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Determining the size of nanoparticles in the example of magnetic iron oxide core-shell systems

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Abstract. The size of nanoparticles is one of the most important factors for their possible applications. Various techniques for the nanoparticle size characterization are available. In this paper selected techniques will be considered base on the prepared core-shell magnetite nanoparticles. Magnetite is one of the most investigated and developed magnetic material. It shows interesting magnetic properties which can be used for biomedical applications, such as drug delivery, hypothermia and also as a contrast agent. To reduce the toxic effects of Fe3O4, magnetic core was covered by dextran and gelatin. Moreover, the shell was doped by fluorescent dye for confocal microscopy investigation. The main investigation focused on the methods for particles size determination of modified magnetite nanoparticles prepared with different techniques. The size distribution were obtained by nanoparticle tracking analysis, dynamic light scattering and transmission electron microscopy. Furthermore, fluorescent correlation spectroscopy (FCS) and confocal microscopy were used to compare the results for particle size determination of core-shell systems.

1. Introduction

Development of nanotechnology opens new possibilities in the design, formulation and manufacture of structures and materials with desirable physicochemical properties. Nanomaterials are considered according to their unique behavior as an innovative solutions in the fields of medical applications, electronic devices manufacturing, sensors, energy storage, daily use devices and so on [1-2]. One of the most important factor which decides of the final application of NPs is the size (mostly denoted by diameter of the particle).

Magnetic iron nanoparticles have been investigated for several decades as possible transporters of a therapeutic substance. The concept is, the possibility to control the direction of flow of particles with magnetic properties, using the external electromagnetic field. The key role plays two iron oxide structures: maghemite γ -Fe_2O_3 and magnetite Fe_3O_4, which exhibit magnetic properties. MNP are usually considered for their use in magnetic separation, diagnostics, including MRI (magnetic resonance imaging) or hyperthermia and tissue engineering [3]. The bioavailability and possible application for biomedical systems [4] of MNP strongly depends on their size and structure. This is one of the major intent to use various techniques for a particle size characterization.

From the definition, one of the dimension of nanosystem should be in the range between 1 – 100nm. In practice the structures larger than 100nm but smaller than 1μm are named as a nanostructures, too. In this paper systems which the size above 100nm will be described also as submicronsized to emphasize and more clearly discuss the difference between the particle size determination techniques. As a representative nanostructures for this case study we chose the core-shell MNP. As the core magnetic iron oxide nanoparticles were prepared with the selected commonly used methods. Biopolymers, such as dextran and gelatin were used as a shell. Fluorescent agents, such as Rhodamine B isothiocyanate (RBITC) and Fluorescein isothiocyanate (FITC) were added for biopolymeric matrix. This brought the opportunities to investigate the fluorescent behavior of CS-MNP with confocal laser microscopy (CLSM) and fluorescence correlation spectroscopy (FCS). In this paper the main differences between selected particle size determination methods will be discussed.

2. Materials and methods

2.1. Preparation of the core-shell nanoparticles

Magnetic iron nanoparticles were prepared with minor changes, according to the methods described by Hyeon et al. [5], Park et al. [6] and B. Gaihre et al. [7]. Next MNP cores were attached by biopolymeric shells based on fluorescent dextran and gelatin [8-9].

2.2. Particle size determination techniques

Hydrodynamic diameters (dH) of the MNPs and CS-MNPs were obtained using Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA). For the DLS and NTA investigations NanoSight NS500 with laser beam wavelength of 405 nm were used. Before the examinations the MNP and CS-MNP were suspended in water.

For structural investigations a Transmission Electron Microscope (TEM) - JEOL JEM-1011 microscope was used in UniHamburg.

Confocal Laser Scanning Microscopic studies were performed using confocal microscope (Zeiss Laser Scanning Microscope LSM780). For the measurements, the chamber coverglass (8 WELL from LAB-TEK) was used and or filled up slide channels (1 μ-Slide from Ibidi) were used. Moreover Fluorescence Correlation Spectroscopic (FCS) measurements were made at the same CLSM with special module ConfoCore3 and software for autocorrelation function (AF) determination.

