MagNaStand

FINAL PUBLISHABLE REPORT

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Coordinator: Uwe Steinhoff, PTB	Tel: +49 30 3481 7419	E-mail: uwe.steinhoff@ptb.de
Project website address: http://www.magnastand.eu/		
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RMG: -		

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1 Overview

Magnetic nanoparticles (MNP) find wide biomedical and technical applications. However, at the beginning of the project, international standards for characterising MNP were not available. This project summarised the metrological knowledge on the characterisation of MNP and brought it into the development of ISO 19807-1 "Nanotechnologies -- Magnetic nanomaterials -- Part 1: Specification of characteristics and measurements for magnetic nanosuspensions" by ISO/TC229 WG4. A close collaboration with the standardisation organisations and the involved European industry, as well as the review of previous EU research projects, ensured the wide acceptance of the new standard. A roadmap for further measurement standards for MNP has been submitted to ISO/TC229.

2 Need

Magnetic nanoparticles (MNPs) have many applications in biomedicine and other technical areas, e.g. as diagnostic tracers and therapeutic agents in cancer therapy; as carriers in cell separation from biological liquids; in magnetic seals, in the damping of audio speakers or in environmental remediation.

Prior to the start of this project, international standards for the definition and measurement of the magnetic properties of MNPs did not exist. This reduced the trust of MNP consumers with regards to the safety, reliability and functionality of MNP products and it hampered the market chances of European MNP producers, mostly SMEs which could not solve this problem with their own resources.

In 2015, the International Organization for Standardization (ISO) began the development of a material specification for MNPs (ISO 19807). A New Work Item Proposal for a standard on "Superparamagnetic beads for DNA extraction" (PG14) was prepared in 2016. ISO had decided to create a series of standards on magnetic nanomaterials, with the beforementioned drafts to be the first members as ISO 19807-1 and ISO 19807-2. In these documents, new magnetic parameters needed to be defined and existing standards needed to be applied to MNPs or products based on MNPs. This project defined, implemented and tested the measurement of magnetic parameters of MNPs and related products.

To finalise the development of ISO 19807-1, parameters needed to be formulated, while measurement methods and techniques had to be developed along with the transmission of the pre-normative scientific knowledge into a suitable form for an industrial standard. This could only be achieved through the effective communication with European stakeholders in MNP applications to represent their interests in the international standard, and with the means to communicate with ISO/TC229 WG4 in an appropriate way, i.e. by participation in committee meetings, interim web meetings, and preparations of draft standards and ballots.

Since 2010, and prior to this project, the European Union has funded more than 90 research projects related to MNPs. Some of those, for example the FP7 project NanoMag or the TD COST Action RADIOMAG, were explicitly focused on pre-normative metrological research. The uptake of these results into the development of ISO 19807-1 greatly enhanced the scientific quality of the new standard.

3 Objectives

The main target of the project was to collect the available knowledge on standardised measurement of MNPs, to create it where it was not readily available, to make this knowledge available for the standardisation of MNPs at ISO, as well as to involve stakeholders from industry and academia in the design and application of standardised measurements and labelling of magnetic nanoparticles. The specific project objectives were:

1. To develop measurement methods and techniques to ensure highly qualified scientific input into the preparation of ISO 19807-1.

   Specifically, this included:

   - collection and preparation of existing pre-normative knowledge on identification and definition of relevant physical parameters of MNPs such as i) saturation magnetisation, ii) crystallite size distribution iii) hydrodynamic size distribution and iv) magnetic interaction between MNPs;
   - definition and description of appropriate measurement methods for MNPs magnetic parameters; specifically, static and dynamic magnetisation measurements and specific loss power; and

- coherent application of existing standards for X-ray diffraction techniques (for example SAXS) for determination of crystallite structure and application of transmission electron microscopy (TEM) and dynamic light scattering (DLS) for MNP size characterisation.

2. To summarise metrological knowledge on MNPs gained in this project according to the “Metrological Checklist” ISO/TC 229 N 673, so that further normative documents covering measurement techniques for MNPs (e.g. static magnetic susceptibility, dynamic magnetic susceptibility and specific loss power) can be prepared.
3. To ensure the take up of results from finalised and ongoing FP7 and Horizon 2020 EU research projects on MNPs e.g. “NanoMag” and “RADIOMAG” into the international standardisation process. This included the definition of terms for magnetic quantities and the compartments of magnetic nanoparticles, actual versions of standard operating procedures (SOPs) for magnetic measurements (static and dynamic magnetisation, determination of specific loss power) and surveys of industrial requirements on standardisation of magnetic nanoparticles.
4. To ensure coordinated participation of European NMIs and stakeholders in ISO’s standardisation process on MNPs to provide the highest impact and the fastest development of the standards. To facilitate the take up of methods and technology developed in the project by technical committees e.g. ISO/TC 229 and end-users e.g. biomedicine, mechanical engineering and environmental remediation sectors.

4 Results

4.1 Development of measurement methods for structural and magnetic parameters of MNPs

Magnetic nanoparticles (MNP) find wide biomedical and technical applications. However, prior to the project, there were no existing standards for characterising this material class available. The EMPIR project MagNaStand aimed at expanding and summarising the metrological knowledge on the measurement and characterisation of MNPs for standardisation purposes. This involved a close collaboration with national and international standardisation organisations, industry, and the uptake of results of previous EU research projects including “NanoMag” and “RADIOMAG”.

A special prerequisite for successful standardisation of advanced materials like magnetic nanoparticles is profound knowledge on characterisation techniques and peculiarities of the respective measurement methods and data analysis.

One of the objectives of the MagNaStand project was therefore the development of advanced measurement methods and techniques to ensure highly qualified scientific input into the preparation of ISO 19807-1. Specifically, this included the collection and preparation of existing pre-normative knowledge on identification and definition of relevant physical parameters of MNPs such as i) saturation magnetisation, ii) crystallite size distribution iii) hydrodynamic size distribution and iv) magnetic interaction between MNPs. Furthermore, the appropriate definition and description of measurement methods for MNPs magnetic parameters, specifically, static and dynamic magnetisation measurements and specific loss power, was an important part of the investigations. Finally, the coherent application of existing standards for different measurement methods like SAXS, TEM and DLS for MNP size characterisation should be demonstrated.

Thus, the MagNaStand project has collected and summarised the pre-normative knowledge on identification and definition of relevant physical parameters of MNPs like saturation magnetisation, crystallite size distribution, hydrodynamic size distribution and magnetic interaction between MNPs.

Synthesis of MNP

To investigate MNP properties thoroughly, the MagNaStand project partner MICROMOD has synthesised a number of multicore MNP-systems in different concentrations: nanomag®-D-spio, perimag®,nanomag®-D, BNF-Starch, synomag® and synomag®-D. Furthermore, two single-core MNP systems, S01018 and S01418 have been synthesised. These MNP systems were thoroughly characterised by different measurement techniques.

Application of existing standards to characterise MNP

Currently, there exist measurement standards for Small Angle X-Ray Scattering (SAXS), Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) which are suitable to characterise nanoparticle samples including MNP. During the project, standard operating procedures (SOPs) for these measurement methods were documented and the samples were analysed by PTB according to the SOP rules.

As an example, the results for synomag®-D are reported here.

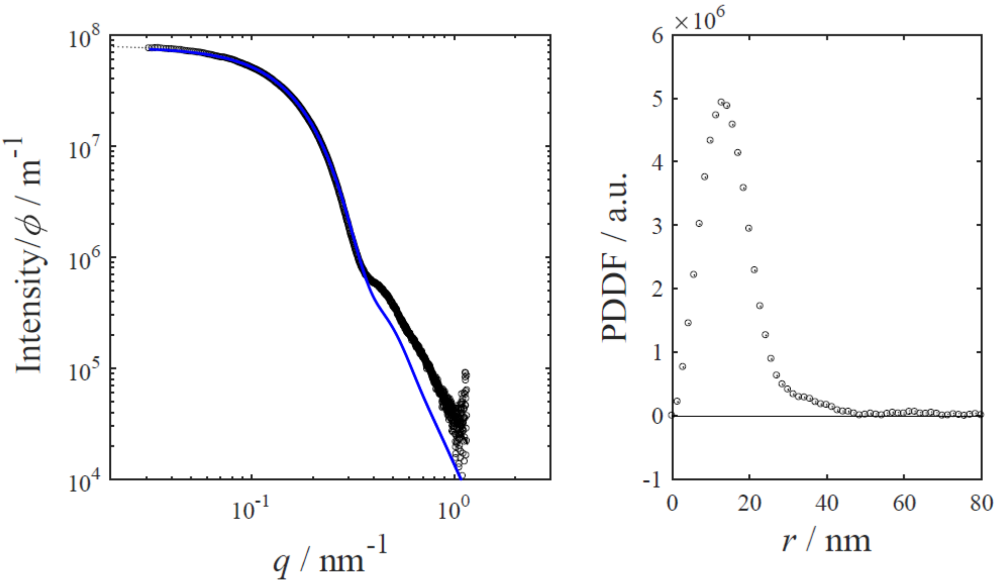

Figure 1: Result of SAXS measurements on synomag®-D: a) intensity over scattering angle and b) pair distance distribution function over scatterer radius. Symbols represent the measurement data, points the extrapolated values and the blue lines the fit curves.

