

Research Article

Continuous synthesis of single core iron oxide nanoparticles for MPI tracer development

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Abstract

The development of suitable tracers with optimized characteristics is a crucial factor to bring the powerful and high-innovative technology of Magnetic Particle Imaging (MPI) further towards clinics. Successful engineering of new magnetic particles for this imaging method requires a deeper understanding of structure-performance relationships. This means that the influence of certain particle characteristics as e.g. core size, crystallinity and agglomeration behavior on the signal spectrum in MPI requires comprehensive investigation. Even though many international research groups work in the field of tracer development for the MPI technology, until now no economic and reproducible synthesis method was found to produce high-performance tracer materials with optimal characteristics concerning their MPI signals as well as their behavior in vivo. In the present work recent achievements in the continuous synthesis of single-core iron oxide nanoparticles with tunable characteristics employing a micromixer set-up are reported.

1. Introduction

Magnetic Particle Imaging (MPI) is a powerful preclinical imaging technology with high potential and a broad range of application fields in medicine. This method has attracted growing interest of international researchers and innovative companies. However, to exploit the full potential of MPI high-performance tracer materials are required. Resovist[®], a former contrast agent for magnetic resonance imaging (MRI), that is not available on the market anymore, is still commonly used as gold standard for magnetic particle imaging. These nanoparticles were initially not developed for MPI, thus their performance is far below the theoretically calculated optimum. It is known that only a small fraction of about 3% contribute significantly to the MPI signal [1].

In literature different approaches to synthesize iron oxide nanoparticles for MPI are reported [2]. Those encompass synthesis strategies as thermal decomposition from organic precursors [3], biotechnological mineralization with microorganisms [4, 5] and controlled clustering from small superparamagnetic iron oxide nanoparticles [6–8]. In particular single-core iron oxide nanoparticles with a distinct size of about 30 nm core diameter - the suspected optimal size for MPI according to theoretical models [1] - are not easily accessible by the common synthesis routes. Thermal decomposition as well as biomineralization can yield those particles, nevertheless they represent relatively sophisticated and time-consuming methods [9–11]. Moreover, the scalability of these techniques and therefore the reproducible production of larger amounts is limited. In summary, until now no economic produc-

tion process for tracer materials with optimal characteristics was found.

The here presented work addresses these issues and aims on a cost-efficient, safe and green synthesis route that enables a controlled tuning of the particle characteristics. The overall goal is to establish a particle library and investigate comprehensively the structure-performance relationship regarding Magnetic Particle Imaging. Besides the synthesis of large single core nanoparticles in the range of 20 nm – 50 nm also the controlled manufacturing of smaller particles and their precise clustering in a continuous and highly reproducible manner could be of great interest to also obtain a deeper understanding of particle-particle interactions and their influence on the MPI performance. All these findings will help to discover the optimal particle system as tracer material.

II. Experimental Part

II.I. Nanoparticle Synthesis

The continuous synthesis of iron oxide nanoparticles was performed by precipitation from aqueous, alkaline solutions of iron salts in a micromixer set-up as previously reported [12, 13]. A schematic representation of the microfluidic mixing device consisting of HPLC pumps (Knauer, Germany) and Caterpillar micromixer (Fraunhofer ICT-IMM, Germany) is shown in Fig. 1.

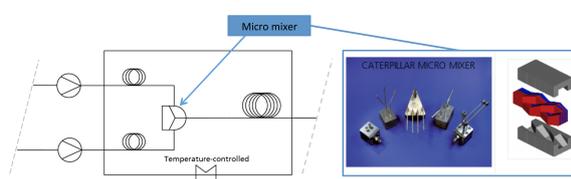


Figure 1: Schematic representation: micromixer set-up.

The particle formation in the micromixing device can be described as a two-stage process; the initial nucleation is followed by particle growth [14]. Varying the process parameters as e.g. temperature, mixing ratios and the residence time, size and morphology of magnetic nanoparticles can be adjusted.

