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Fast detection of SARS-CoV2 Antibodies by the use of critical off-set magnetic particle spectroscopy (COMPASS)

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Abstract

Current bioassays for detection of antibodies or antigens such as ELISA (Enzyme-linked Immunosorbent Assay) are relatively inflexible, expensive and time-consuming. Upcoming methods, such as ACS (AC susceptometry) or MPS (Magnetic Particle Spectroscopy), exploit the magnetization response of functionalized magnetic nanoparticles (MNP) ensembles to assess specific information about the MNP mobility as well as conjugations of chemical or biological compounds on their surface. Both methods have shown promising results in the past but cannot reach the sensitivity of above-mentioned techniques. We used a novel method based on a modified MPS being sensitive to minimal changes in mobility of MNP ensembles. This facilitates robust and easy-to-handle measurements of minimal changes in the diameter of MNPs. As an example, we detected SARS-CoV-2 antibodies binding to the S1 antigen on the surface of functionalized MNPs. Without any purification or incubation, we could show a sensitivity of less than 50 ng/mL of SARS-CoV-2 antibodies in samples.

I. I. Introduction

The measurement of biological molecules, such as for example antibodies, is a highly important issues in medical diagnostics long before the SARS-CoV2 pandemic. Methods like ELISA, real-time-PCR or Loop-mediated Isothermal Amplification (LAMP) are highly efficient methods that can be used for the diagnosis of a huge number of probes, but with these methods the problem is the need of specialized extraction, incubation and washing steps, a dedicated laboratory and machines as well as sophisticated and reliable probe logistics. Immunobased quick tests based on gold nanoparticles and antibodies or aptamers have become familiar to the majority of the cur-

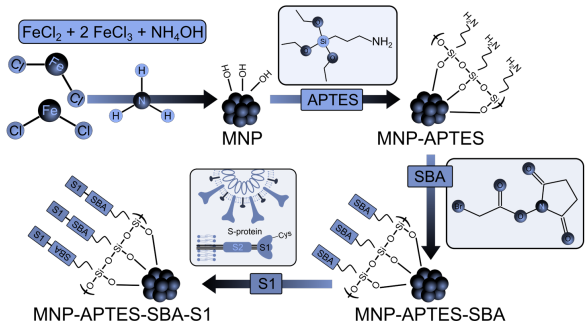

Figure 1: Synthesis of MNP-APTES-S1.

rent population all over the world. They are an example of the high potential of using nanoparticles.

On the other hand, magnetic nanoparticles (MNPs) have gained an enormous interest in many areas. By controlled engineering of specific surface properties, named functionalization, MNPs can be equipped with special features for desired applications, e.g., bioassays for the detection of biomolecules or biomarkers such as antibodies [1, 2]. Here, we report on the synthesis of MNPs (iron oxide NP) dedicated for measuring biological probes with a novel method enabling highly sensitive and robust detection of SARS-CoV2-Antibodies competitive to ELISA in sensitivity. The COMPASS (**C**ritical **O**ffset **M**agnetic **PA**rticle **S**pectro**S**copy) method is based on a critical offset magnetic field effect of MNPs that can be performed at the point of care without extraction, incubation and washing steps [3].

II. Material and methods

The MNPs were produced by alkaline precipitation. Briefly, iron -salts were precipitated by the addition of 25% NH_3 and coated with APTES ((3-aminopropyl) triethoxysilane, Carl Roth, Germany) resulting in nanoclusters with a hydrodynamic diameter of approximately 200 nm [4]. Functionalization was carried out using SBA (N-succinimidyl bromoacetate) serving as a linker for the covalent coupling of proteins or peptides [4] and here the SARS-CoV2-S1 protein to the MNPs surface (Fig. 1).

II.I. ELISA sample preparation and measurement

S1 antibodies (RayBio, Peachtree Corners, GA) were diluted from 1,500 ng/ml to 25 ng/ml. For the measurement, 100 µl sample was added to each well and 100 µl of biotinylated anti-human IgG antibody was added. After Incubation of 1h, washing, adding of horse-radish-peroxidase-streptavidin solution, another 30 min of incubation, washing, adding of TMP One-Step Substrate Reagent, further 30 min of incubation and addition of

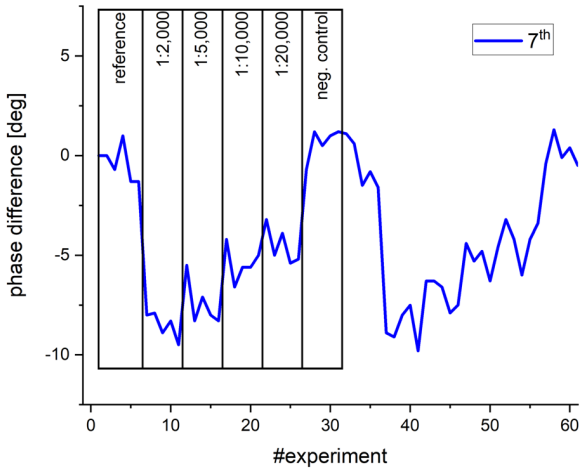

Figure 2: Results of the first MPS experiments. The single experiments of the measuring sequence (ref, 1:2,000, 1:5,000, 1:10,000, 1:20,000 (equaling 50 ng/ml), neg. control) show a clear trend in phase difference of the 7^{th} higher harmonics. Each sample was measured 5 times without any averaging (acquisition time 10 ms each).

stop solution the measurement was performed at 450 nm using a dedicated plate reader.