Table 1 SAXS results for synomag®-D.

iron concentration c_{Fe} [mmol / L]	**volume weighted diameter** d_V [nm]	**size distribution width** σ [1]
7.3	22.7	0.2

There is no distinct minimum visible in the SAXS intensity curve for synomag®-D. The scatterers have a wide size distribution. The reason for this behaviour is the multi-core structure of synomag®-D (c.f. TEM image in Fig. 2). For this reason, only larger diameters were used in the fit of the size distribution, and the values for smaller diameters were extrapolated, as can be seen in the blue line in Fig. a). The resulting volume weighted diameter of 22.7 nm is in the range of the diameter found in the TEM analysis (26.1 nm).

The ISO standard for SAXS cannot be straightforward applied, additional knowledge about the MNP system is necessary to come to a reasonable interpretation of the measurement results.

Table 2 DLS results for different concentrations of synomag®-D.

Name	cFe	Z-Average [nm]	PDI	Z-Average [nm] measured	PDI	Z-Average [nm] from manufacturer	PDI
synomag®-D	0,54	44,3	0,0595	44,7	0,058	45	0,059
	1,1	44,2	0,0603				
	2,7	44,8	0,0507				
	5,5	45,3	0,0628				

The MNP system is stable against concentration changes and the measurement results match the values from the manufacturer. However, this was not the case for all investigated MNP systems. The hydrodynamic diameter of MNP with a stronger tendency for aggregation is not stable under concentration variation. Thus, the ISO standard for DLS measurements has to be applied with special care.

a) b)

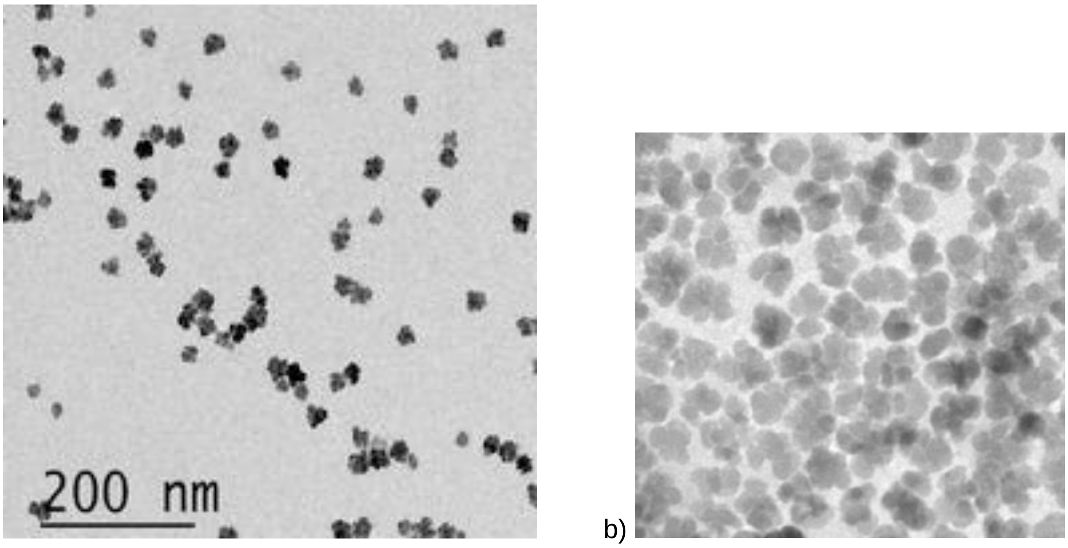

Fig 2. TEM images of synomag®-D: a) standard resolution b) higher resolution

The median TEM diameter amounted to 26.1 nm.

While the TEM analysis was stable for synomag®-D, there appeared problems for other MNP systems, where the borders between crystallites or MNP in aggregates could not be identified.

In summary, existing ISO standards can be used to characterise MNP systems. Special attention should be paid to the aggregation state of the system, which is especially sensitive in MNP systems, because of the magnetic interaction between nanoparticles.

Measurement of magnetic parameters of MNP

The project partners INRIM, UCL, RISE Acreo, RISE, IRM, NPL and PTB have collaborated to document the standard operating procedures (SOPs) for static (DC) and dynamic magnetisation measurements (AC susceptibility). Also, the uncertainty influences for these measurements have been investigated by all project partners (Fig. 3).

For static magnetisation (DC magnetisation) measurements, we could derive the following conclusions:

- Special care has to be applied for the selection of *H* field values, both for the determination of the initial susceptibility and, if applied, for the linear high-field slope correction. A dense linear field distribution in both field regions is recommended.

- However, too many points in the very low field region should be avoided, because the magnetisation curve becomes "non-monotonous", because the set field values cannot be reached by the MPMS measurement system.
- The precision of the initial susceptibility fits depends on the MNP type, as some suspension evidence a linear $M(H)$ behaviour in the low-field range, while others do not. Considerable differences in initial susceptibility values are observed for specimens of different concentration, of the same MNP suspension.
- A general recommendation specifying the field range to be used for determining the initial susceptibility from DC initial magnetisation is inappropriate. The different nature of the samples may result for some in a non-linearity within a pre-defined low-field range for ones while others not.
- Instead, initial susceptibility values may be determined with much higher precision using AC magnetic fields.
- We have used the magnetisation at 5 *T* as a surrogate for saturation magnetisation. The broad dispersion of about 20% which we found in the magnetisation at 5 *T* throughout the concentration range of the same MNP suspension is neither related to the specimen preparation nor to measurement uncertainties. It is suggested that agglomeration of MNPs occurs at high fields resulting in a phase separation and mechanical movement of the particles within the sample cup during measurement. It has to be tested if this indeed occurs, possibly by measuring $M(H)$ slightly below 273.15 *K*.
- A precise determination of specimen weights reduces the uncertainty considerably. The uncertainties of the specimen masses are not negligible for the overall uncertainty budget.
- The measurement of more than one specimen per sample concentration is strongly recommended in order to analyse the dispersion of initial susceptibility and the magnetisation at 5 *T*.

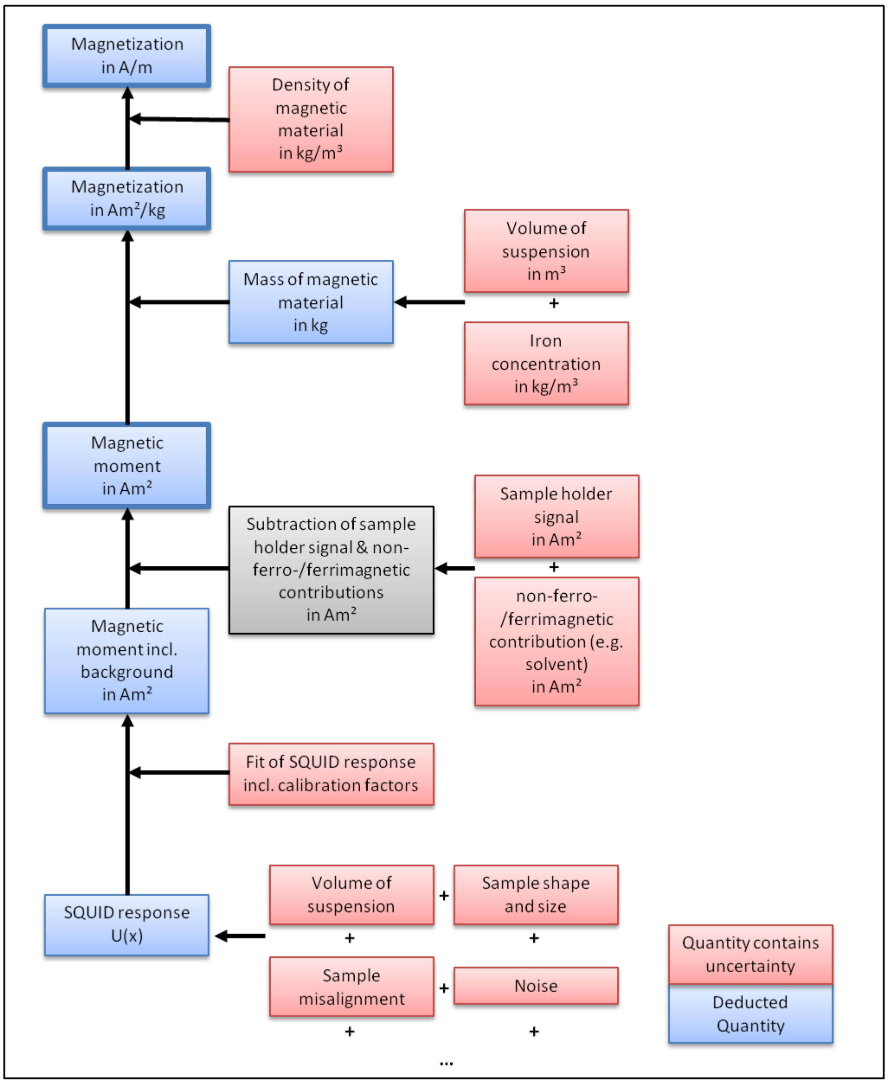

Figure 3: Influence factors affecting the magnetic moment/magnetisation measured at one field point.