II.II. Particle Characterization

Continuously manufactured magnetic particles were analyzed by imaging with Transmission Electron Microscopy (TEM). TEM measurements were performed at a Zeiss Libra 120 on carbon coated copper grids at 120 kV acceleration voltage and the images were taken with a CCD camera. As ensemble method to investigate the particle dispersion in aqueous media analytical centrifugation (Differential Centrifugal Sedimentation, DCS)

was performed with a device from CPS Instruments Inc.. Measurements were performed at 24000 rpm after calibration with a polyvinyl chloride standard (239 nm).

The iron concentration of the particle samples was determined by Prussian Blue staining. Shortly, magnetic particles were dissolved with concentrated hydrochloride acid and subsequently oxidized by H_2O_2 . The formed complexes with potassium hexacyanoferrate(II)trihydrate were measured photometrically at $\lambda = 690 \text{ nm}$ and the iron concentration of the samples were derived employing a calibration curve of Fe(III) standard solutions.

Magnetic Particle Spectroscopy measurements were performed using a commercial Magnetic Particle Spectrometer (MPS) (Bruker, Germany). The MPS measures the non-linear magnetic response of magnetic nanoparticles exposed to an AC magnetic field, thus allowing to estimate the performance of magnetic nanoparticles as a tracer for MPI. For the measurement a PCR tube (Applied Biosystems[®], MicroAmp[®] fast reaction tube with cap) containing a sample volume of $30 \mu\text{l}$ is placed in the detection coil of the MPS system. By exposing the sample to a sinusoidal oscillating magnetic field (amplitude B_{excite} up to 25 mT, frequency f_0 of 25 kHz) the induced magnetization can be measured simultaneously by the coils. In addition, the fundamental excitation frequency f_0 is suppressed by an efficient filter circuitry and gradiometer design of the pickup coil. The Fourier transformation of the detected time signal results the spectral components showing distinctive amplitudes at odd multiples of the excitation frequency f_0 evoked by the magnetic nanoparticles. In this study all measurements were performed at 36.6°C applying $30 \mu\text{L}$ of undiluted MNP samples.

III. Results

The analysis of the continuously manufactured iron oxide nanoparticles from the micromixer set-up reveals that the particles exhibit different sizes (ranging from 15 nm to 55 nm) and different shapes (e.g. spherical or disc-like) depending on the process parameters such as mixing ratio, total flow rate, residence time and temperature. Preliminary experiments have shown that due to the very effective mixing and the extremely short mixing times in the micromixer, the parameters temperature and residence time have the largest effects on the particle characteristics while the influence of mixing ratio and total flow rate are subordinated. Therefore, these two effects are further discussed in the following.

Fig. 2 shows an example of a TEM micrograph of a sample with disc-like nanoparticles.

Preliminary experiments have also shown that disc-like iron oxide particles particularly appear at very short residence times. Disks seem to occur as initial seeds in the micromixer. To investigate the influence of tempera-

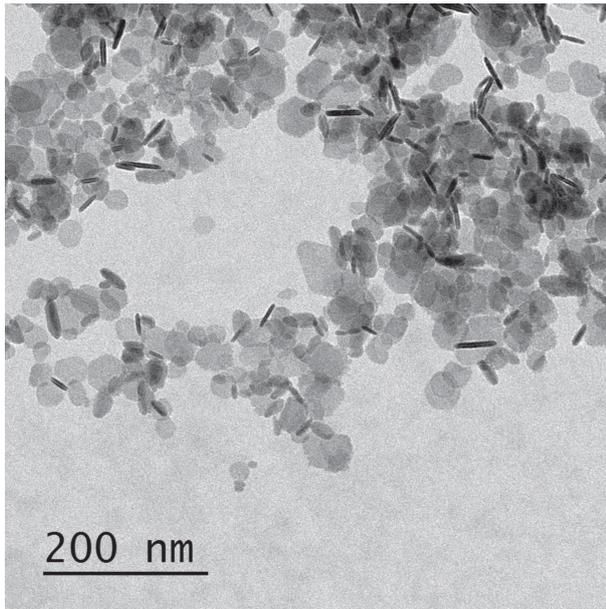


Figure 2: TEM micrograph of a continuously manufactured iron oxide nanoparticle sample with particles displaying disc-like shape.

