

CLASSIFICATION OF ANALYSIS METHODS FOR CHARACTERIZATION OF MAGNETIC NANOPARTICLE PROPERTIES

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Abstract – The aim of this paper is to provide a roadmap for the standardization of magnetic nanoparticle (MNP) characterization. We have assessed common MNP analysis techniques under various criteria in order to define the methods that can be used as either standard techniques for magnetic particle characterization or those that can be used to obtain a comprehensive picture of a MNP system. This classification is the first step on the way to develop standards for nanoparticle characterization.

Keywords: standardization, metrology, magnetic nanoparticle, iron oxide particles

1. INTRODUCTION

During the last years, the number of applications where nanostructured objects are used has strongly increased. Nanostructures are applied for instance in electronics, environment, medicine or cosmetics. In these applications it is utilized that nanoobjects show significantly altered physical properties compared to the properties of macroobjects or bulk materials [1]. Thus, nanostructures give access to novel techniques or they can be used to improve existing applications.

Magnetic nanoparticles (MNPs) are a significant class of these novel nanoobjects and their application in biomedicine is under intensive investigation [2]. MNPs are used for diagnostic applications as contrast agents in imaging [3] or tracers [4], for therapeutic applications such as magnetic drug targeting [5] and magnetic hyperthermia [2], and for *in vitro* techniques like magnetic separation [6],[7] or magnetofection [8]. The key benefit of the use of MNPs is

that due to their magnetic moment, they can be *in situ* manipulated by externally applied magnetic fields. Moreover, the particles can be located and imaged in tissue by noninvasive techniques without applying harmful ionizing radiation.

2. DEMAND FOR STANDARDIZATION OF MAGNETIC NANOPARTICLE CHARACTERIZATION

An open issue in the use of MNPs in biomedical applications is the standardization of their physical properties and conditions of application. Regulatory work is required to guarantee a safe and effective implementation of advanced techniques for application of magnetic nanoparticles in the human body. This comprises a reproducible production of nanoparticles with desired physical and (bio)chemical properties as well as analysis techniques to characterize the particle properties as accurately as needed in a metrologically traceable way. For a practical application, the characterization techniques have to be cost effective, easy to handle and they should provide reproducible results on MNP properties. Within the framework of a European FP7 project "Nanometrology Standardization Methods for Magnetic Nanoparticles (NanoMag)" [9], we have developed a roadmap for the standardization of characterization of magnetic particles to be used in medical applications.

A stakeholder committee was formed by interested members from industry and academia. It is closely connected to the NanoMag project and provides valuable guidance for the standardization process. The committee

represents large companies and SMEs and researchers from different application fields (MNP synthesis, instrument developers and end-users).

It is known from the literature that the static and dynamic magnetic behaviour of MNP systems vary significantly depending on the chemical composition and physical structure of the actual MNP system and on the interplay between the MNPs and the surrounding matrix [10]-[13]. Thus, it is important first to identify classes of MNPs that share common principal behaviours.

In a second step, the physical measurement methods as well as the mathematical models and data analysis methods exploring the properties of the respective MNP classes have to be described.

3. METROLOGY OF MAGNETIC NANOPARTICLES

During the process of standardization of MNP characterization, we first selected MNP systems suitable for the analysis methods. Here, we focused our investigations on a limited number of single- or multi-core superparamagnetic iron oxide nanoparticles that are well established as contrast agents in magnetic resonance imaging (MRI) [14] or have been applied already in magnetic drug targeting, magnetic hyperthermia [15], magnetic separation techniques [16] and magnetic particle spectroscopy [17]. The MNPs are synthesized by the manufacturing partners of NanoMag or purchased from commercial suppliers. Various synthesis processes and a variety of functional groups on the particle surface ensure that a wide range of existing MNP systems is covered [18]. An example for particles, which have been synthesized within the NanoMag project, is shown in Fig. 1 in Transmission Electron Microscopy (TEM) images. The left image shows MNPs with a single magnetite core surrounded by a silica shell. In the right image, multi-core particles are shown where several crystallites are imbedded in the silica coating. If the non-magnetic shell layer of the single-core particles ensures a sufficient distance between the magnetic cores, the magnetic behavior of the single-core nanoparticles can be modeled as those of independent, non-interacting particles. In contrast, in the case of multi-core particles, the dipolar interaction between them has to be considered due to the small distance between the crystallites. These are two main distinctive classes of particles that require separate