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Table 3: Static magnetisation of synomag®-D at 5 Tesla related to the mass of MNP in the sample

MNP name	Concentration [mg/ml]	Magnetisation at 5T in Am^2/kg
synomag®-D	5.6	102.0 ±0.9
synomag®-D	2.7	121.7 ±0.9
synomag®-D	1.1	125 ±1
synomag®-D	0.54	103.0 ±0.9

Also, the measurements at IRM, INRIM, NPL and PTB revealed, that the bulk values for saturation magnetisation of magnetite or maghemite cannot be used when MNP are investigated. Further, it could be demonstrated that some MNP systems are already saturated at 5 T, while for other MNP systems a higher field is necessary to reach saturation. This makes it difficult to define a standardised measurement procedure, since magnetometers with a measurement range above 5 T are sparsely available.

All projects samples have also been analysed by AC susceptometry by RISE Acreo, RISE and PTB. Data and fitting result using the multi-core model for synomag®-D can be seen in Fig. 4.

Fig. 4: ACS results of synomag®-D at a concentration 5.5 mg/ml (size distribution to the right)

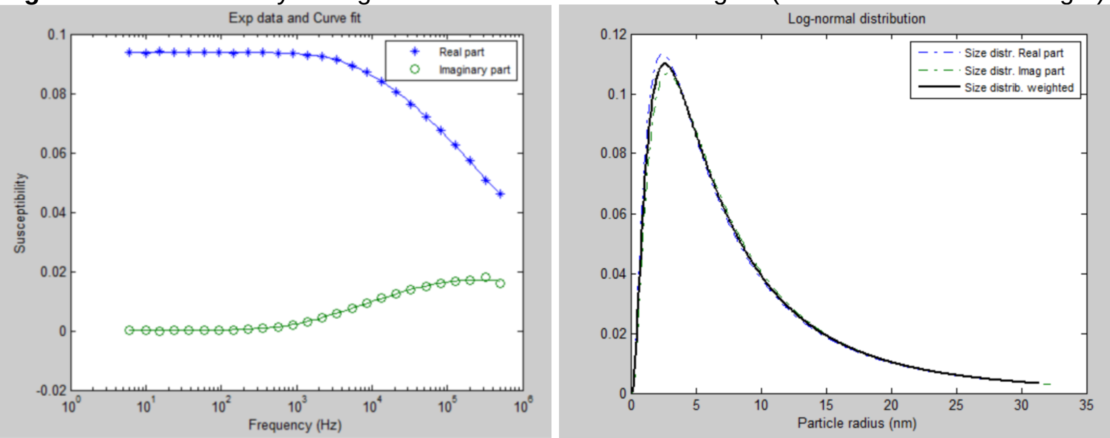


Table 4: ACS analysis (fitting result) using the multi-core model on synomag®-D

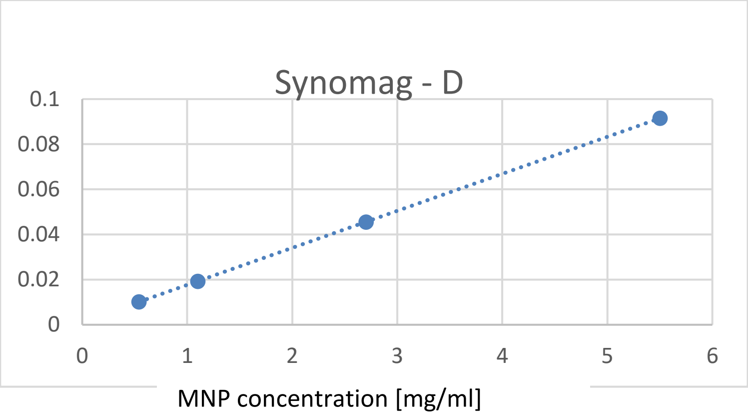
Particle concentration (mg/ml)	**Dm (nm)**	**σ**	**χ_0B**	**χ_high**	**Sum (χ_0B, χ_high)**	**Relaxation frequency (kHz)**
5.5	12.6	2.54	0.084175	0.007327	0.09150	265
2.7	13.2	2.44	0.040911	0.004636	0.045550	230
1.1	13.3	2.48	0.016942	0.002334	0.019276	222
0.54	13.1	2.58	0.008917	0.001256	0.010172	233

Particle sizes from ACS analysis of synomag®-D resembles does not match the obtained particle sizes from DLS analysis. This is a typical behaviour when analysing nanoflower MNP systems.

Extrapolated DC susceptibility (sum of DC parameters in the fitting) versus particle concentration of synomag®-D can be seen below. As can be seen the relationship is almost linear meaning that the MNP systems show negligible particle-particle interactions in the AC susceptibility (within this particle concentration range). The slope corresponds to 0.016724 (ml/mg).

Thus, quantification of MNP samples with synomag®-D by ACS susceptibility is possible.

Fig. 4: ACS results of synomag®-D at a concentration 5.5 mg/ml (size distribution to the right)



Description of interaction between MNP and crystallites

During the project, UCL has also investigated the description of interaction in MNP systems. The complex internal structure of multicore magnetic nanoparticles leads to important functional property differences, which are currently being reported and exploited in scientific and commercial laboratories all over the world.

However, to date there is no standardised nomenclature in use to describe the internal structure of such particles. Perhaps as a consequence of this, in the literature to date it appears that technologists generally report their findings without attempting to relate the functional properties to underlying physical properties.

Project partner UCL has proposed a methodology to describe the internal structure of MNP which was accepted in the consortium. It is an important step towards standardisation of the physical description of multicore magnetic nanoparticles, which we hope may lead to an increased precision in reported work in the field, and in the longer term, better understanding and control of these materials.

Long-term investigation of MNP properties

PTB and RISE have performed a long-term investigation of MNP properties, observing several MNP samples over the course of >2 years. In the result, it could be shown that MNP samples can be stable over this time and that the main variation between measurements was due to sample preparation and operator influence.

Input of the metrological knowledge into the development of new international standards

Using the metrological knowledge gained by the detailed investigations described above, the MagNaStand project has substantially contributed to the initiation of the first series of standard documents on a nanomaterial in the ISO/TC229 "Nanotechnologies" committee. Project partners UCL, RISE Acreo, RISE, UMIT and PTB have sent technical experts to the ISO/TC229 committee.

In June 2019, the first international standard on magnetic nanoparticles, ISO 19807-1 "Nanotechnologies — Magnetic nanomaterials — Part 1: Specification of characteristics and measurements for magnetic nanosuspensions" was issued by ISO/TC229, which was the main goal of this project.

For the next document in the series, ISO 19807-2 "Nanotechnologies — Magnetic nanomaterials — Part 2: Specification of characteristics and measurements for nanostructured superparamagnetic beads for nucleic acid extraction", the MagNaStand project members UCL, RISE, UMIT act as technical experts and, in the case of PTB, as project's co-leader in ISO/TC229. UMIT has been invited to participate in the Chairman's Advisory Group (CAG) of the ISO/TC229 committee and is thus even more involved in the strategic work of the committee.

Via collaboration agreements, MagNaStand has supported technical experts from Luxembourg and Poland to participate in the work of their national standardisation organisations and also to participate as technical experts in the work of ISO/TC229.

With support from the MagNaStand experts from UMIT and PTB, the ISO/TC229 committee has currently initiated a new project ISO 5094 “ISO 5094 Nanotechnologies — Assessment of peroxidase-like activity of metal and metal oxide nanoparticles”.

MagNaStand has regularly communicated its results to the experts of standardisation organisations in France (AFNOR) and Spain (AENOR).

Summary of the key outputs and conclusions

The MagNaStand project has synthesised a number of different magnetic nanoparticle systems and characterised them thoroughly by a number of analysis techniques. Documents on the standardised measurement of magnetic properties of MNPs have been prepared, specifically for saturation magnetisation, AC- and DC-susceptibility (NPL, INRIM, PTB, IRM) and specific loss power (UCL, INRIM, IRM, PTB). The developed terminology was harmonised with the content of the new ISO standard ISO 19807-1. With the help of these standardised measurement protocols, the project was able to establish a procedure for assessment of the long-term stability of MNP samples. A report on measurement methods for MNP, extensive measurement results of the project and a guidance on structural MNP analysis has been prepared by all project partners. Further, the project measurements revealed, that magnetic MNP parameters can be used to monitor the stability of MNP suspensions over a course of more than 2 years. The obtained knowledge was used in the preparation of international standards at ISO level.

