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Physical characterization of biomedical magnetic nanoparticles using multi-detector centrifugal field-flow fractionation

Abstract: The unique magnetic properties of magnetic nanoparticles (MNP) combined with their small size already led to numerous medical applications. Accurate determination of their magnetic properties is a key requirement enquired by users, that is impeded by the ever-present distribution of MNP sizes. Field flow fractionation (FFF) techniques may help to overcome these limitations by first separating the particles before characterization. In this study, we demonstrate the use of centrifugal FFF coupled to online detectors for fractionation, structural, and magnetic characterization of MNP. The primary goal is to establish a reproducible centrifugal FFF (CF3) method for MNP fractionation. We show that CF3 has the same capability as other FFF techniques in resolving the bimodal hydrodynamic size distribution present in the commercial MNP system Resovist® but is faster and more straightforward through its technical approach.

Keywords: field-flow fractionation, magnetic nanoparticles

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1 Introduction

The unique magnetic properties of magnetic nanoparticles (MNP) combined with their small size already led to numerous medical applications. They are used in in-vitro diagnostics for magnetic separation, as contrast agents in in-vivo imaging diagnostics or in tumor therapy [1]. For each of these applications, MNPs with specific structural and magnetic properties must be provided. Reliable determination of their structural and magnetic properties is a key requirement enquired by users, that is impeded by the always existing distribution of MNP sizes. Typically, ensemble methods such as dynamic light scattering (DLS) to extract hydrodynamic diameters or multi-angle light scattering (MALS) to determine MNP core diameters provide acceptable results but with poor selectivity when

applied to mixed particle populations. The same applies to the magnetic characterization of samples with a broad distribution of properties by magnetic techniques like magnetic particle spectroscopy (MPS). This complicates the optimization and development of MNP for a specific application, since structure-property relationships can only be determined blurred by the underlying MNP distribution.

Field flow fractionation (FFF) techniques may help to overcome these limitations by separating particles according to their sized within the range of 1 nm up to 100 µm under mild operating conditions before characterization. All FFF techniques use the same basic principle but with a different selection field (fluid flow, centrifugal force, temperature gradient, or gravity) perpendicular to the main flow transporting the sample through the channel. Due to the counteracting diffusion of the particles, different equilibrium layer heights are formed, so that smaller particles elute first in the faster streamlines. FFF methods are technically challenging and require time and effort for method development. Due to the lack of established protocols, a reproducible FFF procedure has to be developed for a specific MNP system, first [2]. This focuses on selecting a mobile phase, instrument parameters, experimental conditions, and on adjusting sample concentrations to achieve low sample loss, avoid overloading, deliver high-resolution size separation, and prevent sample alteration.

Combining FFF techniques with complementary detectors offers a powerful approach for reliable sample characterization. We recently presented asymmetric flow FFF (AF4) coupled with an MPS instrument and particle size determination for multidimensional characterization of MNP [3]. In contrast to AF4, where particles are separated according to their hydrodynamic size, particles in centrifugal FFF (CF3) are separated according to their mass difference in a channel located inside a centrifuge [4,5]. It promises a straightforward and fast method since it does not require the use of a special semipermeable membrane as in AF4. Herein, we demonstrate the use of CF3 coupled to on-line UV, MALS, DLS, and MPS for fractionation and characterization of MNP. We envisage to establish a reproducible CF3 method for MNP fractionation by systematically investigating the major parameters influencing the separation behavior of MNP such as carrier fluid, rotation speed, and sample concentration. We finally exemplify the

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performance of the CF3 system by fractionation of a commercial MNP sample for biomedical applications.