II.II. Sample preparation for COMPASS

For the detection of S1 antibodies using the COMPASS device, the stock solution of the antibodies was diluted in buffer 1:2 000, 1:5 000, 1:10 000, and 1:20 000 (corresponding to 50 ng/ml for the lowest antibody concentration). An amount of 25 µl of MNP-APTES-S1 dispersion (100 µg Fe/ml) was added in an 0.5 ml Eppen-dorf cap. Subsequently 25 µl of antibody dilution (*S+*) or buffer (*ref*) were added. Samples were directly measured in the COMPASS device after careful mixing by pipetting without any further incubation times.

II.III. COMPASS device

The COMPASS device is a modified MPS device utilizing time-varying (AC) and static (DC) magnetic fields in a specific manner. At specific rations of AC and DC, each higher harmonic shows so-called critical points where a strong phase shift of the signal can be observed [3]. By varying the MNPs diameter, the magnetization response of the particle ensemble changes slightly. This results in a strong phase shift when measuring at a critical point. When using a static offset magnetic field gradient, the acquired signal consists of multiple phase shifts coming from different higher harmonics critical points.

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III. Results and discussion

To evaluate the sensitivity of COMPASS we compared the detection of SARS-CoV2 antibodies to ELISA.

Using the above-mentioned standard SARS-CoV ELISA test (RayBio® COVID19 S1 RBD protein Human IgG ELISA Kit Protocol, RayBiotech, Inc.) according to the manufacturer's instructions, the detection limit was between 25 ng and 50 ng antibodies per ml, but here the incubation time had to be extended to 18 h due to the low signal.

With COMPASS the samples were measured directly after short mixing (app. After 75 sec.). The acquisition time of each measurement was 10 ms. The single experiments (5 repetitions each) of the measuring sequence show a clear phase difference in the 7^{th} harmonics. In first experiments dilutions of 1:2 000, 1:5 000, 1:10 000 and 1:20 000 could be clearly distinguished from the negative control revealing a sensitivity of 50 ng/ml antibody which is comparable to ELISA (Fig. 2).

After hardware modifications, in further experiments the sensitivity could be expanded first to 1:200 000 (5 ng/mL) and later even to 1:500 000 (2 ng/ml) (data not shown) [3].

IV. Conclusions

Current methods of molecular diagnostics like ELISA or PCR are very sensitive but are needing sophisticated machinery, logistics and preparation as well as incubation times and washing steps. Although they are developed for high throughput analysis of enormous sample numbers all these steps are time consuming and the methods are relatively expensive. The COMPASS method is taking advantage of the differences in the magnetic behavior of MNPs that bind a biomolecule e.g. an antibody compared to the pure particles or a negative control, e.g. a non-binding antibody.

The sensitivity of the measurements presented here is in the range of commercially available ELISA tests (50 ng/ml) but we could also show that by hardware adjustment an improvement of 10 times was possible. Nevertheless, it is important to point out that these results were acquired after a sample preparation time below 2.5 minutes without further incubation, washing or other time-consuming steps: In contrary to that the preparation and measurement of ELISA needs several incubation- and washing-steps and a time period of at least around 1h up to overnight without counting time for probe logistics.

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Author's statement

Conflict of interest: Authors state no conflict of interest.
Informed consent: Informed consent has been obtained from all individuals included in this study.

References

[1] Ali, A. et al. *Review on recent progress in magnetic nanoparticles: synthesis, characterization, and diverse applications.* Front. Chem.9, 629054 (2021).

[2] K. Wu et al., *Magnetic nanoparticles and magnetic particle spectroscopy-based bioassays: a 15-year Recap*, Nano Futures, vol. 6(2):022001, Apr. 2022.

[3] P. Vogel et al., *Critical Offset Magnetic PArticle SpectroScopy for rapid and highly sensitive medical point-of-care diagnostics*, Nature Communications, vol. 13(1):7230, Nov. 2022.

[4] Friedrich, B. et al. *Scavenging of bacteria or bacterial products by magnetic particles functionalized with a broad-spectrum pathogen recognition receptor motif offers diagnostic and therapeutic applications.* Acta Biomater 141, 418-28 (2022).