4.2 Preparation of “Metrological Checklists” for measurement of magnetic properties of MNPs

International document standards for measurement methods in the area of nanotechnology are developed by the ISO/TC229 JWG2 “Nanotechnologies – measurement methods” committee. This committee has issued a metrological checklist document, where 10 questions on metrological and technological maturity of a measurement method must be passed, before the development of a new measurement standard in ISO/TC229 JWG2 can be initiated. The MagNaStand project partners INRIM, PTB, NPL, UCL, RISE, UMIT, DIN and BSI have developed a document which adopts the metrological checklist of ISO/TC229 JWG2 to formulate specific questions for the assessment of the state-of-the-art of static magnetisation measurements, dynamic magnetisation measurements and magnetic field hyperthermia for the characterisation of magnetic nanoparticles (MNP). Each question of the adopted checklists was answered in detail, based on the existing literature and on the experience of the authors. Thus, the presented document provides the necessary information for the formulation of New Work Item Proposals at ISO/TC229 JWG2, but identifies also a number of open questions, that have to be answered, before international measurement standards for magnetic properties of MNP can be effectively developed.

The “Metrological Checklist” is a tool of the ISO/TC229 “Nanotechnologies” committee to document the maturity of a new nanomaterial measurement method for international standardisation. The checklists have been used in discussions at ISO/TC229 for the preparation of further measurement standards for MNP. The most likely candidate for a further ISO measurement standard is currently the measurement of static magnetisation and of high-field (saturation) magnetisation.

In a report, all project partners have collaborated to provide a comprehensive overview on the maturity of three different measurement methods for MNP characterisation for international standardisation: static magnetisation measurements, dynamic magnetisation measurements and magnetic field hyperthermia. Each of these measurements is well known for a long time, described extensively in the literature, and applied for scientific and industrial purposes. Nevertheless, critical aspects of a complete metrological infrastructure are still missing. The detailed results are summarised in Table 5 of the present report:

Table 5. Summary of metrological checklists investigated during the MagNaStand project.

Are MNP measurement methods ready for standardization? Summary of metrology check-lists for ISO/TC229 JWG2				
	Checklist question	**Static magnetisation measurement**	**Dynamic magnetisation measurement**	**Magnetic field hyperthermia measurement**
1	Is the measuring equipment or instrument available and used in a number of laboratories that is sufficiently large to justify the development of an ISO standard?	Yes	Yes	Yes
2	Has the method already been validated and have the results of this study been published?	No	partially	partially
3	Is the property measured/assessed by the method (= measurand) unambiguously described?	Yes	Yes	partially
4	Are measurement units defined and in accordance with SI rules? Are the tools required to obtain metrological traceability to the measurement units available?	Yes	partially	partially
5	Has it been clearly indicated whether the measurand is 'operationally or method- defined', or whether the measurand is an 'intrinsic' property?	Yes	No	Yes
6	Has the (material / substance / test sample / test system) subject to measurement, clearly been described, including its state?	Yes	Yes	Yes
7	Is the definition of the (material / substance / test sample / test system) subject to measurement unnecessarily restrictive?	No	No	No
8	Are any quality control tools available to enable the quantitative demonstration of a laboratory's proficiency with the method, e.g. in terms of repeatability or bias? Example: certified or non-certified reference materials (calibrants, quality control materials …), proficiency testing schemes, …	Yes	No	partially
9	Does the document propose a measurement uncertainty budget?	partially	partially	Yes

It becomes clear, that all measurement methods justify the development of a standardised measurement procedure, but some work is still needed for a success of standardisation. The most critical points are validation studies, quality control tools, proper uncertainty budgets, traceability to SI units and a clear description of measurands.

An additional desirable is the development of certified reference materials for magnetic properties of MNP. The availability of such material would greatly support the solution of the open questions mentioned above.

As a consequence of development of metrological checklists, project partner DIN has together with PTB developed a standardisation roadmap for the measurement of MNP which has been proposed for consideration to the ISO/TC229 committee (c.f. Table 6) by project partner BSI, who holds the secretariat of the committee.

Table 6: Roadmap for the development of MNP measurement standards in ISO/TC229/JWG2

Standardization item	**Priority**	**2020**	**2021**	**2022**	**2023**	**2024**	**2025**	**2026**	**2027**	**2028**
DC magnetometry	High	■	■	■						
AC susceptometry	High		■	■	■					
Magnetic field hyperthermia	Medium				■	■	■			
Rheometry with magnetic field control	Medium							■	■	■
NMR	Medium							■	■	■

The roadmap is based on the considerations in MagNaStand project which is summarised the previous EU projects NanoMag and RADIOMAG. Of course, considerations already performed in ISO/TC 229/WG 4 was given a high priority. While the NanoMag and the RADIOMAG scopes were a little bit broader, in MagNaStand it was decided to focus on the characteristics which are specific to magnetic nanoparticles. The roadmap was developed in close cooperation with the industrial stakeholders of MagNaStand.

The characteristics for which no standardised test methods are available and their respective test methods more or less follow the characteristics given in ISO/TS 19807-1 are defined in Table 7:

Table 7: Magnetic characteristics of MNP suspension samples as defined in ISO 19807-1

Characteristics	**Measurement method**
Field dependent magnetic moment of superparamagnetic MNP	
AC susceptibility (volume, mass, molar)	AC susceptometry
DC susceptibility (volume, mass, molar)	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
Magnetic moment	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
Magnetization (volume, mass, molar)	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
Saturation magnetisation	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
MNP internal structure: Interaction between crystallites	
Single core/multi-core magnetic nanoparticles	A combination of TEM, XRD, DCM and ACS. One or few of them might be sufficient.
Magnetothermal effects	
Specific loss power	Magnetic field hyperthermia measurement

Pyromagnetic coefficient	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
Curie temperature	DC magnetometry (using a vibrating sample magnetometer or a SQUID magnetometer or any other magnetometer)
Magnetohydrodynamics (only for MNP nanosuspensions)	
Magnetoviscosity	Rheometry with magnetic field control
Magnetic relaxivity	NMR

Table 7 shows that all 11 characteristics could be determined if standards for the following 5 test methods were available:

- DC magnetometry
- AC susceptometry
- Magnetic field hyperthermia
- Rheometry with magnetic field control
- NMR

DC magnetometry and AC susceptometry are considered highest priority in the ISO/TC 229/JWG4 roadmap. So, this priorisation was adopted for the proposed roadmap. As magnetic field hyperthermia is considered highest priority in the NanoMag project it is given 2nd priority and rheometry with magnetic field control and NMR can be given 3rd priority. A parallel development of maximum 2 test standards was considered realistic. That leads to the roadmap shown above which has been submitted to ISO/TC 229/JWG 2.

The initiative was highly welcomed by the committee and a special discussion session for the roadmap was scheduled for the ISO/TC229 meeting in Washington in May2020, where PTB should have given a presentation of the roadmap. However, the global COVID-19 crisis resulted in the cancellation of that meeting, which was transferred to an online event. In the face of this situation, the discussion of the roadmap for MNP measurement methods was postponed to the October 2020 meeting of ISO/TC229.

In addition to the roadmap for standardisation of MNP, the MagNaStand project has also developed a roadmap for an industrial metrology network, describing the main needs and also economical opportunities in the metrology of MNP. In order to understand the economic opportunities of MNP standardisation, it is necessary todescribe the different roles where entrepreneurial activity can contribute to further development of the field. While an economic analysis is beyond the possibilities of the MagNaStand projects, we sketched the steps for a standardised metrological infrastructure, that are necessary from a scientific point of view.

Considerations for ring comparisons to harmonise measurement methods and to assess the uncertainty of measurements

In order to assess the current level of accuracy in the measurement of magnetic properties of MNPs and MNP based products, it is necessary to perform ring comparisons among a number of proficient laboratories. In very few cases, these ring comparisons have been performed already for static magnetisation, specific loss power in magnetic field hyperthermia and AC susceptibility. Throughout, the results have indicated that a further harmonisation of measurement protocols is necessary and that the full uncertainty budget for each measurement method has to be established and used in the assessment of the accuracy of such measurements. Regular ring comparisons are the only way to elucidate systematic deviations between the measurements of different laboratories, since they will be hidden in repeated measurements performed by only one lab. Thus, an experimental assessment of the measurement uncertainty performed by repeated measurement in only one lab will systematically underestimate the true uncertainty of the measurements.

The state of metrological traceability of magnetic measurements in key laboratories

A number of key laboratories, preferably in the national European metrology institutes, have to secure the metrological traceability of their measurement devices to the transfer standards for SI units. This is the only possibility to assure coherence of the measurements of magnetic properties of MNPs with the generally accepted and required metrological system that has been established in Europe and is used in all areas of industry.

So far, the procedures how to link magnetic measurements of MNP samples by calibrated measurements to SI units are not established and the details are unknown. The establishment of metrological traceability is key for the development of a proper metrological network for magnetic MNP properties and should have the first priority.

European candidate organisations to pave the way towards this goal are the MagNaStand partners from Belgium, Germany, Italy, Sweden and UK, as well as the project collaborators from Luxembourg and Poland; others might follow within a short time. However, for the foreseeable future, it cannot be expected, that SI-traceability for magnetic MNP properties can be established in all European countries. For that reason, European countries with more advanced measurement methods should take over the role of key laboratories also for those countries, that don’t have the technological capabilities. It is already known, that industrial MNP production and application has considerable economic implications also in countries that don’t have the relevant metrological infrastructure at a national level (=in the NMI), e.g. in Norway, Austria, Romania or Latvia.