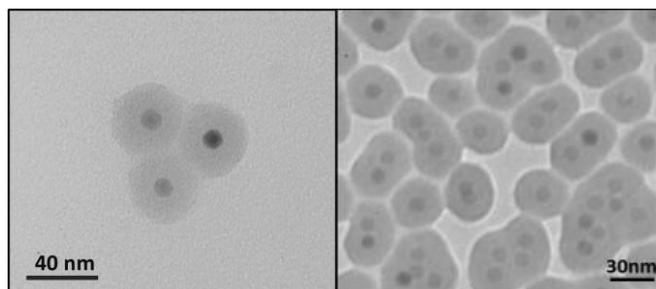


Fig. 1: TEM images of single-core (left) and multi-core (right) magnetic nanoparticles.

descriptions of the MNP system properties and separate approaches to standardization.

MNPs are complex physically, chemically and even biologically active systems. They require monitoring of a large number of different physical properties in order to achieve a reproducible and safe application in a biomedical environment. Their most important parameters are: hydrodynamic size distribution, nanocrystal size distribution, aggregate size distribution, particle shape and morphology, crystal structure, surface coating thickness, chemical composition, binding efficiency, particle concentration in a suspension, magnetic coercive field, magnetic saturation field, total magnetic moment, spin structure, initial magnetic susceptibility, critical temperatures, magnetic relaxation time, effective anisotropy, particle surface charge and specific energy absorption rate. Depending on the application of the MNPs, further parameters may be specified.

We apply a number of common analysis techniques to investigate structural MNP properties as well as their static and dynamic magnetic behavior under influence of external magnetic fields and temperature changes. We also investigate application-oriented methods that are closely linked to existing biomedical applications (e.g. magnetic separation or magnetic hyperthermia). In particular, we investigated whether different analysis techniques provide consistent physical parameters of the particles, bearing in mind that the results of different techniques are interrelated with each other.

A large number of methods exists that can be utilized for nanoparticle characterization. Every method varies in complexity and may determine, directly or indirectly, a limited number of particle parameters. To evaluate the suitability of a method to be chosen as a basic/standard characterization method, the following aspects are important:

- **Standardization of the measurement technique**
Are traceable standards and procedures available? How sensitive is the method?
- **Sample amount**
What is the typical sample amount required for the analysis?
- **Throughput**
What is the typical time required for sample preparation, measurement and data analysis?
- **Ease-of-use**
What level of user expertise is required for sample preparation, measurement and data analysis?
- **Availability**
Is the method widely available? What is the planning effort before a measurement can be performed? How is the method regarded in the scientific community (standard method or basic research tool)?
- **Low cost**
Is the technique commercially available? What is the cost of an instrument? What is the cost for a single measurement?

Partners of the NanoMag consortium being experts in the field of nanoparticle research have answered these questions

for a number of analysis techniques. Based on this, a comprehensive description of measurement methods has been created to identify the methods to be used as standard methods for MNP characterization and those to be used to obtain a more detailed picture of an MNP system.

3.1. Results

The measurement techniques for MNP have been classified into methods for structural and magnetic characterization as well as application-oriented methods. It should be noted that some techniques provide information in several categories (marked by *).

