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Superparamagnetic Iron Oxide Nanoparticles

An Evaluation of the Sonochemical Synthesis Process

Abstract: The direct detection of the spatial distribution of Superparamagnetic Iron Oxide Nanoparticles (SPIONs) as a tracer for Magnetic Particle Imaging (MPI) enables three-dimensional functional images with high spatial and temporal resolution. The commercially available tracers have not been developed primarily for MPI. Therefore, they do not sufficiently contribute to the desired image quality. Hence, optimizing the SPIONs during the production process is of interest. A peculiarity of the here presented synthesis method – the alkaline coprecipitation – is that this process takes place under ultrasonic control. The use of ultrasound creates extraordinary reaction conditions through sonochemical phenomena, such as formation, growth and implosive collapse of cavitation bubbles within a liquid. In addition, the ultrasonic waves and the oscillation of the medium improve the mixing process and thus ensure the homogenization during the synthesis. The objective of this study is the variation of ultrasonic frequencies and the type of used dextran as coating material, to provide SPIONs with better performance for MPI and more suitable properties for in vivo application. The focus of the optimization is to increase the magnetite core size while simultaneously reducing the hydrodynamic size. The experiments have shown that both, the ultrasound frequency and the molecular weight of used dextran, influence the properties of the SPIONs.

Keywords: Alkaline Coprecipitation, Magnetic Particle Imaging, Magnetic Particle Spectroscopy, Photon Cross Correlation Spectroscopy, Sonochemical Synthesis, Superparamagnetic Iron Oxide Nanoparticles, UV-Vis Spectroscopy

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1 Introduction

The superparamagnetic iron oxide nanoparticles as a tracer for MPI have to exhibit a high nonlinear magnetic response with

low energy dissipation in contrast to high energy dissipation of the magnetic particles used in hyperthermia [1]. The guarantee of uniform physical and chemical properties of SPIONs is essential for their medical application. The homogeneous and stable suspensions of monodisperse SPIONs can achieve this desired uniformity.

The SPIONs described in this contribution consist of a nonmagnetic dextran (see Figure 1) shell and a magnetite (Fe_3O_4) core. The thickness of the spherical particle shell – which contributes to the hydrodynamic diameter – has, in addition to the prevention of agglomeration, a particular relevance for suitable biomedical applications. However, the magnetic properties are crucial for imaging and depends heavily on the core size and the chemical composition of the SPIONs.

Figure 1: Chemical structure of dextran – polysaccharide derived from the condensation of glucose – consisting of α -1,4 or α -1,6 glycosidic linkages [2]

2 Materials and Methods

2.1 Synthesis under ultrasonic control

Alkaline coprecipitation is used as a representative of a bottom-up method for the production of the SPIONs because it offers the following advantages: a cost-effective synthesis with high yields, a high chemical reaction rate, a relatively low synthesis temperature and the specifically coated SPIONs in suspension without the need for a special equipment. On the other hand, coprecipitation is characterized by a broad particle size distribution and therefore requires intensive post-processing of the synthesized SPIONs [3].

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An alkaline environment is created allowing the formation of iron(II) and iron(III) hydroxides (1).

The 7.5 % ammonia solution is added using a syringe pump with a flow rate of 99 mL/h. In order to control particle growth and thereby influence the size and shape of the particles, the temperature is kept below 4 °C (via an ice bath). Nevertheless, an ammonia/ammonium chloride buffer is formed in the flask to prevent a significant change in the pH value.

In the next step (2), the chemical mixture is heated up to 80 °C and kept at this temperature for at least one hour to force the elimination of water with simultaneous growth of magnetite (Fe₂O₃) cores.

The particles produced in this way are characterized by particularly good stability in the suspensions [4–5].

The reaction vessel, usually a three-necked flask, remains in the ultrasonic bath during the entire reaction. The frequencies 25 kHz and 130 kHz were generated with two different ultrasonic baths Transonic TI-H (Elma Schmidbauer, Germany).

2.2 Determination of iron concentration

The iron concentration of the SPIONs is determined photometrically using UV-Vis Spectroscopy.

