International Journal on Magnetic Particle Imaging
Vol 9, No 1, Suppl 1, Article ID 2303061, 4 Pages

Infinite Science Publishing

Proceedings Article

Magnetic particle spectroscopy for monitoring the cellular uptake of magnetic nanoparticles: Impact of the excitation field amplitude

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Abstract

Magnetic particle spectroscopy (MPS) is a fast and accurate quantification method based on the detection of the nonlinear magnetic susceptibility of magnetic nanoparticles (MNPs) exposed to an oscillating magnetic field of moderate amplitudes. MPS has been shown to be a robust and effective method for characterizing tracers and monitoring cellular uptake of MNP. The magnetic excitation fields of some tens of millitesla used in MPS measurements might lead to unwanted, field-induced changes in the MNP sample (e.g., chain formation, aggregation, etc.) having adverse effects when using MPS for quantification and characterization of MNP. Here, the effect of the magnetic excitation field amplitude and exposure time is investigated and suggestions are provided for MPS measurements with reduced field induced impact on MNP in biological environments.

I. Introduction

Magnetic particle spectroscopy (MPS) is a powerful, sensitive method to quantify magnetic nanoparticles (MNPs) in biological environment [1]. MPS is based on the detection of the nonlinear magnetic AC-susceptibility and has been established as a fast and straightforward method for tracer characterization, originally. Additionally, MPS is capable for monitoring the uptake of MNP by cells [2]. The use of magnetic excitation fields of several millitesla in MPS measurement generally increase the risk of unwanted, field-induced changes of the MNP sample (e.g., chain formation, aggregation, etc.) [3]. So far, such effects of MPS measurements have not been studied in detail. In this work, we investigate field-induced changes during monitoring the uptake of two different MNP systems by THP-1 cells. By using different exposure duration and amplitudes of the magnetic excitation field in MPS the impact of dynamic magnetic fields on the MPS signal of MNP will be investigated and recommendations for MPS measurements of MNP in biological environment will be given.

II. Material and methods

This chapter describes the experimental methods and materials that have been used in this work.

II.I. Magnetic nanoparticles and Media

We used two commercial MNP systems, Synomag® (LOT: 12121103-01, Syn-COOH-30nm) and Synomag®-D (LOT: 12821104-01, SynD-PEG-50nm) (*micromod Partikeltechnologie GmbH*, Rostock, GER). Both MNP have the same core diameter of about 15 nm [4]. The cores of Synomag® MNPs consist of several cores, therefore

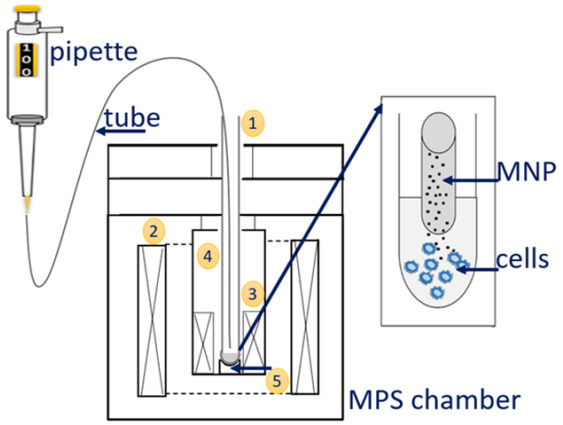

Figure 1: Scheme of continuous MPS measurements. The MNP are pipetted through a tube directly to the THP-1 monocytes in the glass tube "1", which is in the measuring chamber of the MPS. The MPS chamber "4" consists of positioning pin of the sample holder "5", receiving coil "3" and excitation coil "2".

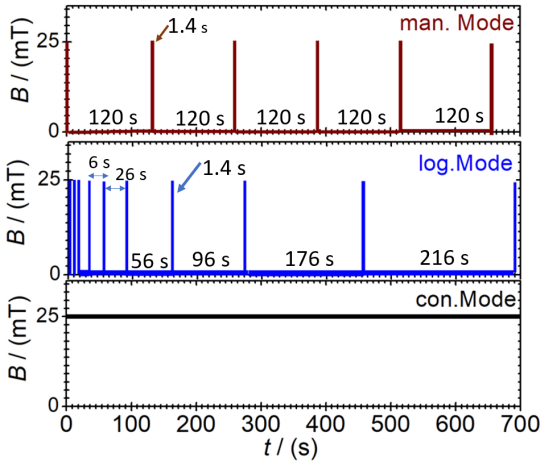

Figure 2: Three different measurement modes to measure the influence of the exposure duration of cell sample. In the continuous mode the sample is exposed permanently to the excitation B, while in the logarithmic mode only B is switched on for 1.4 s of MPS signal acquisition distributed logarithmically over the interval of 700 s. In the manual mode the sample is removed by hand from the MPS detection coil system after each measurement for about 2 min after the MPS acquisition of 1.4 s.

they can be described as multicore MNPs (MCMNPs) [5]. The MNP systems deviate in coating and functional groups. Compared to the PEG-dextran coated Syn-PEG-50nm, the MNP system Syn-COOH-30nm has a citrate coating [4].

