

Proceedings Article

Development of novel nanoparticles for MPI

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Abstract

A modified procedure for preparation of iron oxide nanoparticles was used to produce nanoparticles suitable for magnetic particle imaging (MPI). Cationic and anionic maghemite particles were prepared and coated by poly(N-(2-hydroxypropyl)methacrylamide)-co-N-(2-(hydroxyamino)-2-oxo-ethyl)-2-methyl-prop-2-enamide. Bare and coated particles were characterized by magnetometry, dynamic light scattering, MR relaxometry, transmission electron microscopy and tested *in vitro* in a field-free-point MPI scanner. The nanoparticles were compared to Resovist and to differently oxidized particles dedicated for magnetic resonance imaging (control NP). The cores of particles had an average diameter 8.0 nm (cationic ones) and 8.7 nm (anionic ones), hydrodynamic diameter of coated particles in water colloids was 88 nm, and zeta potential +52 mV or -60 mV for cationic or anionic particles, respectively. Cationic particles provided lower signal-to-noise ratio than Resovist during MPI, while that of anionic particles was higher by 25 %. Also signal dispersion to surrounding voxel was smaller. The worst results (lowest signal-to-noise ratio, high signal dispersion) were reached in the case of control NP.

I Introduction

Magnetic particle imaging (MPI) is a novel noninvasive imaging technique introduced by Gleich *et al.* [1]. The method enables spatial detection of a suitable magnetic tracer. Image quality depends both on the instrumentation, and on the physical properties of the tracer. Most of the currently used tracers are based on superparamagnetic iron oxide nanoparticles (SPIONs).

Although some superparamagnetic MRI contrasts are used as tracers for MPI, they may not be always suitable. Compared to MRI, MPI requires no or low hysteresis at measuring frequencies (hysteresis might be related also to Néel and Brownian relaxations), and narrow size distribution. The particle properties are related both to core structure and particle coating [2].

We tested nanoparticles prepared according to a novel protocol, coated them and subjected them to a

row of physical measurements. The particles were then compared to Resovist (a commercially available tracer) and similar maghemite nanoparticles used for MRI [3].

II Material and methods

II.1 Preparation of maghemite nanoparticles

Aqueous $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ solutions (Sigma-Aldrich, Prague, CZ) were used for preparation. The solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.2 M, 100 mL) was treated with aqueous ammonia 0.5 M, 100 mL (Sigma-Aldrich). Then $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ solution (0.2M, 55 mL) was added. The dispersion was poured into aqueous ammonia (0.5 M, 250 mL) and stirred for 1 hour. Black magnetite particles were formed. The supernatant was poured out and the sedimented particles were purified by addition of wa-

ter followed by magnetic separation. Supernatant was poured out, and the nanoparticles were dispersed in water (20 mL). Cationic colloid ($\gamma\text{-Fe}_2\text{O}_3^{\oplus}$) was obtained by addition of HCl (0.2 M, 9 mL, Lachner, Neratovice, CZ) whereas anionic colloid ($\gamma\text{-Fe}_2\text{O}_3^{\ominus}$) was prepared by addition of trisodium citrate dihydrate solution (0.1 M, 12 mL, Sigma-Aldrich) before oxidation with H_2O_2 (3 %, 6 ml). Both colloids were filtered through 0.45 μm filter and their concentration was set to 60 mg/ml.

Zeta potential was +52 mV (cationic particles), or 60 mV (anionic ones).

For comparison, particles prepared by a different procedure described in [3] by oxidation by sodium hypochlorite, with zeta potential $\zeta = -55$ mV were used (control NP).

Both cationic and anionic magnetic colloids, and control NPs were coated by poly(N-(2-hydroxypropyl) methacrylamide)-co-N-[2-(hydroxy-amino)-2-oxo-ethyl]-2-methyl-prop-2-enamide (poly(HPMA-co-HAO)). Introduction of amine, carboxylic acid and hydroxamic acid functional groups to the structure enabled to reach stability at various pH, high ionic strength or protein presence.

The uncoated and coated nanoparticles were characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), magnetometry, and MR relaxometry.

A field-free point MPI scanner was used for in vitro tests. A simplified scanning protocol was used for nanoparticle testing. Calibration for each sample (8 μL sample, FOV 10x10x6 mm³, matrix 5x5x3, DF = 8 mT, SF = 2.5 T/m) was followed by scans of the same sample in different positions within FOV. A signal-to-noise ratio, signal dispersion, and displacement was evaluated by an in-house script ISNER (Matlab, MathWorks, Natick, MA, USA).

Resovist[®] used as a reference was treated similarly.

III Results and discussion

TEM images showed spherical shape and confirmed narrow size distribution of both uncoated and coated particles. The cores (uncoated particles) had an average diameter 8.0 nm ($\gamma\text{-Fe}_2\text{O}_3^{\oplus}$) or 8.7 nm ($\gamma\text{-Fe}_2\text{O}_3^{\ominus}$) compared to slightly smaller control particles (6 - 7 nm, prepared according to [3]) or Resovist (4.2 nm, [4]).

The hydrodynamic diameter of both $\gamma\text{-Fe}_2\text{O}_3^{\oplus}$ and $\gamma\text{-Fe}_2\text{O}_3^{\ominus}$ colloid samples in water was 88 nm. The control particles showed similar value (85 nm), while Resovist is smaller (45 - 62 nm).