(1) Techniques for structure, chemical composition and particle size determination

- Scanning Electron Microscopy (SEM)
- Transmission Electron Microscopy (TEM)
- X-Ray Diffraction (XRD)
- Neutron Diffraction (ND)*
- Dynamic Light Scattering (DLS) + Zeta potential
- Asymmetrical Flow Field-Flow Fractionation (AF4)
- Small-Angle X-ray Scattering (SAXS)
- Small-Angle Neutron Scattering (SANS)*
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
- Mössbauer Spectroscopy*
- X-ray Absorption Fine Structure spectroscopy (XAFS)

(2) Techniques for magnetic measurements

- DC Magnetometry, magnetization vs. field and temperature (DCM)
- Cavity-based Ferromagnetic Resonance (FMR)
- AC magnetic Susceptibility vs. frequency (ACS vs. f)
- AC magnetic Susceptibility vs. temperature (ACS vs. T)
- Magnetorelaxometry (MRX)
- Magnetic Particle Spectroscopy (MPS)
- Rotating Magnetic Field (RMF) (phase lag φ vs. f)

(3) Application oriented techniques

- Magnetic Separation (MagSep)
- NMR Relaxivity (R1R2)
- Magnetic Hyperthermia treatment
- On-chip AC Susceptometry (Chip-ACS)

Note that DLS and Zeta potential measurements provide different MNP properties, however some benchtop instruments offer to measure both of them within the same device.

Fig. 2 shows as an example the detailed assessment of Dynamic Light Scattering (DLS) and Neutron Diffraction (ND). Here, the rating of the method (range:1-5) is plotted against different categories. Equal weight was given to all questions within a category. A high score means that the method has potential to serve as a common technique for particle characterization and therefore it should be standardized. A high number can result for example from low measurement costs, from a high throughput or from possibilities to provide fundamental particle properties that

can be measured only with this method. A low score means that the method is highly advanced and can be used to determine additional, rather than fundamental particle parameters.

