Table 1). For this fraction, hydrodynamic diameters of 83 and 84 nm were determined by AC susceptometry and MRX, respectively. While the moderate SHP of the original ferrofluid correlates relatively well with the hysteresis losses measured by VSM, the fraction with $d_h = 82$ nm shows much larger losses determined by calorimetry than extrapolated from hysteresis. This may be explained by the high frequency of calorimetrical measurements in comparison to the nearly six orders of magnitude slower VSM measurements. The sample with the relatively small mean size probably contains a considerable

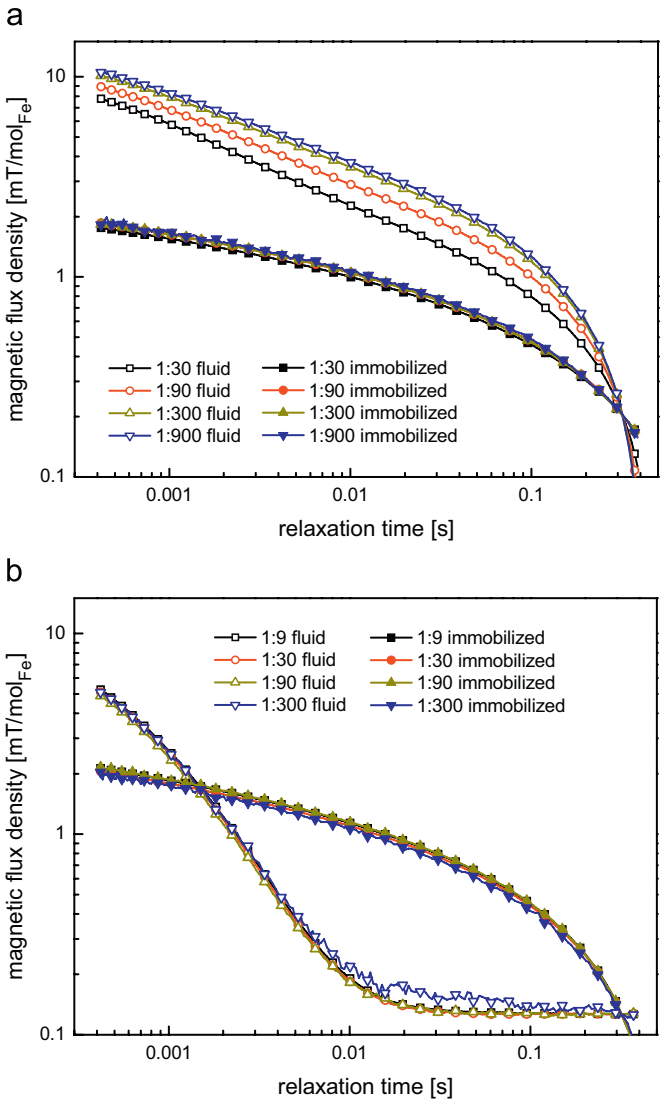

Fig. 3. MRX curves of the original fluid (a) and the sample with the highest SHP (b). Curves for measurements with fluids (open symbols) as well as immobilized particles (full symbols) are shown. The dilution factor ferrofluid to de-ionized water was changed from 1:9 to 1:900.

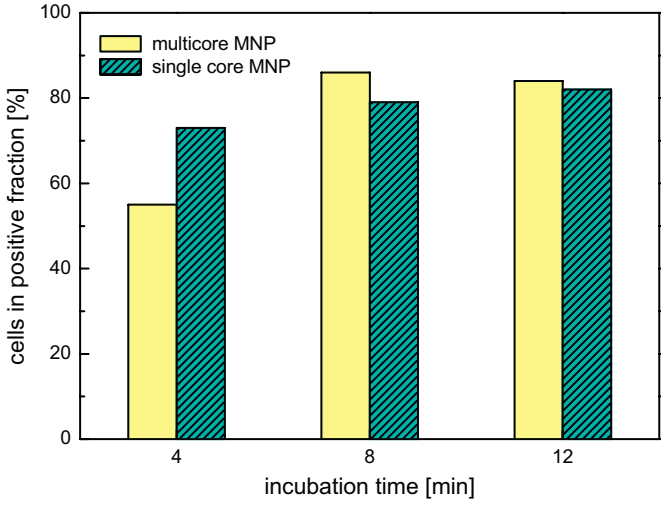

Fig. 4. Uptake of MCNP in MCF-7 tumor cells as a function of the incubation time. For comparison data of single core MNP obtained in an earlier investigation [20] are shown (hatched bars).

amount of smaller particles which relax during the quasistatic VSM measurement contributing negligibly to hysteresis.

After dilution with de-ionized water, the particles of the original fluid show a tendency to aggregation due to the relative high amount of larger particles in the fluid. Accordingly, the relaxation curves in MRX measurements (Fig. 3a) show a shift of the amplitude for different dilutions, but no changes for the investigation of immobilized particles. For the fraction with the highest SHP, no significant changes of their properties after strong dilution by de-ionized water and BSA buffer were found (Fig. 3b). This means a good stability against sedimentation. Due to the wider cluster size distribution and a higher amount of larger clusters in the original fluid, this sample shows a slower Brownian relaxation than the sample with the highest SHP. For both samples, a relatively high relaxation amplitude was determined by MRX compared to the values of commercial ferrofluids.

The interaction of the MCNP with human cells was analyzed with the breast cancer cell line MCF-7. The tumor cells are labelled rapidly with the nanoparticles. Within 4 min more than 50% of the cells are detected in the positive fraction (Fig. 4). Prolonged incubation lead to an increase of cell content in the positive fraction up to 85%. These data are in good correlation to previous results with CMD-coated MNP with a mean diameter of 10 nm [20]. The data show that MCNP are a suitable tool for cell separation.

3. Discussion

In the XRD measurements, the peak position of the (4 4 0) reflex was used to distinguish between the magnetic phases maghemite and magnetite. The obtained diffractogram show a relatively broad single peak between the theoretical angles for maghemite and magnetite. This means that the resulting particles presumably consist of solid solutions of both phases (one resulting peak). However, it is also possible that particles consist of a mix of maghemite and magnetite particles (two separated peaks). In principle, a differentiation between these both cases is feasible, but due to the peak broadening of the small particles, a superposition of both peaks is possible. For a better determination of the phase composition, another analytic method is needed, e.g. Moessbauer spectroscopy.

The SHL of the immobilized particles measured by VSM are relatively high in comparison to commercial samples. However, the curves of the original fluid as well as all fractions show a similar course of SHL over the field amplitude which does not reflect the strong differences between the fractions and the original fluid observed for the SHP of the fluid samples. Hence, the SHP values are affected by another relaxation mechanism beside the hysteresis losses—the Brownian relaxation. For heating applications of the MCNP in medicine (hyperthermia), the Brownian losses are mainly suppressed due to a relatively high degree of binding of the particles to the tumor tissue which means a diminishing of the SHP [21] that may result in an insufficient temperature increase in the tumor region [22]. The effect of particle immobilization on the SHP of MCNP has to be investigated in more detail in further experiments.

Investigations on the cellular uptake of the MCNP show that these particles interact with tumor cells in a similar way as single core nanoparticles with similar core diameters do. Initial analysis was carried out with breast cancer cell line cells only, thus further studies with other cell types are needed in order to assess the potential of MNCP for differentiation of tumor cells from other cells, e.g. leukocytes. The amount of adsorbed or incorporated particles and thus the magnetic moment of the magnetic material physically connected to the cell is sufficient for a magnetic separation of the cells with commercial separation units.

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