The coating polymer substantially improved suspension stability. Polydispersity index (PI) was 0.13 for $\gamma\text{-Fe}_2\text{O}_3^{\oplus}$ and 0.16 for $\gamma\text{-Fe}_2\text{O}_3^{\ominus}$ coated nanoparticles. The suspensions were stable in the range of pH 4 to 10.

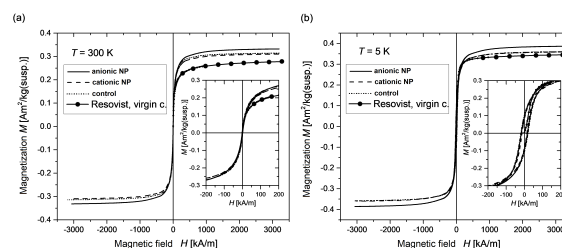


Figure 1: Hysteresis loops of the different samples at (a) 300 K and (b) low 5 K. The magnetization values are based on the weight of the suspension with the concentration of 4.4 mg (Fe_2O_3)/mL that was dried for the measurement (the data for Resovist were scaled to correspond the same concentration).

III.I Magnetometry

The hysteresis loops of anionic $\gamma\text{-Fe}_2\text{O}_3^{\ominus}$, cationic $\gamma\text{-Fe}_2\text{O}_3^{\oplus}$, control particles, and Resovist at room and low temperatures are depicted in Fig. 1 together with virgin curves of Resovist supplemented for comparison. The low-field details of the loops show anahysteretic behavior at 300 K (Fig. 1a), whereas hysteresis with coercivity of ≈ 20 kA/m is observed for all samples at 5 K (Fig. 2b).

Although static loops do not provide a complete picture, they demonstrate high magnetization of newly synthesized particles, which is advantageous for MPI. However, to assess relaxations on the relevant time scale, which have a crucial impact on the MPI signal, magnetic studies, namely AC susceptibility measurements, should be performed at frequencies corresponding to those used at MPI scanners.

III.II Magnetic particle imaging

A simple measurement protocol was introduced for nanoparticle testing. The used method represents a phenomenological approach for evaluation of the tracer, which may assess practical usability of the tracers, albeit it did not include exact evaluation.

Signal-to-noise ratios (S/N) of the tested samples (coated cationic, anionic, control nanoparticles, and Resovist) are summarized in the Table 1. While cationic nanoparticles reached lower S/N than Resovist, the anionic ones reached a higher signal than Resovist. Low S/N was found also in a control sample (differently oxidized nanoparticles).

Similarly, signal dispersion to surrounding voxels (data not shown) was higher in the case of cationic particles and lower in anionic ones compared to Resovist.

Signal dislocation (relative maximum signal outside the correct position) represented a serious problem for samples containing the control iron oxide particles. In this case, we observed complete displacement (i.e., the highest signal was localized in a voxel corresponding to an empty space).

Table 1: Signal-to-noise ratio and signal dislocation in MPI image of the tested samples containing 8 μL of anionic $\gamma\text{-Fe}_2\text{O}_3^\ominus$ nanoparticles, cationic $\gamma\text{-Fe}_2\text{O}_3^\oplus$ nanoparticles, control particles, and Resovist.

Nanoparticles	S/N	Signal dislocation (%)*
$\gamma\text{-Fe}_2\text{O}_3^\ominus$ coated	120 ± 11	20 ± 14
$\gamma\text{-Fe}_2\text{O}_3^\oplus$ coated	49 ± 13	70 ± 28
Control coated	84 ± 38	Complete dislocation**
Resovist	100 ± 15	29 ± 9

*100 % corresponds to the same signal intensity in a random voxel as is found in the correct position.

**The signal in the actual sample position was in several cases 0, which ruled out relevant calculation of the ratio.

Interestingly, substantial differences were found between anionic and cationic particles, although the core structure should have been the same. Neither TEM nor DLS revealed any substantial differences in their size or tendency to aggregate. Anionic particles performed better than Resovist, but not the cationic ones; we speculate that difference in charge might be responsible for different interactions in the water suspension, which might affect Brownian relaxation time. Estimation of relaxation times will be a subject of further research. We assume that the coating might decrease absolute value of zeta potential, however, it needs to be experimentally verified to explain this peculiar result.

The control NPs prepared according to [3] reached the worst results. Low S/N, high signal dispersion to the surrounding voxels or even signal dislocation was observed. Several phenomena may contribute to this: smaller size of the cores, different arrangement of the cores, which possibly affected Néel relaxations. Although they were reported as a superb contrast agent for MRI [3], our findings disqualified them as an MPI tracer.

IV Conclusions

The presented synthesis led to slightly bigger $\gamma\text{-Fe}_2\text{O}_3$ cores with a narrow size distribution than the previously used method of preparation [3], which is advantageous for MPI. The charge of the particles together with the poly(HPMA-co-HAO) coating contributed to suspension stability. The coating ensured good stability at broad range of pH, which would be necessary for possible future in vivo applications. Usability was confirmed by a row of physical tests and by a simple in vitro MPI measurement. Anionic particles provided better results than commercially available tracers.

Author's Statement

Research funding: Czech Science Foundation - Project No. 19-02584S, Ministry of Education, Youth and Sports of the Czech Republic - Czech-BioImaging LM2015062, National Sustainability Program II (Project BIOCEV-FAR LQ1604) Conflict of interest: Authors state no conflict of interest

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