ture on the synthesis in the micromixer device a series of experiments was performed using the example of disc-like particles. Fig. 3 shows analytical centrifugation measurements from three samples manufactured at different temperatures ($T_1 = 40^\circ\text{C}$, $T_2 = 60^\circ\text{C}$ and $T_3 = 80^\circ\text{C}$) but consistent dwell time of only 12 seconds while maintaining all other process parameters. A temperature increase of 20°C leads to an increase of about 3 nm of the average equivalent sphere diameter in this case. Corresponding MPS measurements demonstrated an increase of the signal amplitude with increasing particle diameter as expected (see Fig. 4). This example emphasize that a few nanometers in diameter can already have a significant effect on the particles' performance as MPI tracers. Nevertheless, the MPI signal spectra of the investigated disc-like samples are clearly below the signal spectrum of Resovist[®]. Apparently disc-like iron oxide nanoparticles in this size range do not provide sufficient MPI performance. For successful MPI tracer development further investigation on the structure-performance relationship is of great importance as this builds the basis to find the optimal reaction conditions to synthesize high-performance MPI tracers.

In a second series of experiments the influence of residence time in the microfluidic device was investigated. For this study process parameters resulting in samples of spherical nanoparticles were selected. Dwelling time was hereby varied from one to five minutes by adjusting the total flow rate maintaining all other process parameters (mixing ratio 1:1, 60°C reaction temperature).

With decreasing total flow rate and therefore increas-

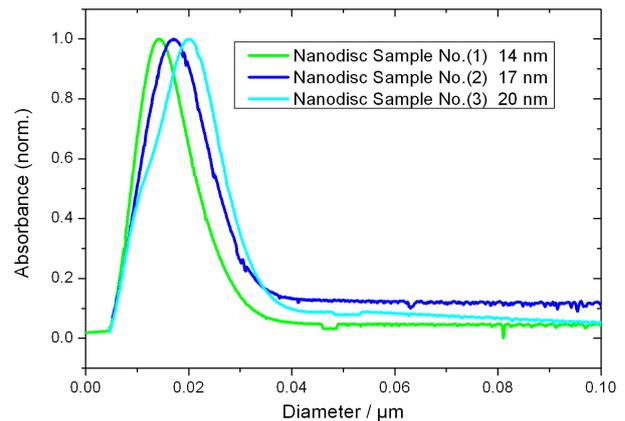


Figure 3: Continuously manufactured disc-like magnetic nanoparticles: size analysis via analytical centrifugation shows equivalent sphere diameter.

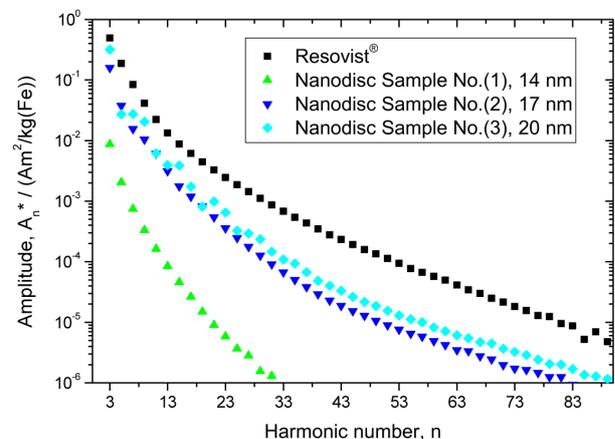


Figure 4: MPS signal spectrum of continuously manufactured disc-like magnetic nanoparticles measured at $B_{\text{excite}} = 25$ mT. For comparison the spectrum of Resovist[®] as clinically approved magnetic nanoparticle system is shown.

ing residence time an increase of average core diameters of the obtained particles was observed.

Due to the very effective mixing in the micromixer even at relatively low flow rates (e.g. total flow rate of 3 ml/min) nucleation occurs immediately and is not or only marginally influenced by the total flow rate. The particle growth period during the subsequent dwell therefore defines the particle size.

Analysis of this set of experiments with TEM reveals predominately spherical particles with average core diameters of the main fraction between 21 nm and 55 nm (Fig. 5). Particles diameter increased with decreasing total flow rate in a roughly linear correlation. Interestingly, in this case, MPS measurements show opposite structure-performance relationship. Here, MPS signal amplitudes decrease with increasing average core diameter (Fig. 6).