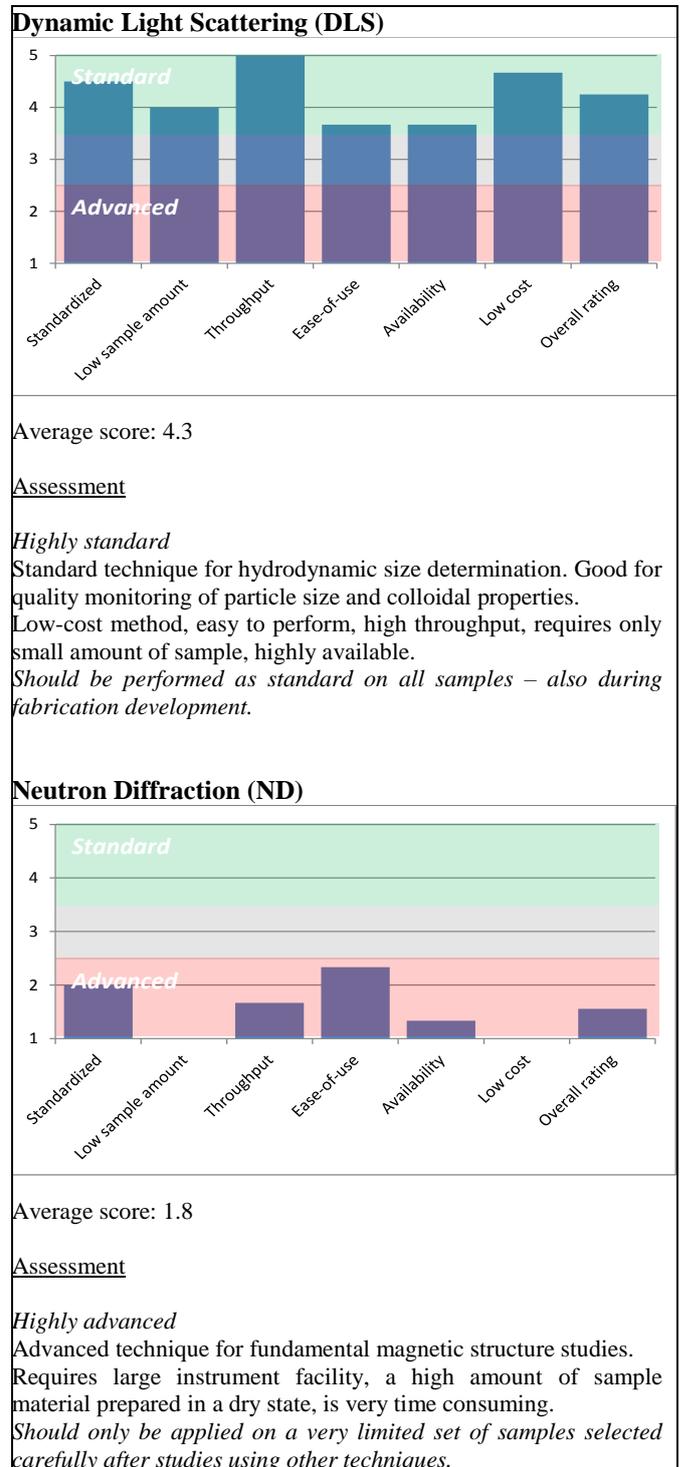


Fig. 2: Scoring of DLS and ND as MNP analysis methods. The high score for DLS for MNP shows that the method is a candidate to be standardized. On the other hand, ND is a technique that can be used to obtain a comprehensive picture of the particle properties.

The high score for DLS is due to the fact that reference samples of monodisperse particles with certified sizes are available (e.g. from NIST) and that an ISO standard (ISO 22412:2008) exists for the application of DLS to determine the average particle size and the polydispersity index [19]. In addition, DLS is a very fast measurement – the complete time for the sample preparation, the measurement and data analysis is in each case below 10 minutes - and the cost for an analysis is low, ~30-40 €.

On the other hand, ND measurements are carried out at beam line facilities for which intensive planning is needed in advance. The time for the sample preparation, the measurement and the data evaluation is in the order of hours. The required sample amount for an analysis is at least several hundred of milligrams. Furthermore, the cost for ND is very high, i.e. about 6000 € per day. There exists no ISO standard for measurement of nanomagnetic material by ND, although ND has been standardized for other purposes (ISO/TS 21432:2005, Standard test method for determining residual stresses by neutron diffraction).

From the ranking in the different categories it becomes clear that DLS is a widely available method which can be applied for assessment of MNP properties in a standardized way. ND for measuring MNP is not a good candidate for a formal standardization process due to limitations of the method that allow only a limited number of measurements. However, it is a valuable tool for fundamental research and it delivers valuable particle properties for an extensive characterization of the particle system.

In Tab. 1, an overview of the assessed methods is given. The methods are classified into standard methods being suitable for standardization of MNP and advanced techniques with research character. Intermediate methods could potentially serve as standard methods. However, their results have to be correlated with those obtained by other techniques. Note that some methods belong to several categories (e.g. Mössbauer and ND providing both structural and magnetic information).

Table 1: Result of assessment of methods

	Standard method	Intermediate method	Advanced method
Structure, composition, size	DLS, SEM, TEM, AF4, SAXS, ICP-MS	XRD, Mössbauer	XAFS, ND, SANS
Magnetic properties	DCM, ACS vs. f, ACS vs. T	FMR, MRX, MPS, RMF, Mössbauer	ND, SANS
Application oriented methods	MPS, MagSep, Hyperthermia, R1R2	Chip-ACS	

In this overview it is shown that the structural characterization of the particle core is performed by the low-throughput methods SEM, TEM and SAXS. It might be

useful to combine these methods with high-throughput methods like DCM and ACS that also provide information on the particle core. Advanced techniques such as ND or SANS are highly suited to characterize both structural and magnetic properties of MNPs. However, their limited throughput prohibits their application as standard methods for particle characterization.

It should be noted that the assessment of the methods should be taken as the assessment of the potential of each method to serve as a standard method. This assessment is based on the experimental infrastructure within the NanoMag project and on the experience of the project partners. With gaining experience during the project runtime the final recommendation may differ from this initial assessment.

3.2. Practical aspects

The overview in Tab. 2 shows for each method the typical amount (i.e. not the minimum) of magnetic material and the required sample form. The average cost for a typical single measurement is also given. It could be feasible for some applications to combine low-costs and low-mass methods in order to derive a reasonable sample characterization.

Table 2: Classification of different methods for magnetic particle characterization regarding required sample amount and form as well as cost for the measurement. The key to the sample form is: P = powder; D = colloiddally stable suspension, S = solid (particles immobilized in matrix). The cost of single measurement is coded as: €: 100€ or less; €€: 100-500€; €€€: >500€; LF = Large Facility.