It is an opportunity for the European industry, to take part in the establishment of SI-traced measurement laboratories for magnetic MNP properties, both on a national level, but also within other organisations.

The need for development of reference materials for magnetic properties of MNPs

So far, there exists no accepted reference material for magnetic MNP properties. A reference material is characterised by being homogeneous and stable with respect to a certain material characteristic. This can be a physical quantity like initial magnetic susceptibility, but it can also be a performance characteristic like colloidal stability. For reference materials with defined physical properties, it is desirable to have a certified measurement of this property available, together with an indicated uncertainty. This class of materials is called certified reference material (CRM). CRMs are indispensable for the development of harmonised measurement routines, for the evaluation of key comparisons and for the assessment of the proficiency of a lab for performing custom measurements within a quality assurance system.

Currently, big manufacturers of MNPs use their in-house reference materials for magnetic properties. Smaller companies and academic institutes are relying on other commercial MNP products as quasi-reference materials, without a knowledge on the actual variation of the material properties from batch to batch.

The establishment and commercialisation of a reference material for magnetic MNP properties is an opportunity for the European industry that will gain more importance in the future.

Certification of measurement services – an economic opportunity

Typically, industrially accepted testing laboratories operate under a strict quality control and management system. Such a system is described in ISO/IEC 17025:2017 “General requirements for the competence of testing and calibration laboratories”. In the biomedical area, it might be necessary to consider also the more focused ISO 15189:2012 “Medical laboratories — Requirements for quality and competence”. After implementation of this quality standards, testing laboratories can gain accreditation by national accreditation services which operate under mutual recognition agreements provided by the International Laboratory Accreditation Cooperation (ILAC).

This process ensures, that the test certificates issued by accredited laboratories can be trusted and that the quality of the test is acceptable and continuously monitored.

In the current situation of lacking or incomplete standardisation, it is not possible that a measurement service can gain accreditation for testing of magnetic nanomaterials. It is a striking finding, given the scale of economic importance of magnetic nanoparticles, that not any laboratory in the world can issue qualified test certificates for magnetic properties of magnetic nanoparticles.

The only way to change this situation is the development of harmonised measurement standards, which must be accompanied by the development of reference materials for magnetic properties of magnetic nanoparticles. Also, it will be necessary to develop new measurement devices for magnetic properties of MNP which can process many samples in a reasonable time with sufficient accuracy and at a reasonable price.

It is clear, that this situation offers several opportunities for MNP manufacturers, developers of measurement devices and testing service providers. On the other hand, accreditation cannot be achieved if only one of the open tasks is solved. The operation of an accredited testing service for magnetic properties of magnetic nanoparticles requires a certain minimum level of standardisation and technological development of measurement procedures, devices and reference materials, simultaneously. This complex situation is the reason why these services are not available so far.

MagNaStand partners provide pilot laboratories for measurement of magnetic MNP properties

At some institutions, pilot laboratories are going forward and offer testing services for magnetic properties of magnetic nanomaterials on a high scientific level, although they can currently not issue testing certificates which are qualified according to ISO 17025.

The first example is the magnetic testbed which is operated by the project partner RISE (https://www.ri.se/en/what-we-do/expertises/magnetic-expertise). RISE offers characterisation of magnetic nanomaterials by a number of measurement methods. A speciality is the measurement of complex AC susceptibility, which is performed using the specialised Dynomag device (https://www.ri.se/en/what-we-do/services/dynomag-system-and-high-frequency-hf-ac-susceptometer.

A second example is the core facility “Metrology of Ultra-Low Magnetic Fields” which is operated by project partner PTB in Berlin (https://www.ptb.de/cms/en/ptb/institutes-at-ptb/geraetezentrum-8-2.html).

There, with financial support by the Deutsche Forschungsgemeinschaft (DFG), PTB grants external scientists from universities, from international metrology institutes and from companies access to its know-how and to its equipment with instruments for the measurement of ultra-low magnetic fields.

By basic characterisation measurements a variety of MNP parameters can be acquired by PTB, such as saturation magnetisation, magnetic moment distribution, hydrodynamic and core diameter distribution, effective anisotropy and dynamic susceptibility. Additionally, considering especially the biomedical in-vivo application, further parameters of MNP can be examined, in particular changes of magnetic properties due to interaction with the physiological environment, aggregation behaviour in physiological media, binding capacity to biomolecules and blood half-life and clearance.

Further MNP measurement services for external users are planned by project partners UCL and MICROMOD. The actual implementation was delayed by the COVID-19 crisis.

Need for calibration services for measurement devices for magnetic properties of MNPs

With the development of new ultra-sensitive high-throughput measurement devices for the magnetic properties of magnetic nanoparticles, it can be safely predicted that these devices will be installed in larger numbers in the industry, because of the huge economic impact that magnetic nanoparticles and the products based on them have already now. For a safe and reliable operation of these devices, it will be necessary to have a calibration service, which is able to certify the initial qualification of a device to perform the intended measurements with an acceptable uncertainty. Further, such measurement devices need regular checking and surveillance of their measurement functions as well as calibration in order to maintain a high quality of the measurement results.

Specialised companies offering such a testing and calibration service for the measurement devices, which can only be performed using dedicated instruments, procedures and reference materials, will find an economically viable field of activity there.

Custom determination of MNP concentration and distribution in engineered objects, in biological specimens, at workplaces, in the environment and in waste products

In addition to the characterisation of the magnetic nanoparticles or products based on them, there exists a huge demand for quantification of magnetic nanoparticle amount and their local state in certain technical, biological and other environments. Once the measurement principles for rapid and accurate characterisation of magnetic nanoparticles are known, they can be used and extended for non-invasive imaging and quantification procedures of magnetic nanoparticles in larger specimens and objects. Examples are engineered parts containing MNP, biological specimens like cell cultures, organs ore even complete animals or humans, samples from workplaces, the environment or from waste materials.

A number of devices is already on the market for such purposes, but also here, harmonised measurement procedures are not available. This creates a further obstacle for the development of the procedures themselves, but also for the wider application of magnetic nanoparticles, since important information about the MNP distribution at workplaces or in organisms is not available today because of the lack of appropriate measurement instruments and methods. Especially regulatory agencies rely on the submission of such data to assess the safety of new nanoparticle-based products and they tend to block new developments when the required data are not available.
Clearly, this area offers huge opportunities for device manufacturers and commercial measurement services. Standardisation is one of the key factors which will lead to economic viability of this activities.

Summary of the key outputs and conclusions

Metrological checklists according to the requirements of ISO/TC229/JWG 2 have been prepared for static magnetisation measurements, dynamic magnetisation measurements and determination of specific loss power in magnetic hyperthermia. The analysis revealed that all three measurement methods have reached a high degree of accepted measurement methods which would justify the development of a measurement standard. Open question that need to be solved before standardisation were also discussed.

Based on these findings, the MagNaStand project has developed a roadmap for development of measurement standards for MNP and submitted the document to ISO/TC229/JWG2. A further discussion of the roadmap document at ISO was delayed due to the COVID-19 crisis.

The standardisation roadmap was amended by a guidance document describing the needs and opportunities of an industrial metrological network for MNP metrology.

4.3 Uptake of previous results from FP7 and Horizon 2020 EU research projects on MNPs for ISO standardisation

In a new field such as nanotechnology, the foremost interest is the definition of terms and characteristics. The selection of important MNP characteristics should be based on reviews of the scientific work on MNPs and of economically relevant MNP based applications.

Prior to this project, two pre-normative European projects have been outstanding in this field: The EU FP7 project NanoMag, devoted to metrological groundwork on MNP standardisation and the EU COST Action RADIOMAG, that dealt with the standardisation of the measurement of MNP hyperthermia performance. Both projects have been revisited and summarised during the MagNaStand project. The former NanoMag partners RISE Acreo (NanoMag coordinator), UCL, MICROMOD, NPL and PTB summarized this project. The former RADIOMAG partners IRM (RADIOMAG coordinator), RISE Acreo, UCL, NPL, INRIM and PTB summarized the RADIOMAG network activity. Further, UMIT and PTB have evaluated the European CORDIS database for previous and running EU-projects concerned with MNP. In addition to the pure database work, they have also performed a survey of researchers involved with MNPs. The main results are provided here.

Economic importance of MNP

Liquid suspensions of magnetic nanoparticles (MNPs) are used in many technical areas like loudspeakers, mobile phones, vacuum sealings, metal separation and water remediation. In the biomedical applications, MNPs play a very important role in in-vitro diagnostics for the separation of cells, bacteria, viruses, protein, nucleic acids and other compartments from blood and body liquids. MNPs are used as contrast agents in Magnetic Resonance Imaging, as well as tracers in Magnetic Particle Imaging and sentinel lymph node detection as well as in MNP based therapies, where they act as heating agents in magnetic field hyperthermia or as drug carriers in magnetic drug targeting and magnetic gene therapies.

The economic impact of MNP based biomedical products of European companies alone amounts so far to more than 2 billion € per year. The largest part of this economic impact is generated by in-vitro diagnostics applications. Another large application field is the use of nanostructured iron oxide in pigments for cosmetics, structural engineering, and many other purposes. The annual production of those pigments alone in the European Union is more than 100,000 tons per year. Obviously, this creates a demand for international standards on the main characteristics of MNPs and the respective measurement procedures.