II.II. Cell line

The experiments were performed with human tumor cells (THP-1 monocytes) that are isolated from patients with monocytic leukemia. The cells were purchased from ATCC® (*Wesel*, GER). The cultivation was performed in a cell culture flask in a suspension of RPMI medium 1640 (*Invitrogen*, Dreieich, GER) inside an incubator (37°C, 5 % CO_2). The culture medium was supplemented with 10 % fetal calf serum (FCS) (*Biochrom*, Berlin, GER) and 1 % penicillin-streptomycin (*Invitrogen*, GER). The THP-1 cells were loaded with an MNP concentration of 1 mmol/L. Phosphate-buffered saline (PBS) (Gibco, Dreieich, GER) was used to dilute the MNP.

II.III. Magnetic particle spectroscopy (MPS)

In MPS, a sinusoidal excitation field of an amplitude B at a fixed frequency f_0 is applied to a MNP sample. Due to the non-linear magnetization of the MNP, the measured response can be decomposed by Fourier transformation into distinct odd multiples of f_0 (i.e., higher harmonics A_i). The amplitudes A_i of the odd harmonics are directly proportional to the absolute MNP content in a sample and this can be employed for MNP quantification. The third harmonic A_3 is often used to quantify MNP in biological systems as A_3 carries the highest signal of all harmonics (whereas the first harmonic at f_0 is filtered out to get rid of interferences with the oscillating basic excitation field). A_3* is the A_3 amplitude normalized to the absolute iron mass of a sample. The shape of the spectrum is characterized by the concentration independent ratio A_5/A_3 and serves as a fingerprint of the dynamic magnetic behavior of MNP. From MPS measurements at B=12 mT, a specific third harmonic amplitude of A_3*= 3.4±0.05 Am^2/kg(Fe) and A_3*= 6.1±0.03 Am^2/kg(Fe) as well as a spectrum shape of A_5/A_3= 12.6±0.07 % and A_5/A_3 = 22.1±0.05 % is determined for Syn-COOH-30nm and SynD-PEG-50nm, respectively [4]. For MPS monitoring of the cell uptake, THP-1 cells were first centrifugated for 1 min, the supernatant was discarded, the residual cell pellet was taken up in 100 µL of PBS (37°C), the suspension was pipetted into a glass tube and placed in the MPS excitation (Fig 1, "2") and detection coil system Fig. 1, "3"). After starting a sequence of repeated MPS measurements the MNP system (c(Fe)=1 mmol/L) in 100 µL PBS was injected into the glass tube (Fig. 1, "1") via a tubing system. The solutions were mixed for resuspension of the MNP in the cell solution.

Different MPS measurement modes (continuous, logarithmic, and manual mode) were investigated to study the influence of the exposure duration of the excitation field. During the continuous mode (*con. mode*), the MNP-cell sample was continuously exposed to the magnetic excitation field B (see Fig. 2, bottom).

In the logarithmic mode (*log. mode*), only during 1.4 s of data acquisition the excitation field B is switched on while no field applied in between. First, three MPS measurements were carried out in a row, followed by

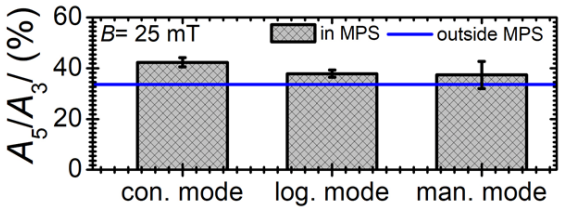

Figure 3: Comparison of A_5/A_3 for the three different measurement modes with the corresponding reference sample incubated outside the MPS. The uncertainty was calculated from a triplicate replicate for each measurement mode.

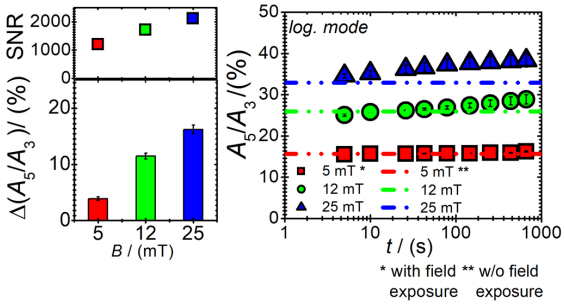

Figure 4: Percentage change of A_5/A_3 (related to a cell sample without excitation field exposure) (right, bottom) and SNR as a function of field strength B = 5, 12, and 25 mT by using the log. mode (right, top). The change of A_5/A_3 for the three field strengths of 5, 12 and 25 mT is compared to the reference sample incubated outside the MPS (left). The uncertainty was calculated from a triplicate replicate for each field strength.