2 Materials and Methods

2.1 Magnetic nanoparticles and media

Four different MNP systems (Tab. 1) were investigated with respect to their suitability for determination of the temporal offset of the detector signals and calibration of the size determination by MALS. Another system, Resovist® (RV, Bayer HealthCare, GER) was selected to investigate the applicability of the CF3 method. RV is an aqueous suspension of iron oxide particles coated with carboxydextran. Since the RV contains two particle populations (single and multicore MNP), it is ideally suited for multi-detector FFF analysis. Details of origin, coating, and some physical parameters of the MNP samples are summarized in Tab. 1. All chemicals used in this study were of analytical reagent grade. Deionized water (ddH₂O) containing FL-70 detergent (Fisher Sci., USA) and sodium chloride (NaCl, Merck, GER) was used as carrier liquid for fractionation.

2.2 Fractionation system

The mass sensitive separation of MNP was performed using a commercial CF3 system (Postnova Analytics GmbH). In CF3, the particles are driven towards the accumulation wall by centrifugal forces applied as vertical field. Due to the diffusion, the concentration $c(x)$ of the particles decreases exponentially at distance x from the accumulation wall of the channel: $c(x) = c_0 e^{-x/l}$, where c_0 is the analyte concentration at the accumulation wall ($x=0$) and l is the center-of-gravity distance from the accumulation wall at equilibrium [4,5]. The parameter l can be expressed by the dimensionless retention parameter

$\lambda = l/w$, where w is the channel thickness. For CF3, λ can be expressed as: $\lambda = 6kT / (\pi|\rho_p - \rho|w^2 r \omega d^3)$, where $T=293$ K is the temperature of the medium, k is the Boltzmann constant (1.38×10^{-23} kg m² s⁻² K⁻¹), ρ_p is the particle density, ρ is the solvent density, r is the rotor radius, ω is the angular rotation frequency, and d is the equivalent spherical MNP diameter. The retention parameter λ represents the link between theoretical and measurable experimental parameters through the retention ratio R_s , which is defined as the ratio of void time t_0 and analyte retention time t_r . R_s is related to λ by [5]:

$$R_s = 6 \lambda \coth\left(\frac{1}{2\lambda}\right) - 12 \lambda^2. \quad (1)$$

The optimal range for R_s should be approximately $0.03 \leq R_s \leq 0.2$ to obtain fast separation with adequate resolution [2]. To assess the performance of the CF3 method, the sample recovery R_w is determined according to: $R_w = m/m_0$, where m is the recovered mass after fractionation and m_0 is the injected sample mass. The recovery R_w was determined from the UV signal.

2.3 Detectors

The in-line detector array consisted of a UV detector for concentration determination, a MALS (both Postnova, GER) and DLS detector (Zetasizer NanoZS, Malvern, UK) for size determination, and a magnetic particle spectrometer (MPS-3, Bruker BioSpin, GER) for magnetic characterization. Angular dependent scattering profiles of MALS were used to obtain the root-mean-square radius, commonly named radius of gyration r_g . The core diameter d_c was calculated by the following equation: $d_c = \sqrt{20/3} \cdot r_g$. The hydrodynamic size d_{hyd} was determined by means of DLS measuring the fluctuations of the scattered light at an angle of 173° to determine the diffusion coefficient D , which is inversely proportional to d_{hyd} . In MPS a sinusoidal excitation field of $B=25$ mT amplitude at a frequency $f_0=25$ kHz is applied to an MNP sample. Due to their non-linear magnetization, the measured response of the MNP contains

Table 1: Summary of the five different MNP types used in this work. Given are the name, the ID used in the graphs together with the name of the supplier and the coating of the MNP. Furthermore, the hydrodynamic diameter d_{hyd} (z-average) and polydispersity index (PDI) obtained by DLS and characteristic MPS parameters A_3^* normalized to iron amount and shape parameter A_5/A_3 as determined for the stock suspension are presented. The numbers in parentheses denote the uncertainty of the last digit, e.g. 58.3(1) reads as 58.3±0.3 nm.