The pre-normative EU NanoMag project

The NanoMag project was funded by the EU FP7 research program in the years 2013 – 2017 and had a budget of about 11 M€. The NanoMag project objectives were to standardise and harmonise ways to measure and analyse the data for MNP systems. NanoMag brought together leading experts in: synthesis of magnetic single- and multi-core nanoparticles, characterisation of magnetic nanoparticles, and national metrology institutes. The project defined standard measurements and techniques which are necessary for defining a magnetic nanostructure and quality control. NanoMag was focused on biomedical applications, for instance bio-sensing (detection of different biomarkers), contrast substance in tomography methods (Magnetic Resonance Imaging and Magnetic Particle Imaging) and magnetic hyperthermia (for cancer therapy).

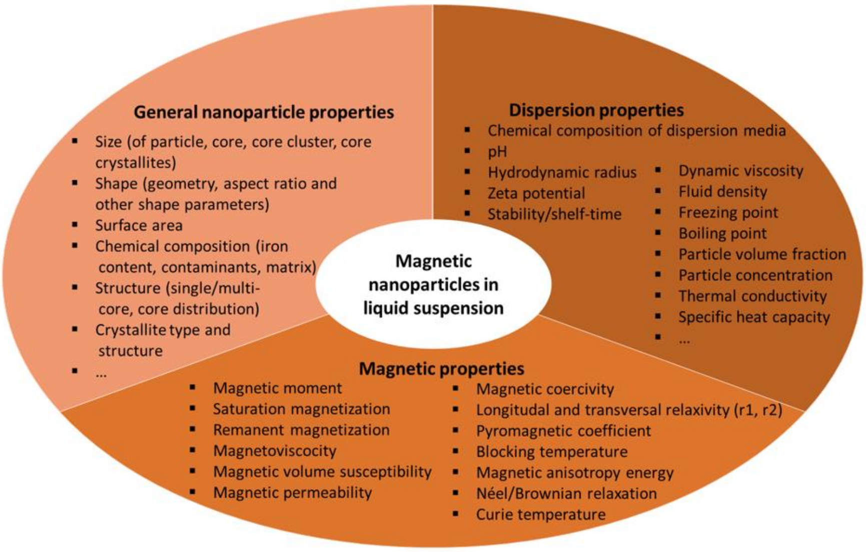


Fig. 5. Important parameters of liquid MNP suspensions that can be standardised. Result of the NanoMag project.

Initially, NanoMag was designed as a pre-normative project for the preparation of measurement standards. When the International Organisation for Standardisation started a normative activity for material properties of MNP suspensions, NanoMag members entered the ISO committee TC 229 "Nanotechnologies" as technical experts and made substantial contributions to ongoing ISO standardisation work in the field of MNP systems.

The knowledge of the NanoMag experts has been summarised in four electronic-learning modules that can be accessed via the internet from the NPL website:

https://training.npl.co.uk/course/magnetic-nanoparticles-standardisation-and-biomedical-applications/

To support MNP standardisation the NanoMag project has:

- developed a clearer nomenclature to describe the structure and magnetic properties of MNPs and MNP ensembles (see Fig. 1);
- performed surveys of measurement methods for MNPs and their pros and cons, classification of these methods including also classification of different types of MNPs (single-core, multicore, nanoflowers);
- used Monte-Carlo simulations to explain the experimental results, which have led to improved modelling of MNP magnetic properties, especially the dynamic magnetic behaviour; and
- performed initial round robin measurements (using the same analysis methods on the same MNP samples but in different laboratories utilising the developed standard operating procedures (SOPs)) using different types of MNP systems.

A standardisation roadmap developed during the NanoMag project forecasted the development of measurement standards for MNP to start in the year 2021.

EU Transdomain COST Action RADIOMAG

The EU COST Action RADIOMAG was a network of over 140 scientists in the field of tumour therapy and it was dedicated to research on multifunctional nanoparticles for Magnetic Field Hyperthermia and Indirect Radiation Therapy. The RADIOMAG project duration was from 2014 – 2018. An important task of RADIOMAG was the harmonisation and standardisation of the assessment of heat generation by MNP in an alternating magnetic field, since this is the basic principle in Magnetic Field Hyperthermia.

Magnetic Field Hyperthermia, especially in combination with radiotherapy, has been demonstrated as an effective tool to slow down or stop tumour growth and to support anti-cancer therapy in difficult tumour cases like glioblastoma. Pilot studies in humans are underway in a number of clinics in Europe. In April 2019 a new magnetic field hyperthermia treatment centre for brain tumours has been opened at the Independent Public Clinical Hospital No. 4 (SPSK 4) in Lublin, Poland.

These continuous developments have also created a niche market for several small and medium-sized enterprises for manufacturing magnetic field hyperthermia test devices. These devices are mainly used by academics testing in-vitro and ex-vivo the efficiency of MNP suspensions to deliver heat. The relevant physical quantity is the Specific Loss Power (SLP). For calorimetric determinations, the SLP is deduced from time vs. temperature curves T(t), measured with the SLP test device.

The RADIOMAG activities concerning the standardised characterisation of MNP for magnetic field hyperthermia focused on SLP measurements:

1. Survey amongst RADIOMAG members on available SLP test devices/setups and their field/frequency combinations;
2. Development of a standard operating procedure for the calorimetric SLP determination, i.e. from T(t) measurements;
3. A comparative SLP determination on water-based ferrofluids between 21 participating laboratories (SLP ring test) and evaluation of SLP calculation methods;
4. Study of the field dependence, i.e. SLP(H); and
5. Design of a possible calibration sample for SLP test devices.

The results showed that a large majority of groups determine the SLP from calorimetric measurements with non-adiabatic setups, also commercially available on the market. In contrast, only a few producers exist for non-calorimetric devices using AC hysteresis and a single laboratory used a “home-made” nearly adiabatic setup.

The RADIOMAG work demonstrated that there are no common procedures available for carrying out T(t) measurements in magnetic field hyperthermia setups. Typically, different laboratories use their own individual best-practice protocols, or follow instructions given in the SLP test device manual, in case of commercial setups. RADIOMAG has therefore developed a SOP for measurements, including a questionnaire for instrument specific parameters.

Furthermore, RADIOMAG performed a ring test on the determination of SLP values, where the same MNP formulation was investigated by 21 different laboratories. The results showed a significant variation of quantitative SLP values, even for an identical MNP suspension. Further analysis of these results is still ongoing.

Search of the EU CORDIS database for research projects that are relevant for MNP standardisation

The MagNaStand has evaluated the Community Research and Development Information Service (CORDIS) database on research projects funded by the EU. The aim of this activity was to provide a short summary of the most important EU FP7 and H2020 projects on MNPs. The search terms are explained in Table 8.

The search of the CORDIS website was conducted using all search terms of Table 1 in quotation marks and connected by Boolean OR operators (i.e. “*magnetic *particle” OR “*magnetic *bead*” OR etc.).

Afterwards the result was filtered by the homepage’s “Refine by:” option. Only results of the “Content Type: Project” which were part of “Programme: Horizon 2020” or “Programme: FP7” were downloaded in tabular form as CSV-files and - for the full description of the project objectives – as PDF-booklets. The search resulted in a total of 108 EU research projects with summarised project budgets of roughly € 267 million. In comparison, a search in the Research Portfolio Online Reporting Tools (RePORT) of the U.S. Department of Health & Human Services using the same search terms from Table 1 resulted in 214 projects with a total budget volume of $ 67 million. A close inspection of the search results revealed that despite the wide range of the search terms, a number of relevant EU projects concerned with MNPs that were already known to the authors were not captured. They were later added manually to the final list of relevant EU projects concerned with MNP. The final total number of EU projects concerned with MNP was 118 with a total budget of € 348 million.

Table 8. Search terms in the CORDIS database for identification of relevant EU projects for MNP standardisation.

Search term	Description
*magnetic *particle*	Since the standardisation of magnetic nanoparticle characteristics is our main goal, it is only natural to find all projects containing any variant form of "*magnetic *particle*" (e.g. "superparamagnetic nanoparticles").
*magnetic *bead*	Some research groups use the notation "bead" instead of "particle". Otherwise, the reason to use this search term is the same as "*magnetic *particle*".
iron oxide nanoparticle	Iron oxide nanoparticles are by far the most commonly used magnetic nanoparticles.
superparamagnetic*	A salient feature of MNPs is their superparamagnetic behaviour.

An online survey of researchers involved in MNP projects

The MagNaStand project has compiled a list of contact addresses of the leaders of the identified EU MNP projects, enhanced by contacts from the NanoMag and RADIOMAG networks. Altogether, over 100 European researchers were asked to participate in an online survey on MNP standardisation. We have received 32 responses, of which the most relevant results are summarised below in a question-answer (**Q**/**A**) scheme.

