Journal of Magnetism and Magnetic Materials 380 (2015) 266–270

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Hydrodynamic and magnetic fractionation of superparamagnetic nanoparticles for magnetic particle imaging

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ARTICLE INFO

Article history:
Received 30 June 2014
Accepted 16 August 2014
Available online 30 August 2014

Keywords:
Superparamagnetic nanoparticles
Asymmetric flow field–flow fractionation
Magnetic particle spectroscopy
Magnetorelaxometry
Magnetic separation
Magnetic particle imaging
Resovist®

ABSTRACT

Resovist® originally developed as a clinical liver contrast agent for Magnetic Resonance Imaging exhibits also an outstanding performance as a tracer in Magnetic Particle Imaging (MPI). In order to study the physical mechanism of the high MPI performance of Resovist®, we applied asymmetric flow field–flow fractionation (A4F) and static magnetic fractionation (SMF) to separate Resovist® into a set of fractions with defined size classes. As A4F based on an elution method separates MNP according to their hydrodynamic size, SMF fractionates a particle distribution by its magnetic moment. The obtained fractions of both separation techniques were then magnetically characterized by magnetorelaxometry measurements to extract the corresponding effective magnetic anisotropy and hydrodynamic size distribution parameters. Additionally, the MPI performance of each fraction was assessed using magnetic particle spectroscopy. With both separation techniques fractions (normalized to their iron amount) an MPI signal gain of a factor of two could be obtained, even though the distribution of effective anisotropy and hydrodynamic size were significantly different. Relating these findings to the results from magnetic characterization allows for a better understanding of the underlying mechanisms of MPI performance of Resovist®. This knowledge may help to improve the design of novel MPI tracers and development of separation methods.

1. Introduction

The properties of magnetic nanoparticles (MNP) are strongly dependent on their size, which often exhibit a broad distribution. To this end, in many applications only a small proportion of particles contribute to the desired magnetic effect. This applies in particular to the novel imaging modality magnetic particle imaging (MPI) which is based on the nonlinear magnetization properties of MNP, the so called tracer and allows for background-free imaging of MNP distributions in living organisms with high spatial and excellent temporal resolution [1]. Besides the development of applicable MPI scanner systems and methods for image reconstruction, the optimization of MNP properties are of prerequisite for MPI image quality at present [2]. This is due to the fact that both, sensitivity and spatial resolution of MPI are strongly influenced by the magnetic characteristics of the tracer, particularly the MNP core size and anisotropic contributions, i.e. shape and crystal structure [3].

So far, Resovist® has been mostly used as MPI tracer as it exhibits surprisingly high signal performance. Furthermore, it has the advantage to be clinically approved for in-vivo MR imaging and is therefore widely tested. Nevertheless, Resovist® is far away from being optimized for MPI. Studies revealed that this sample exhibits a bimodal size distribution, consisting of small primary particles, some of which form stable aggregates (multi-core particles) [4]. Therefore, the isolation of this large sized particle population responsible for the high MPI signal is of great interest. Several attempts were made to separate Resovist® magnetically [5,6] and characterize the obtained fractions with regard to MPI performance [7–9]. Furthermore, asymmetric flow field–flow fractionations (A4F) were performed to gain insight into the distribution of hydrodynamic sizes of Resovist® as this method gently produces fractions of narrow hydrodynamic size distribution and permits highly reproducible and accurate size evaluations [4,10,11]. In addition, A4F was also shown to be suitable for the preparation of fractions with different magnetic properties [12]. In the present work, we compare the impact of both fractionation

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http://dx.doi.org/10.1016/j.jmmm.2014.08.057
0304-8853/

methods on MPI signal enhancement of Resovist® with respect to structural differences. To estimate the distribution of hydrodynamic sizes and to evaluate the separation process samples were measured by dynamic light scattering (DLS) and magnetorelaxometry (MRX) in liquid state. In addition, MRX measurements on samples with immobilized MNP were performed to gain information about the distribution of effective magnetic core sizes and anisotropy energies E_A which determine the responsiveness of MNP to the MPI excitation field. This is of essential interest as Ferguson et al. [13] experimentally found an MPI tracer size optimum at 20 nm. Conversely, simulations from Weizenecker et al., taking into account the anisotropy contributions, showed that larger MNP are expected to perform better in MPI [3]. They found 25 nm to 30 nm particles at $E_A/k_BT=1.7$ and 1.2, respectively, to be responsible for Resovist®'s superior MPI performance.

For the assessment of MPI performance of the individual fractions we used a magnetic particle spectrometer (MPS), which can be considered as a zero-dimensional MPI scanner.

2. Materials and methods

2.1. Samples

The separations were performed on DDM128 (Meito Sangyo, Japan) an aqueous suspension of iron oxide nanoparticles coated with carboxydextran which is a precursor of the clinical formulation Resovist® [14]. Suspension material was first centrifuged gently at 3000 g for 1 min to remove highly aggregated particles and the supernatant with a final iron concentration of 411 mmol/L was denoted as sample of initial state.