First, the coating of the SPIONs, which consists of dextran, is split by treatment with concentrated hydrochloric acid (conc. HCl) (3). This leads to the cleavage of the dextran molecules by acid hydrolysis from dextran to glucose.

A color change from brownish-black to yellow is observed because the iron is no longer in oxide form, but instead again in the soluble form of Fe²⁺ (4) or Fe³⁺ (5).

Since Fe²⁺ oxidizes rapidly in air to Fe³⁺, this oxidation is prevented by adding a reducing agent – the 6 % ascorbic acid (6). Therefore, prior to the complex formation, the existing Fe³⁺ must be reduced by this reducing agent; it is reflected in the immediate discoloration of the sample.

The addition of the acetate buffer ensures the pH optimum adjustment at 4.5. The divalent iron reacts in acidic solution with 1,10-phenanthroline (Ph) to form a concentration-dependent, intensively red-orange ferroin complex [Fe(Ph)₃]²⁺ (see Figure 2), connecting Fe²⁺ ion with three molecules of the chelating agent (Ph) [6–7].

Figure 2: Formation of ferroin complex [8]

After completion of the sample solution, its absorption is determined photometrically at the wavelength of 510 nm.

3 Results and Discussion

The evaluation is carried out with respect to hydrodynamic diameter, magnetic performance in MPI and iron concentration. Due to the wide particle size distribution of the synthesized SPIONs, the produced suspensions are magnetically separated and consequently centrifuged with the relative centrifuge force of 352.837 g (6,000 RPM) for 99 minutes (EBA 20, Hettich, Germany). Finally, the SPION fractions are extracted according to the graduation of the centrifugation tubes within the framework of post-processing the synthesized suspension. Four different fractions were separated (see Tab. 2 and 3). In addition, the hydrodynamic diameters of the particles were determined directly after the synthesis process (raw particles).

Table 1: Evaluation methods and the used instruments

Method	Model	Manufacturer
Photon Cross Correlation Spectroscopy (PCCS)	NANOPHOX	Symphatec, Germany
Magnetic Particle Spectroscopy (MPS)	MPS 1.0	ForkLabs, Germany
UV-Vis Spectroscopy	UV 1800	SHIMADZU, Japan

3.1 Hydrodynamic diameter via PCCS

The cuvettes for PCCS-measurement were filled with 50 μL SPION suspension and 2 mL deionized water and then tempered at the temperature of 25 $^{\circ}\text{C}$ in the water bath. The refraction was adjusted to 1.34 and viscosity to 0.89 mPas. The measured data was evaluated intensity-weighted by Nanophox (Non Negative Least Square -NNLS- was selected as an evaluation mode because of its capability to detect multimodal distributions).

Table 2: Measured hydrodynamic diameters [nm] of SPION fractions via PCCS. Comparison of four different approaches with two different ultrasound frequencies and two different dextrans

SPION fractions*	25 kHz** 70***	130 kHz** 70***	25 kHz** 10***	130 kHz** 10***
raw	159.72	189.12	145.13	180.55
0 – 2 mL	160.30	172.28	103.64	123.17
2 – 6 mL	111.81	105.92	59.03	72.32
6 – 10 mL	93.98	94.73	57.56	63.16
10 – 14 mL	82.36	77.78	52.08	58.57

* SPION fractions extracted according to the graduation of the centrifugation tubes

** Ultrasound frequency used by the synthesis

*** Molecular weight of used dextran [kDa]

The thickness of the nonmagnetic shells was expected to depend primarily on the dextran type. However, the evaluation of the different experiments revealed that the correlation is more complex. The raw particles synthesized at 130 kHz have larger hydrodynamic diameters than their equivalents synthesized at 25 kHz for both types of used dextran. The frequency of 130 kHz provides a more intensive mixing of the reactants. Due to the vibrations caused by the ultrasonic transducer there is an increased reactant motion, therefore several dextran molecules may form bonds to the magnetite cores. This is reflected by the increase in dextran shell thickness. For the smaller molecules (Dextran MW 10,000) the effect is stronger than for the dextran with higher molecular weight (Dextran MW 70,000), since the smaller molecules are easier to move by high-frequency ultrasonic waves. The observation that the particle size increases by a more intensive mixing process is confirmed by the results of Baldyga et al. [9] and the interpretation of Phillips et al. [10].