Method	Typical required amount of solid sample [mg]	Sample forms (typical)	Cost of single measurement
Structure, composition, size			
SEM/TEM	<0.1	D	€€€
XRD	50-100	P	€€
ND	>200	P	LF
DLS	0.4	D	€
AF4	0.2	D	€€
SAXS	0.1	D	€€
SANS	>100	P, D	LF
ICP-MS	0.1	P, D	€€
Mössbauer	50	P, D	€€
XAFS	1	P, D, S	LF
Magnetic measurements			
DCM	0.1	P, D, S	€
FMR	0.1	P, D, S	€€
ACS vs. f	0.1	P, D, S	€
ACS vs. T	0.1	P, D, S	€€
MRX	0.1	P, D, S	€
MPS	0.1	P, D, S	€
RMF	0.1	(P), D, (S)	€

Method	Typical required amount of solid sample [mg]	Sample forms (typical)	Cost of single measurement
Application-oriented methods			
MagSep	<0.1	D	€
R1R2	0.1	D	€
Hyper-thermia	4	(P), D, (S)	€
Chip-ACS	<0.1	D	€

4. STANDARDIZATION OF MAGNETIC PARTICLE CHARACTERIZATION

Fig. 3 displays the overall process of standardization of magnetic nanoparticles within the NanoMag project. The next steps in the standardization process will be the definition of protocols for the sample preparation, standard operating procedures and physical models for the basic analysis methods. Based on this, the partners compare their analysis results and uncertainty budgets of the relevant physical parameters are established. Here, the results are compared between partners using the same analysis technique as well as the comparison of the particle properties obtained by different measurement modalities. We also test our procedures on various types of MNPs to ensure that most of the existing MNP systems are covered.

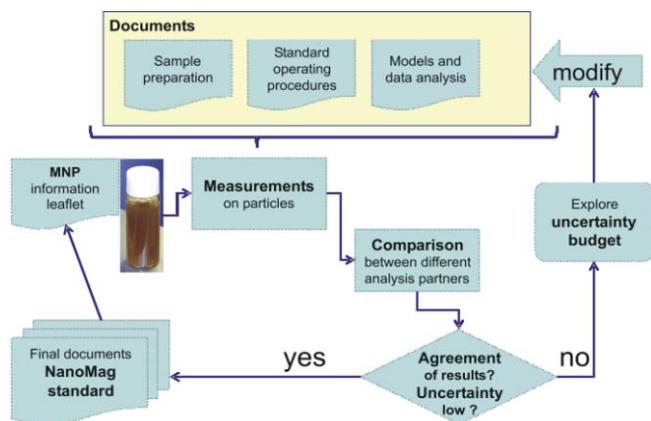


Fig. 3: Schematic view of the standardization process.

For the case that there is no agreement between the results or that the uncertainty is not sufficiently low, we will modify the sample operating procedures and the used analysis models.

This iterative process of performing the particle characterization according a defined protocol and the subsequent comparison of results between the partners is continued until the agreement of results allows setting up procedures for thorough and standardized MNP characterization with lowest uncertainty.

These procedures are the basis for a future normative document of a standardized MNP description. In 2015, the ISO organization started a process of developing a new ISO standard [20], where the knowledge gained from the

standardization process described here will play an important role in the discussion of the final document.

5. CONCLUSION

Our objectives are to standardize, improve and redefine existing analysis methods for magnetic nanoparticle characterization. The used analysis methods allow the exploration of structural and chemical particle properties, their static and dynamic magnetic behaviour under influence of external magnetic fields. Additionally, all the methods considered here are closely connected to existing biomedical applications. Within the standardization process, we have classified current, typically used analysis methods according to their potential to serve as a basic technique for the characterization work. We have identified a number of commonly used techniques, which have to undergo a standardization process with respect to the properties of MNPs. The techniques identified as advanced methods enable the investigation of more fundamental particle properties and thus, they provide valuable means to obtain a comprehensive picture of the particle system. The experience from the present analysis of characterization methods for MNP will provide valuable arguments in an ongoing formal ISO standardization process.

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