sample	ID	supplier	coating	d_{hyd} / (nm)	PDI	A_3^* / (Am ² /kg(Fe))	A_5/A_3 / (%)
Resovist®	RV	Bayer Healthcare	Carboxy dextran	58.3(1)	0.29(5)	8.67(3)	41.31(1)
HK116F3	HK	Charité	Carboxy methyl dextran	72.2(4)	0.19(4)	22.29(4)	34.05(2)
NL60	NL	Universität Hamburg	Siliciumdioxid	49(2)	0.12(2)	4.55(8)	6.44(6)
SHP 25-50	S25	Ocean Nanotech	carboxylic acid	58(2)	0.13(6)	20.62(3)	41.56(1)
SHP30-10	S30	Ocean Nanotech	carboxylic acid	70.48(7)	0.14(1)	10.96(5)	22.16(3)

odd multiples of f_0 (i.e. higher harmonics A_i). The third harmonic amplitude A_3 is directly proportional to the absolute MNP content and can be used for MNP quantification. To compare different MNP systems, the specific amplitude A_3^* is calculated; that is the A_3 amplitude normalized to the absolute iron mass of the sample. The shape of the spectrum is characterized by concentration independent ratio A_5/A_3 describing the dynamic magnetic behavior of the MNP.

3 Results

3.1 Calibration

The different MNP systems (HK, NL, S25, and S30) were compared and characterized regarding their suitability for temporal offset correction and calibration. S25 was selected because of its narrow (hydrodynamic) size distribution, as observed by the DLS parameter PDI, combined with a sufficient high magnetic signal A_3^* (Tab. 1). S25 was used to calibrate the UV detector and the MALS to obtain concentration $c(\text{Fe})$ and core size d_c of the MNP in the subsequent fractionations. Finally, the detector offsets between UV, MALS, MPS and DLS were determined and corrected for all subsequent experiments (Fig. 1).

Figure 1: Normalized elugrams of S25 to determine the temporal offset between UV, MALS, MPS and DLS detector. The given numbers indicate the elution volume at peak maximum, where I represents the intensity of the detector signal.

3.2 Medium

The composition of the medium has a decisive influence on the fractionation success in all FFF techniques. Typically, NaCl is used to reduce the repulsive forces between the charged particles allowing them to more closely approach the channel wall and to achieve a better separation. The addition of surfactants such as FL-70 is used to inhibit agglomeration and promote stability. By integrating the calibrated UV signal (to $c(\text{Fe})$) we found that 100 % of the injected sample is recovered when the concentration of NaCl is less than 1 mmol/L and

the concentration of FL-70 is less than 1 vol-%. Interestingly, using pure ddH₂O 100 % sample recovery was measured by UV as well. Furthermore, a better resolution of the two particle populations present in RV was achieved (Fig. 2). Therefore, ddH₂O was used for all subsequent experiments.

Figure 2: UV signal of RV depending on elution volume for different NaCl concentrations and for pure water.

3.3 Rotation velocity

The most important instrumental parameter affecting the fractionation success in CF3 is the rotation speed v_{rot} . To determine the most suitable rotation velocity for MNP fractionation, v_{rot} was varied from 100 min⁻¹ up to 4900 min⁻¹. In Fig. 3 the retention R_s for RV as a function of v_{rot} is shown together with the calculated R_s values assuming compact MNP of 13 nm and 18 nm core size. For $v_{\text{rot}} < 1960$ min⁻¹ R_s could not be determined due to insufficient peak separation. At $v_{\text{rot}} < 1950$ min⁻¹, the larger fraction of RV (multicore MNP) is not sufficiently separated from the smaller fraction eluting first. For $v_{\text{rot}} > 2000$ min⁻¹, R_s was found to be in the optimum range for FFF (between 0.2 and 0.03). Therefore, for further fractionations $v_{\text{rot}} = 4900$ min⁻¹ was chosen since it allows best separation of the two populations in RV.

Figure 3: Retention R_s depending on the rotation velocity for determination of a suitable rotation velocity for RV. The grey shaded area highlights the expected R_s value for MNP sizes between 13 nm and 18 nm resulting from (1).