Q. 01: Which of the following were your application areas of MNP? **A. 01**: 32 biomedical applications, 14 MNP synthesis, 6 environmental applications, 9 pharmaceutical applications, 6 other.
Q. 02: What was the Technology Readiness Level (TRL) of your MNP project? **A. 02**: 22% TRL1 – basic principles observed and reported, 19% TRL2 – technology concept and/or application formulated, 30% TRL3 – characteristic proof-of-concept, 23% TRL4 – component validation in lab environment, 2% TRL5 – component validation in relevant environment, 2% TRL6 – prototype demonstration in relevant environment, 1% TRL7 – prototype demonstration in application environment, and 0 in TRL8 – complete system in test and demonstration and TRL 9 – complete system in successful operation.
Q. 03: What kinds of materials for MNP were used during the project? Please specify core- and coating material. A free-text answer was possible. **A. 03**: Magnetite (13) and maghemite (9) was the most common core material, other ferrites (Barium-Ferrite, Nickel-Ferrite, Nickel-Cobalt-Ferrite, etc.) (10) played also a role. The coating material came from these material groups:

- Polymer/Organic which included plastics, polysaccharides and organic acids (53)
- Metal/Alloy which included metals, metalloids and alloys (10)
- Biofunctionalised which included bacteria and proteins (7)
- No coating for uncoated MNPs (1)

Q. 04: In which environment(s) were the MNP used? **A. 04**: 43% laboratory, 34% in-vitro (cell cultures), 25% in-vivo.
Q. 05: Properties of the applied magnetic field: specify field strength and frequency during your MNP application. **A 05**.: see Fig. 6

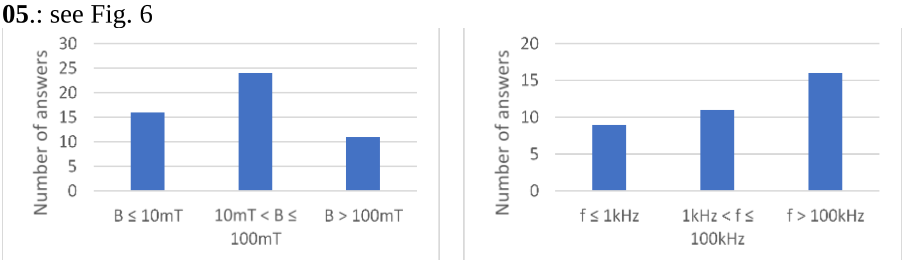


Fig. 6. Magnetic field strength and frequency during the MNP application.

Q. 06: Did you use a static magnetic field or an alternating magnetic field? **A.06**: 25 alternating magnetic field, 18 static magnetic field
Q. 07: What are the sources of the MNP characteristics that you used, please rank. **A. 07**: Summary rank: 1. own measurements, 2. literature values 3. technical data sheet, 4. custom measurement.
Q. 08: Please rate the importance of proposed characteristics for your project (1=low, 5=high). **A.08**: see Fig. 7

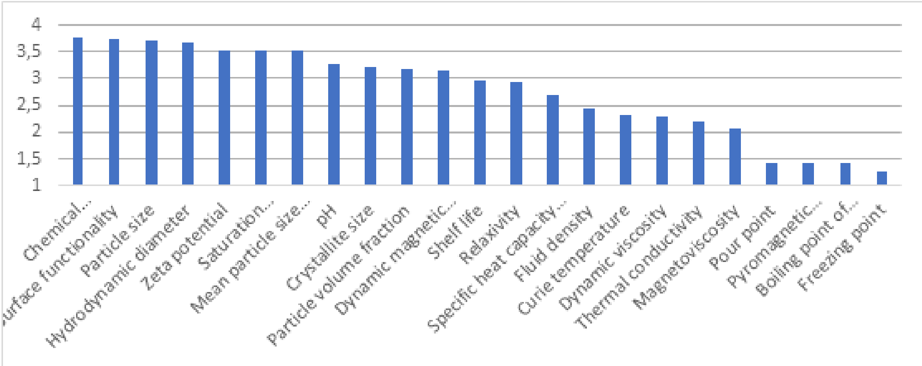


Fig. 7. Importance of different MNP parameters.

Q. 09: Please enter the approximate value range of the characteristics. **A. 09**: see Fig. 8

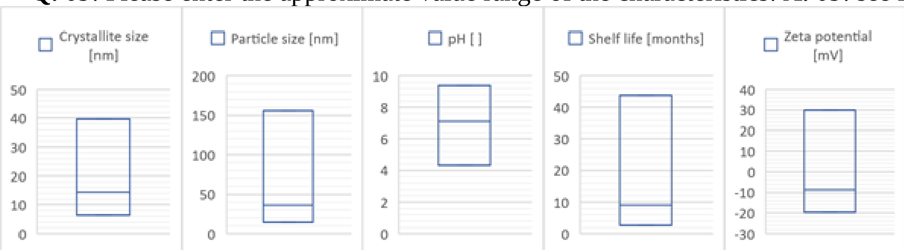


Fig. 8. Mean value ranges for several MNP parameters (minimum, typical and maximum).

Q. 10: Did you encounter any serious problems during the measurement process of important characteristic properties? **A. 10**: 64% no, 21% unclear measurement procedure, 15% measurements were not reproducible.
Q. 11: Are you aware of reference laboratories, where you could check or cross-validate your own measurement results? **A. 11**: 50% yes, 37% no, 13% no answer.
Q. 12: Please rank the most important measurement technique to characterise MNP. **A. 12**: 1. transmission electron microscopy (TEM), 2. magnetorelaxometry (MRX), 3. Hysteresis loops (DC magnetometry), 4. ZFC/FC curves (temperature dependent DC magnetometry) and 5. X-ray powder diffraction (XRD).
Q. 13: What are the most important characteristics? **A. 13**: 36 particle size, 27 saturation magnetisation, 13 hydrodynamic size, 9 specific absorption rate, 8 stability of suspension, 8 chemical composition, 6 biological properties, 5 dispersity, all other <5.
Q. 14: Did you use a standardised measurement protocol or were there any other standardisation aspects in your project? **A. 14**: 50% no, 25% yes, 25% no answer
Q. 15: Did you encounter any problems with the used MNP due to erroneous/unspecified characteristics? If yes, please elaborate. **A. 15**: People were dissatisfied with numerous things involving erroneous or unspecified characteristics of MNPs. Most of the complaints can be summarised by stating that the information provided by manufacturers was incomplete. Complaints involved missing expiration dates, wrong or missing concentration values, wrong or missing magnetite/maghemite indications, unknown surface compositions, stability change over time and recommended storage conditions. Several participants also described problems reproducing previously obtained or published results.
Q. 16: How would you rate the current state of standardisation of MNP? (1=low, 4=high). **A. 16**: see Fig. 9.

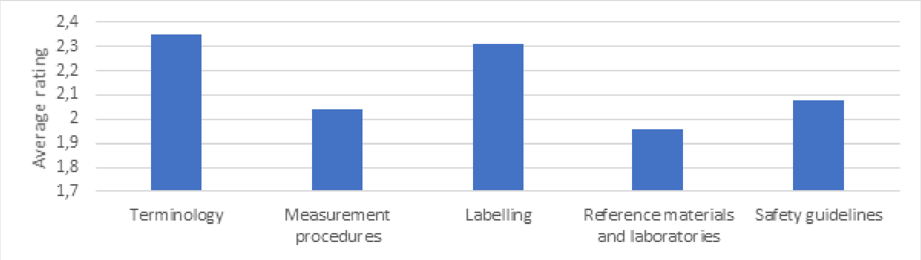


Fig. 9. Rating of current standardisation of different aspects of MNP handling.

The survey, although it is still based on a limited data base, shows clearly the wide range of applications and parameters that have to be considered when the standardisation of MNP is intended. It reveals that EU funded MNP research so far is restricted to very early or early Technology Readiness Levels. This might be one reason that the need for standardisation has been underestimated so far. On the other hand, the survey demonstrates that even in early research in scientific labs people often encounter problems with non-standardised parameters and procedures and non-reproducible measurement results. The need for further standardisation is clearly demonstrated. The survey gives valuable hints, which measurement methods and parameters are most important and would most benefit from standardised procedures.

Summary of the key outputs and conclusions

The MagNaStand project has performed a review of previous EU projects concerned with MNP, with a special emphasis on the pre- and co-normative EU projects NanoMag and RADIOMAG. Further projects have been identified in the CORDIS database.

We found in total 118 research projects and a budget of € 348 million that the European Union has invested into MNP related projects since the FP7 program. The pre- and co-normative EU projects NanoMag, and RADIOMAG, followed by this MagNaStand project, have made decisive steps to improve the MNP standardisation. Currently, Europe is an important contributor to the standardisation of magnetic nanomaterials that is performed in the ISO/TC229 “Nanotechnologies” committee. The efforts for harmonisation are based on surveys and other interactions with academic and industrial stakeholders.

We have presented the detailed content of the review and of a survey among involved researchers at conferences and in a publication in order to stimulate the discussion on the most urgent needs in MNP metrology. In addition to the basic description of MNP that is now been developed at ISO, standard operating procedures for magnetic measurements, calibrated measurement devices and certified reference materials for magnetic MNP properties have been identified as desirable outcomes of future MNP standardisation research.