Deionized water containing 0.2% (v/v) FL70 detergent (Fisher Sci., USA) was used as carrier liquid for A4F. Triton X-100 diluted in water to a final concentration of 0.05% (v/v) was used as a wetting agent to prepare the SMF column. For the elution of the particles carboxydextran solution (0.25 g/mL) was used to prevent aggregation of the fractions due to detachment of the stabilizing coating. All liquids were degassed prior to use.

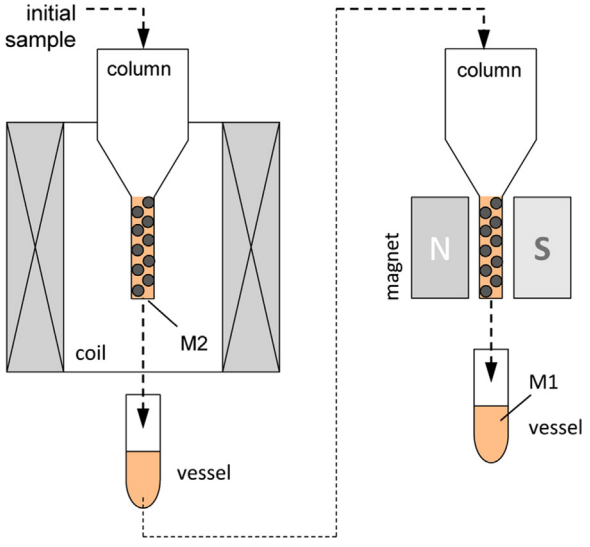

Fig. 1. SMF procedure: In the presence of a magnetic field of $B=12$ mT (generated by a coil) Resovist® was rinsed through a separation column (filled with soft magnetic spheres). The eluted negative fraction was collected for the following separation step. After the magnetic field was decreased down to 1.5 mT the retained MNP of larger magnetic moments were washed out (fraction M2). Subsequently the second separation was performed on the negative fraction of the previous step now in an increased magnetic field of 500 mT. The smallest MNP not attracted were collected (fraction M1).

For magnetic measurements (MRX, MPS) a sample volume of 30 μL sample volume was filled in PCR tubes (fast reaction tube with cap, Appl. Biosystems, USA). Additionally, MNP were immobilized by adding mannitol solution (7% w/v) to the sample before freeze-drying for the purpose to preserve the sample volume and uniform distribution of MNP.

The iron concentration of each sample was determined by photometry using MNP dissolved in hydrochloric acid and stained by Perl's Prussian Blue reaction.

2.2. SMF

Magnetic fractionation was performed using a commercially available separation column (MS column, Miltenyi Biotec, Germany). The columns capture bed consists of soft magnetic iron spheres to create local magnetic gradients in an applied magnetic field. To generate small magnetic fields up to 12 mT we used a 10 layer copper coil (200 mm height, 125 windings per layer). For a large field strength of 0.5 T a commercial separator (MiniMACS™, Miltenyi Biotec, Germany) was used. Before fractionation the separation column was washed with degassed Triton X-100 solution to prevent clogging and to ensure homogeneous flow conditions.

To separate large magnetic moment MNP 400 μL of the sample of initial state were poured onto the column during the presence of a small magnetic field of 12 mT. After collecting the negative fraction the column was gently washed with 200 μL of Triton X-100 solution. Subsequently, the magnetic field was decreased down to 1.5 mT and the majority of the captured MNP, fraction M2, were eluted using 200 μL of carboxydextran solution. The separation procedure was then repeated with an increased magnetic field strength of 500 mT using the negative fraction of the previous separation. Small magnetic moments not captured by the magnetic force were eluted within the negative fraction M1 (Fig. 1).

2.3. A4F

For hydrodynamic size separation of the Resovist® particles we used A4F which is based on an elution method where the hydrodynamic diameter d_{hyd} of an MNP is related to its retention time t_r within a separation channel with a rectangular cross section, where two perpendicular forces act on MNP. While the longitudinal flow V_{Vol} carries the MNP through the separation channel, the cross flow V_x moves the particles towards the bottom of the channel which consists of a semi-permeable ultrafiltration membrane. Due to the smaller diffusion coefficient larger particles are accumulated in an average distance closer to the membrane than smaller particles. Hence, due to the parabolic flow profile of the narrow channel larger particles, moving in slower flow lines, are eluted later (see Fig. 2). This can be approximately described by Giddings equation [15]:

$$t_r = \pi\eta\, d_{hyd} h \exp(2)/(2k_BT)V_{Vol}/V_x \quad (1)$$

where k_BT is the thermal energy, η is the viscosity of the carrier liquid, d_{hyd} is the hydrodynamic diameter of the MNP, h is the channel height, and V_{Vol} is the volumetric flow rate through the channel.

The A4F unit (Postnova Analytics GmbH, Germany) consisted of an AF2000 focus system (PN 5200 sample injector, PN 7505 inline degasser, PN 1122 tip and focus pump). As a supplemental feature the A4F unit was equipped with a slot outlet technique for increased sensitivity and elevated concentration of the resulting fractions [10].