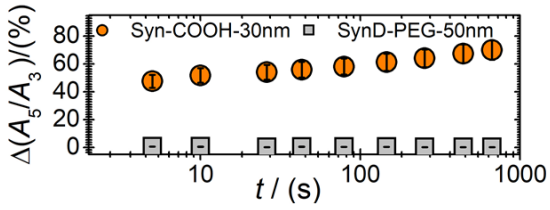

Figure 5: Percentage changes of A_5/A_3 at B=12 mT (related to sample in PBS without cells) using the log. mode for Syn-COOH-30nm and SynD-PEG-50nm. The uncertainty was determined from triplicate replicates for each MNP system.

seven MPS measurements at time points on a logarithmical scale (see Fig. 2, middle). In the manual mode (*man. mode*), the MNP-cell sample was likewise exposed to *B* only 1.4 s measurements intervals, but here a pause of 120 s was set during which the cell-MNP sample was removed from the MPS device and gently shaken to reduce chain formation after the field exposure. All three modes had a total duration of 700 s. In addition, for each measurement mode, we investigated the influence of field strength at *B*=5, 12, and 25 mT on the MPS signals of the cells. For comparison to the cell sample used in the MPS device, a second sample was incubated outside the MPS without any field exposure. This second sample was measured after the time of 700 s by MPS at the same field strength that was used in one of the measurement modes. From this, we determined the percentage change of A_5/A_3 between the cell sample exposed to the excitation field and the sample incubated outside the MPS without field exposure. In addition, to assess the field influence, the signal-to-noise ratio (SNR) was also calculated from the amplitude A_3 of a MNP sample divided by limit of detection amplitude $A_{3,\ LOD}$= 3 · standard deviation + mean value of a series of 10 blank measurements without MNP.

III. Results and Discussion

III.I. Impact of the excitation field exposure duration

First, we investigated the impact of the excitation field exposure duration on the MPS signal of MNP in cells with different measurement modes. Figure 3 shows the harmonic ratio of A_5/A_3 for three different exposure duration, by using the *con. mode*, *log. mode*, and *man. mode*, see Fig. 2. These measurements were performed with the MNP system Syn-COOH-30nm at a field of *B*=25 mT after each corresponding measurement mode.

As can be seen in Fig. 3, the field exposure duration of the MNP during the cellular uptake shows a significant influence on the MPS signal of MNP. The A_5/A_3 is increased to 42.35 % when the sample is permanently exposed to the highest excitation field of 25 mT (*con. mode*) compared to a sample that was incubated outside the MPS (A_5/A_3=33.57 %, see blue line Fig. 3) without field exposure. Lower deviations are observed for shorter exposure durations (*log.* and *man. mode*). Since the cell sample during the *man. mode* was taken from the MPS device after each measurement and was shaken, we observe a higher uncertainty for the MNP-cell sample than for the other two modes. The treatment of taking the MNP cell sample from the MPS device and shaking it apparently affects the cell behavior and thus the MPS reproducibility. We therefore preferred the *log. mode* for the subsequent experiments.

III.II. Impact of the excitation field strength

To investigate the effect of the excitation field strength, the cell sample was measured using the *log. mode* at different excitation fields of *B* = 5, 12, and 25 mT. Figure 4 (bottom) shows the percentage change of A_5/A_3 between the two cell samples and the SNR (top) for the three field amplitudes. In Fig. 4 (left), the change of A_5/A_3 for the three field strengths of 5, 12 and 25 mT is compared to the reference sample incubated outside the MPS.

10.18416/ijmpi.2023.2303061

It can be seen that as the field strength increases, the changes of A_5/A_3 also increase. The smallest percentage change of A_5/A_3 is obtained at a field strength of 5 mT whereas at the highest field strength of 25 mT a more pronounced impact on the cell sample is observed. At the same time, we extract from Figure 4 (top) that the higher the field strength the better the SNR. A high SNR and at the same time a field strength that does not affect the MNP cell sample are recommended, therefore a field strength of 12 mT seems to be a good compromise that was chosen for the subsequent kinetic cell experiments. For a reliable analysis of the uptake kinetics of MNP by THP-1 cells, they were injected into the cell suspension in MPS and measured at 12 mT in *log. mode*. The resulting kinetics of A_5/A_3 (compared to a sample without cells) for the Syn-COOH-30nm and SynD-PEG-50nm MNP systems are shown in Figure 5.

It was found, that SynD-PEG-50nm, which shows no cellular uptake [4], exhibits a percentage change of less than 1 %. In contrast, Syn-COOH-30nm, which is taken up by cells, shows an increase in A_5/A_3 immediately after the first few seconds. This shows that MNP change in their magnetic behavior due to the uptake mechanism of the cells and that the measurement of the concentration independent A_5/A_3 ratio can be used to study the cellular uptake kinetics.

IV. Conclusions

We tested different MPS measurement procedures to determine their capability in monitoring the cellular uptake of MNP system. We found that in MPS, the excitation field strongly affects the dynamic magnetization behavior of MNP during a kinetic study, and therefore adjusted the MPS measurement sequence accordingly. With the developed measurement method, we are able to investigate the kinetics of cellular uptake of MNP.

Acknowledgments

Research funding: This project was partially funded by the German Research Foundation (DFG) within the research grant “CellMPI” (455706279) and the collaborative research centre “Matrix in Vision” (SFB 1340/2 2022, no 322486779, projects A02 and B02).

Author’s statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration and has been approved by the authors’ institutional review board or equivalent committee.

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