3.2 Magnetic response via MPS

Magnetic Particle Spectroscopy (MPS) can be interpreted as Magnetic Particle Imaging (MPI) without spatial encoding. The magnetic excitation frequency of the MPS used here is set

to match the situation in MPI such that the magnetic response can be used to predict the SPIONs' behavior in MPI. The measurements were executed at standard conditions: magnetic excitation field strength of 20 mT with a sinusoidal wave of 25 kHz which forms the first odd harmonic in the particle response. The measurement sequence includes 10 periods with 12,500 repetitions.

As it is difficult to show all the MPS-measurements, therefore one exemplary measurement of the synthesized SPIONs with dextran 10 and ultrasound frequency of 25 kHz is presented in Figure 3 and will be further discussed in Conclusion.

Figure 3: Odd harmonics of the amplitude spectrum of SPION fractions (25 kHz, Dextran 10) and Resovist® (concentration normalized values)

3.3 Iron concentration via UV-Vis Spectroscopy

As described in section 2.2, the photometric determination of the iron concentration was realized forming a 1,10-phenanthroline complex.

Table 3: Determined iron concentration [mol/L]

SPION fractions*	25 kHz** 70***	25 kHz** 10***	130 kHz** 70***	130 kHz** 10***
0 – 2 mL	0.342	0.229	0.344	0.187
2 – 6 mL	0.094	0.129	0.086	0.100
6 – 10 mL	0.066	0.110	0.074	0.088
10 – 14 mL	0.041	0.105	0.047	0.090

* SPION fractions extracted according to the graduation of the centrifugation tubes

** Ultrasound frequency used by the synthesis

*** Molecular weight of used dextran [kDa]

The SPIONs with dextran 10 have more uniform iron concentrations across the fractions, compared to differences in concentrations of SPIONs with dextran 70. The smaller size of dextran 10 is likely to result in a more homogeneous distribution of the reactants in the reaction volume.

Nevertheless, the concentrations of the SPION fractions are significantly lower (in range of 0.3 mol/L) than the iron concentration of Resovist® (0.5 mol/L) as the “gold standard” for MPI. Usually, higher concentration of magnetite leads to better resolution of the images obtained through MPI. The concentration of the dialyzed fraction can be concentrated via Spectra/Gel® Absorbent. On the other hand, the stability of the concentrated samples must be observed over a long time period.

4 Conclusion

The fact that the obtained results with respect to the ultrasonic frequency are not clearly interpretable stems from the interaction between the size and amount of cavitation bubbles formed due to the energy conservation law.

In the case of the objective set out here, the application of the low frequency ultrasound tends to be more suitable, since the deteriorated mixing process leads to the desired smaller hydrodynamic diameters. However, local supersaturating zones are considered as a support factor for nuclear growth according to the LaMer model [11]. Compared to a high ultrasound frequency of 130 kHz, a more inhomogeneous reactant distribution with local maximum supersaturations is achieved at the lower frequency of 25 kHz. In such zones, crystal growth preferably occurs. The wide particle size distribution with respect to the cores confirms the syntheses with dextran 10 at 25 kHz. In addition, the larger cavitation bubbles at the lower frequencies release more energy and form extraordinary reaction conditions with respect to temperature and pressure, further favoring the formation of the larger magnetite cores.

In comparison to Resovist®, higher odd harmonics in the amplitude spectrum are visible for the synthesized samples, enhancing the image resolution (see Figure 3). However, a part of the processed SPIONs with dextran 10 at 130 kHz exhibit an appropriate magnetic behavior for MPI. This means that the

syntheses at a higher frequency also produce suitable particles for MPI. However, these are only apparent after the post-processing with a very small yield.

The SPIONs with dextran 10 present no disadvantages compared to dextran 70. On the contrary, their reduced size is particularly attractive for in vivo applications. After a visual inspection, there is no indication for an increased probability of agglomeration. However, long-term stability studies have not yet been performed.

Author Statement

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