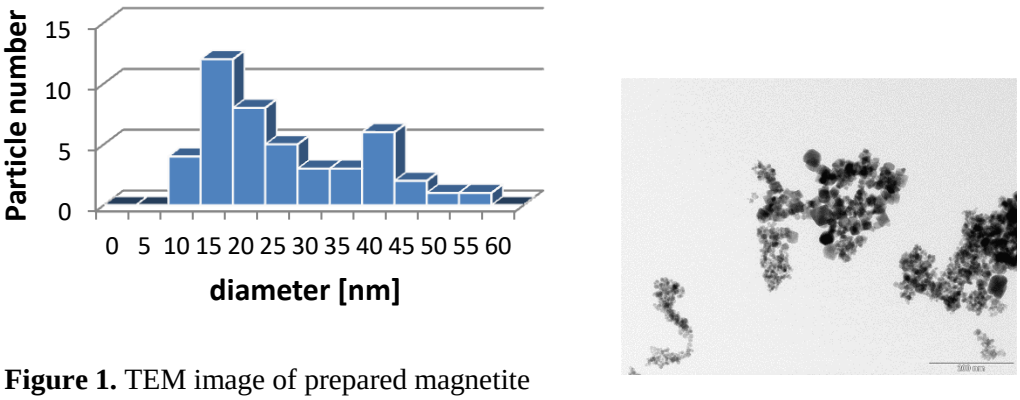

Figure 1. TEM image of prepared magnetite nanoparticle (right side) and particle size distribution from TEM (left side)

3. Results

Iron oxide nanoparticles were synthesized using 3 methods. The modification was conducted using dextran and gelatin as a shell for iron oxide particles. The fluorescent agent, which was a small volume of rhodamine or fluorescein labeled dextran or rhodamine labeled gelatin, was added to the biopolymer shell in order to enable confocal microscope investigations of the particles.

For this paper only selected and representative samples have been chosen of MNP – magnetic cores and MNP-CS –magnetic cores with fluorescent biopolymeric shell. TEM images (Figure 1 and 2a,c) of the prepared samples, confirmed that the synthesized magnetic nanoparticles (MNP) with a diameter in the range from 5nm to 50nm. Smaller particles exhibited lower polydispersity than those with the larger diameter (see Figure 2a). The cores (MNP) were next modified by covering them with labeled and unlabeled dextran and gelatin. Previously synthesized cores were mixed with the 5% solution of dextran or gelatin and vigorously stirred for several hours. Then the mixture was centrifuged several times and dialyzed overnight. The TEM images confirmed the successful preparation of the core-shell system for hydrophilic MNP.

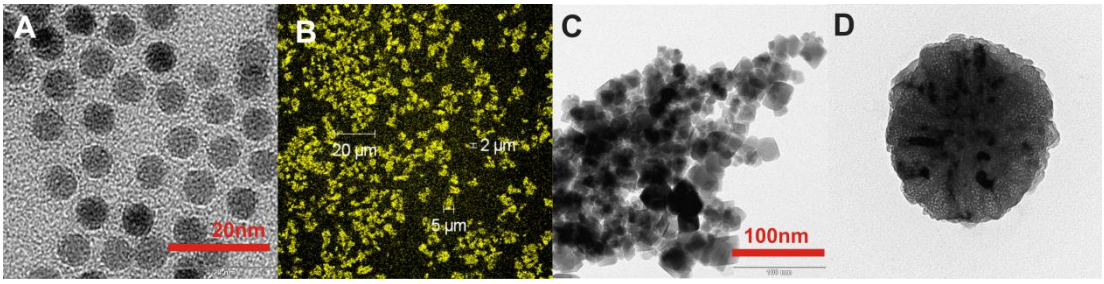

Figure 2. TEM and CLSM images of core and core-shell MNP nanoparticles: A. MNP-core with low polydispersity (TEM), B. MNP-CS with high polydispersity (CLSM), C. MNP-core with high polydispersity (TEM), D. MNP-CS with high polydispersity (TEM)

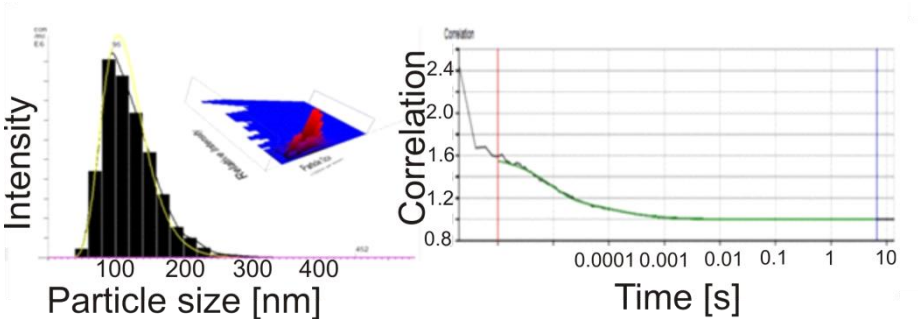

Figure 3. Particle size determination of MNP-CS, on the left: NTA anlaysis (2D plot: black bars – distribution, yellow line – main peak and 3D plot: relative intensity versus particle size) and DLS (pink line); on the right FCS measurement of core-shell systems.