5 Impact

The work on the development of measurement methods for the definition of physical and magnetic parameters of MNPs, as well as on metrological questions of nanomagnetism, has been presented in 4 open access peer reviewed publications and 5 conference proceedings. It was also presented at 22 international conferences and workshops, including at the International Conference on the Scientific and Clinical Applications of Magnetic Carriers 2018 and the 10th International Conference of Fine Particle Magnetism in 2019.

In July 2017, the project held the first industrial stakeholder workshop in Berlin the audience about the consequences of standardisation of magnetic nanomaterials and to invite external stakeholders to share their interests with project representatives who can bring these views into the standardisation process. A second stakeholder workshop was held in November 2018, where the details and implications of the new ISO standards were discussed with industrial representatives from several industrial user groups.

The project has reached a wider audience with interest in nanotechnology by placing 2 articles on MNP standardisation in the Bionanonet newsletter.

Impact on industrial and other user communities

This project summarised European expertise and the interests of European stakeholders in contributing to the finalisation of ISO 19807-1 “Magnetic nanomaterials -- Part 1: Specification of characteristics and measurements for magnetic nanosuspensions” in June 2019 and the ongoing ISO 19807-2 “Magnetic nanomaterials -- Part 2: superparamagnetic beads for DNA extraction”. Within this project, reports were prepared on the physical description of the main characteristics of MNPs, standard operating procedures for the preparation and measurement of samples, metrological aspects of the measurements (e.g. uncertainty budgets for specific methods), and a survey of the specific needs of European industry, academia, national metrology institutes and research centres.. There are several industrial areas where the new ISO standards on MNPs are expected to create impact:

- Manufacturers of MNPs

  Working according to an ISO standard for MNPs, the manufacturers can monitor MNP quality and market their products according to the definitions in the ISO standard. This improves consumer trust with regards to the reliability, functionality and safety of the MNP products and thus enhance market chances. Partner MICROMOD has updated their application brochures and their product catalogue according to ISO 19807-1 to reflect the standardised description of MNP products in their user communication.

- Manufacturers of products containing MNPs

  A considerable part of the European MNP industry relies on superparamagnetic beads containing MNPs for in-vitro diagnostic applications. Although MNPs are in these cases only compartments of complicated high-technology products, their final performance depends also on the magnetic characteristics. The project contributions to the new standard ISO 19807-2 “Specification of characteristics and measurements for nanostructured superparamagnetic beads for nucleic acid extraction” is of special importance for this industrial group. The project partners have been in intensive discussions with a number of European manufacturers who brought their perspective into ISO standardisation.

- Other commercial MNP users

  These are companies that purchase the MNPs from other sources and apply them for biomedical or technical purposes. They have an interest in the proper specification of the magnetic and other MNP properties and to clearly understand what a parameter in a product statement means and how it is measured. A reliable specification of magnetic MNP parameters opens new application fields for MNPs. In addition, the exact definition of MNP parameters is crucial for all regulatory aspects of products or technologies involving these nanomaterials.

The MagNaStand project has developed and distributed a “Guidance document for European industry and other stakeholders on the characterisation of MNPs with reference to the development of ISO 19807 and further ISO standards involving MNPs”.This document describes a framework enabling marketing of MNPs with an internationally accepted statement on defined and guaranteed magnetic and other MNP properties and the necessary steps towards this scenario.

Impact on the metrology and scientific communities

A significant activity within this project was the input of knowledge and production of content for ISO 19807-1 “Magnetic nanomaterials -- Part 1: Specification of characteristics and measurements for magnetic nanosuspensions'”, which defines the important parameters of MNPs and the measurement methods to obtain them. This ISO standard was published in June 2019. Thus, the project contributed to enhance the comparability and reliability of scientific reports on MNP properties. The ISO standard on MNP supports the acceptance of definitions, the consequent usage of SI units, and the implementation of standard operating procedures and a reliable uncertainty analysis, for the most common characterisation methods for MNPs. Noticeably, a recent publication referred to the importance of the new ISO standard in the context of determination of iron concentration (R. Costo, 2018, DOI:10.1007/s00216-018-1463-2). Working in a standardised environment ensures the efficient use of research resources. By providing SOPs for reliable measurement methods, e.g. for the specific loss parameter in magnetic hyperthermia, the project directly supports the development of new MNP-based therapeutic approaches for fighting cancer. Currently, magnetic hyperthermia using MNPs is being intensively researched as a new tumour therapy. The temperature rise in the tumour tissue during magnetic hyperthermia depends crucially on the specific loss parameter, which is now defined in ISO 19807-1. A comparable and precise quantitative determination of this parameter is important for safety and efficacy of the new therapy. Project partner PTB offers now a service for external customers for determination of MNP parameters like static and dynamic magnetisation or specific loss power in magnetic field hyperthermia. Measurement results are specified according to the rules in ISO/TS 19807-1.

The project partner RISE offers a technical service of magnetic AC susceptibility measurements for customers according to ISO/TS 19807-1 [https://www.testbedsweden.se/en/test-demo/rise-magnetic-testbed].

Impact on relevant standards

The project has made an immediate impact by contributing to the initiation of a series of standards on magnetic nanomaterials at ISO/TC229. Although ISO is an international organisation with members from many countries over the world, it relies on active participation of the experts. MagNaStand members participated since the beginning of the project in four ISO/TC229 meetings and many web meetings of the experts on magnetic nanomaterials. Numerous comments of the national standardisation organisations have been prepared in the UK, Sweden, Austria and Germany in order to improve the draft ISO standards on magnetic nanomaterials. This was the first series on any nanomaterial and has been a direct output of the project. The main impact of this project on the actual standards is the contribution to the standard ISO 19807-1 “Magnetic nanomaterials -- Part 1: Specification of characteristics and measurements for magnetic nanosuspensions” , which was issued in June 2019, and the draft standard ISO 19807-2 “Magnetic nanomaterials -- Part 2: Specification of characteristics and measurements for nanostructured superparamagnetic beads for nucleic acid extraction”. For the preparation of ISO 19807-2, project partner PTB has taken over co-leadership at ISO/TC229 WG4, together with an expert from SAC China. A new standardisation project ISO 5094 “Assessment of peroxidase-like activity of metal and metal oxide nanoparticles” was initiated with the support of MagNaStand members. The project had collaboration agreements with partners from Luxembourg and Poland and supported them to become experts in their national standardisation organisations as well as in ISO/TC229. Furthermore, the consortium exchanged information on the standardisation efforts for MNP with the respective NMIs and SDOs in Bulgaria, Croatia, Czech Republic, France, Hungary, Luxemburg, Poland, Romania, Slovakia, Slovenia and Serbia and Spain. The project has submitted a roadmap for measurement standards to ISO/TC229, which will impact the future work of the committee.

Longer-term economic, social and environmental impacts

The standard ISO 19807-1 on the characteristics of MNPs, that reflects both the available scientific knowledge and the needs of industry and society, will greatly enhance the safe and effective application of magnetic nanoparticles. After the finalisation of the material specification ISO 19807-1, further standards on specific MNP material and measurement methods, such as the standard on superparamagnetic beads, will follow.

<u>Wider economic impact</u>

MNPs already have a wide range of application possibilities, including:

- to promote the separation of cells, proteins or DNA from biological fluids;
- to act as therapeutic agents or drug carriers in a number of new cancer therapies;
- to be used as tracers or labels in biomedical imaging;
- to support the cleaning of water, waste-water or contaminated soil;
- to provide magnetic damping in loudspeakers; and
- to provide vacuum seals as magnetic fluid O-rings for rotary shafts.

The standard ISO 19807-1 for magnetic nanoparticles will enhance and increase industrial applications by providing safety and reliability in the interaction between the particle manufacturer and the consumer. This safety of operation will open new application areas for MNPs.

<u>Wider social impact</u>

Especially in the biomedical area, patients suffering from cancer or other diseases will benefit from the new therapy approaches based on MNPs like magnetic hyperthermia, magnetic drug targeting or trans-membrane magnetofection of DNA. MNPs are currently being investigated as labels or tracers in several diagnostic modalities like MRI, MPI and others. It is already proven that MNPs can help in the early detection of cancer and inflammatory diseases of the heart, the vessels and other organs. An industrial stakeholder of the project, Blusense Diagnostics from Denmark, has developed an MNP based fast COVID-19 antibody test for point-of-care usage with CE-mark. Another project stakeholder, Medisieve Ltd. from the UK, enhanced their MNP based magnetic blood filtration system to treat COVID-19 patients in severe conditions. The international standard on MNP characterisation and measurement will speed up such developments while also ensuring the reliability, reproducibility and safety of the MNPs involved. Ultimately this will lead to a better treatment of patients, to the prolonging of life and to maintaining the quality of life of patients.

Wider environmental impact

The trust of consumers in the safety and reliability of new nanomaterial classes is decisive for the wider acceptance of this emerging new nanotechnology. Standardised measurement procedures will help to control the safety aspects during synthesis and particle shipment as well as the degradation of the MNPs after their intended use. Standardized characteristics and measurement techniques are needed to monitor the complete life cycle of MNPs.

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