3.4 Concentration

We found that MNP concentration $c(\text{Fe})$ (of the injected volume) has a significant influence on the separation performance of RV. At high $c(\text{Fe}) > 30 \text{ mmol/L}$, the two particle populations in RV could not be resolved (Fig. 4). Furthermore, at very low concentrations $c(\text{Fe}) < 5 \text{ mmol/L}$, the measured signals of the other detectors were too small to allow accurate characterization (inset Fig. 4). To enable the best possible separation of particle populations, while maintaining high measurement signals in MPS, a concentration of $c(\text{Fe}) = 10 \text{ mmol/L}$ is most suitable for investigation of RV.

Figure 4: MPS (top) and UV (bottom) elugrams of RV separated at different MNP concentrations $c(\text{Fe})$. At high $c(\text{Fe})$ insufficient separation of the two particle populations occurs. At low $c(\text{Fe}) < 10 \text{ mmol/L}$ MPS detector signals are dominated by noise (black line, upper graph).

3.5 Fractionation

For characterization of RV using CF3 we selected ddH₂O as carrier liquid, a rotation velocity $v_{\text{rot}} = 4900 \text{ min}^{-1}$, and an iron concentration $c(\text{Fe}) = 10 \text{ mmol/L}$. In this way, we were able to provide an optimal separation of the two particle populations

Figure 5: UV (top, red), MALS (bottom, red), MPS (top, black), and DLS (bottom, black) elugrams during CF3 of RV. With the help of CF3, a separation of the two particle populations in RV was achieved. The complementary detectors show the broad distribution of properties (size, magnetism) in RV.

in RV (Fig. 5, UV signal). The size determination by MALS and DLS (Fig. 5, lower graphs) resulted in $d_{\text{hyd}} = 22 \text{ nm}$ for the small particle population (since size determination by MALS is only possible from $d_c \approx 20 \text{ nm}$, the small fraction of RV could not be resolved) and $d_c = 21 \text{ nm}$ and $d_{\text{hyd}} = 46 \text{ nm}$ for the large particle population. Due to the significantly higher magnetic moments of the MNP in the larger population, a larger A_5/A_3 value of 45 % than for the small particle population with $A_5/A_3 = 32 \%$ was measured by MPS (Fig. 5, MPS). These values agree with previous fractionations of RV using AF4 [3].

4 Conclusion

We showed that using the multi-detector CF3 method, MNP can be accurately fractionated into their component populations. With the in-line multi-detector platform, the corresponding size and magnetic properties can be determined for each fraction, regardless of whether the presence of a monomodal or multi-modal distribution. Compared to established separation methods, such as AF4, method development with CF3 is much simpler and faster because the sample does not interact with the separation channel. In contrast to AF4, where the sample is pressed against a special semi-permeable membrane, no interaction occurs in CF3. For this reason, adjusting the ionic strength in the carrier medium does not alter the CF3 separation performance. We conclude that for the bimodal MNP system RV the same separation performance and information gain is achieved with both FFF techniques, CF3 and AF4. Using CF3 the MNP quality attributes (size, magnetism) can be accessed even faster and thus support the research and development of biomedical MNP applications.

Author Statement

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References

- [1] Krishnan KM, Fundamentals and Applications of Magnetic Materials, Oxford University Press, 2016.
- [2] Gigault J, Pettibone J, Schmitt C, Hackley V. Rational strategy for characterization of nanoscale particles by asymmetric-flow field flow fractionation: A tutorial. *Anal. Chim. Acta* 214;809:9–24. <https://doi.org/10.1016/j.aca.2013.11.021>.
- [3] Löwa N, Radon P, Gutkelch D, August R, Wiekhorst F. Hyphenation of Field-Flow Fractionation and Magnetic Particle Spectroscopy. *Chromatography* 2015;2:655–68.
- [4] Giddings JC, Yang FJF, Myers MN. Sedimentation field flow fractionation. *Anal. Chem.* 1974;46:1917–24.
- [5] Janka J, Chmelik J. Focusing in Field-Flow Fractionation. *Anal. Chem.* 1984;56:2481–248