Detailed TEM imaging shows that the shape and possibility of agglomeration of a particle depends on the synthesis method. The most regular spherical shapes were obtained by the methods described in [5-6]. The highest polydispersity was determined after the synthesis from iron fluorides (see Figure 2) and after core-shell structures preparation with biopolymeric dextran and gelatin shell (see Figure 2B and 2D).

To confirm the particle size, hydrodynamic diameters were obtained with two techniques: Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS). Even these two methods use the same Stokes-Einstein relation between the diffusion of the particles, hydrodynamic diameter and viscosity, the obtained results of dH are different [10]. Representative results are present in Table 1 and Figure 3(left). As it is presented, the hydrodynamic diameters are larger than determined from NTA. It is caused by the fact that the diffusion coefficient determined in NTA is independent on the molar concentration of the investigated particles (for more physical and mathematical aspects see [10-12]). Comparing TEM and NTA results it is proved that some of the samples exhibit high polydispersity.

Figure 2 (right) shows the autocorrelation function (AF) of MNP-CS which is determined with FCS technique. From the calculations after fitting the FCS results to the mathematical model, the final hydrodynamic diameter was around few nanometers. This results are outstanding from the TEM, NTA and DLS investigations. Nevertheless, in FCS technique only fluorescent part (particles) have been "seen". That indicates, that the size of the particles of fluorescent biopolymeric shells was obtained. Moreover, it is suggested that in the investigated sample the signal from core-shell MNP systems is too weak. Furthermore, it confirms that not whole the MNP cores where covered by the fluorescent shell and a lot of labelled biopolymer is in the sample.

Table 1. Representative results from NTA and DLS measurements of MNP and MNP-CS

Sample description		d_{DLSNS} [nm]	$d_{NTAmean}$ [nm]	$d_{NTAmode}$ [nm]	SD [nm]
core	shell				
MNP04	---	290	208	154	74
MNP02	dextran FITC M=6kD	99	122	95	40

4. Discussion

Particle size determination is one of the crucial step for their future applications. Nowadays a lot of techniques are available for nanoparticle characterization. Nevertheless, during the analysis of the same sample, such as in this study, some differences of the value of the particle diameters occur. Bell et al. [13] have been prepared critical evaluation of the emerging techniques for submicrometer particle sizing, using as a model silica particles. In this paper, additional technique like FCS was suggested for nanoparticle and submicron sized particles characterization. Moreover, the investigations present the results of fluorescent systems.

In general no best techniques have been developed so far and the results using different investigation methods often give different values of the particles size. The deep size and structure analysis of the nanoparticles is possible by the TEM. The size, shape and the particle size distribution can be confirmed. On the other hand, only selected area of the measured grid is investigated, which caused that lower number of the particles population is characterized. Moreover, usually for TEM the sample is dried or freeze which may cause the structure changes in some systems (i. e. hydrocolloids or polymers).

The highest concentration (the largest population) of the particles can be used in DLS (according the described techniques). With DLS, there is no limit for the solution in which particles are suspended. Unfortunately, DLS is sensitive for the dust and the impact of larger structure – in some cases, signal from the small particles can be screened by the larger particles. NTA shows better the relation between the population of two or more different particle sizes than DLS and single particle tracking is possible. With NTA fluorescent behavior of the investigated system can also be confirmed (depending on the laser wave length). For online investigations of the interaction in the system, confocal microscopy is recommended. It is usually used for the micron structures analysis and brings information (without size analysis) where the nanostructures accumulate or agglomerate creating large structures. CLSM plays a key role for biological, cell and food system investigations [14].

Typical confocal microscope with dedicated detector (in Zeiss LSM 780 - ConfoCore3 module) can be successfully used as a tool for fluorescence correlation spectroscopy (FCS) [15]. Our previously described investigations proved and experimentally validated with a simple procedure, allowing accurate FCS measurements for submicron sized particles characterization [16-17]. In the present study, results bring an information about the MNP-CS particle size and behavior.

5. Conclusions

Particle size, especially in the nanometer range, plays a key role for the final applications of newly designed material. Various techniques for nanoparticles characterization are available, but it is recommended to use more than one. Moreover, FCS might be successful applied as fluorescent

particles size determination and possible contamination of fluorescent agents in comparison of TEM, SEM and DLS techniques. Knowing the opportunities and weaknesses of presented size determination technique it is possible to choose the optimal solution dedicated for measured system.

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