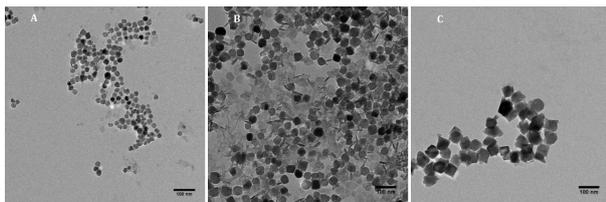


Figure 5: Continuously manufactured spherical magnetic nanoparticles: TEM images showing different core sizes of the samples.

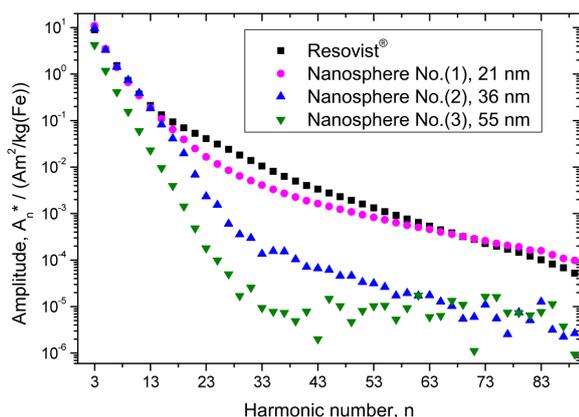


Figure 6: MPS signal spectrum of continuously manufactured spherical magnetic nanoparticles measured at $B_{\text{excite}} = 25$ mT. For comparison with clinically approved MNP the spectrum of Resovist[®] is shown as well.

Further investigations on the stability of the particle dispersion using differential centrifugal sedimentation indicated significant differences in the aggregation status of the three samples. These DCS measurements (data not shown) clearly demonstrated agglomeration of the two samples with particles of larger core diameters (Nanosphere Sample No (2) and No (3)), while the sample with the smallest nanoparticles (21 nm) seems to be stabilized as single cores. Obviously, clustering of the larger particles leads to a fast decay of the signal spectra. Here, the MPS measurements demonstrate the importance of sufficient particle stabilization.

It was already discussed before that different particle systems displaying clusters of particles with smaller cores have recently been described in literature as promising candidates for MPI tracer materials [7, 8]. This shows that the highly controlled clustering is also an interesting approach that could lead to high performance tracer materials. However, the clustered particles need to be colloidal stabilized as well at the end of the production process to prevent further particle growth or degradation.

The best MPS signal performance in the present study was measured for Nanosphere Sample No (1) with 21 nm

average core diameter showing high signal amplitudes and a flat spectrum. These are key parameters for tracers used in MPI. The third harmonic amplitude of this sample $A_3^* = 7.3 \text{ Am}^2/\text{kg}(\text{Fe})$ was in the range of the signal amplitude of Resovist[®] $A_3^* = 9 \text{ Am}^2/\text{kg}(\text{Fe})$.

In summary, these results show that the continuous micromixer-based synthesis route enables tuning particle characteristics in a certain range. In further experiments, the defined goal is to find the parameters to obtain particles of the desired core size of about 30 nm and further improve particle stability.

Furthermore, also particles with smaller core sizes are of interest, particularly to study effects of particle-particle interactions on their MPI performance. Therefore, the adaption of the continuous process to achieve a controlled particle clustering could also be of great importance. The incorporation of hydrophobic iron oxide nanoparticles in supramolecular structures build from block copolymers has already been demonstrated by our group [15] and might be a promising basis for further developments regarding controlled clustering in a continuous manner.

IV. Conclusion

In the present study we demonstrated the huge potential of a continuous micromixer-based synthesis strategy to provide tunable magnetic nanoparticles of different sizes and morphology for MPI tracer development. The investigated samples showed different structure-performance relationships depending on the stability of the dispersion and the average particle core size.

One of the spherical nanoparticle samples with an average core diameter of 21 nm showed a promising MPS signal with a high amplitude and a slow decay. This sample is going to be investigated also on a MPI scanner. Further optimization studies on the synthesis including the comprehensive analysis of the influence of different process parameters on the particle characteristics as well as investigations on potential steric stabilization strategies are in progress.

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