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# The spike protein of SARS-CoV-2 can be detected by electrochemical impedance spectroscopy using antibody desorption from iron magnetic nanoparticles

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**Abstract:** SARS-CoV-2 is a matter of concern. Here, biosensors were prepared using iron magnetic nanoparticles containing antibodies against the receptor binding domain (RBD) of the spike protein. Antibodies were adsorbed to nanoparticles in three configurations, including direct adsorption without functionalization (DANPs). Nanoparticles were added to a glassy carbon electrode and connected to an electrochemical cell. Electrochemical impedance spectroscopy and ELISA experiments indicated that antibodies were desorbed from the DANPs upon the addition of the RBD. DANPs-based biosensors produced linear curves with decreasing charge transfer resistance due to the removal of antibodies. Thus, a detection method can be based on antibody desorption.

**Key words:** Biosensor, electrochemical impedance spectroscopy, spike protein, receptor binding domain, COVID-19, SARS-CoV-2.

## INTRODUCTION

The number of deaths due to COVID-19 reflects the importance of detection methods. The golden standard method is RNA amplification (Filchakova et al. 2022). However, biosensors are more useful (Suleman et al. 2021). Biosensors use biological recognition connected to an electrochemical device (Mendes et al. 2018). The analysis is based on electrochemical signals upon interactions of the electrode with specific analytes, where the signal is enhanced by nanoparticles. One of the most used nanoparticles for biosensor assembly is iron magnetic nanoparticles (MNPs) given their biocompatibility and low cost. Examples of biosensors using this system can be found in the literature (Rocha-Santos 2014).

Some biosensors use immobilized antibodies attached to the working electrode (Filchakova et al. 2022). Desorption of the biological recognition element, thus the antibody, is an undesirable phenomenon, as it interferes with quantification. Here, we evaluated 3 different configurations for the development of a biosensor to detect the spike (S) protein of SARS-CoV-2. We observed that, for a device based on antibodies against the receptor biding domain (RBD) of the S protein, the desorption phenomenon that occurred upon antigen binding gives a linear response for increasing concentrations of the antigen. Therefore, a biosensor could be designed based on antibody desorption.

## MATERIALS AND METHODS

Monoclonal antibodies against the RBD of the S protein of SARS-CoV-2 were from Rheabiotech,

Campinas – SP, Brazil. A sample of the antigen, RBD domain of S protein was kindly given by Rheabiotech. Iron magnetic nanoparticles were produced in the laboratory (Lu et al. 2007). Anti-mouse IgG-peroxidase antibody, produced in rabbit was purchased from Sigma-Aldrich. Fraction V, Bovine serum albumin (BSA) and glutaraldehyde 25% solution were also purchased from Sigma-Aldrich.

Suspensions of 100 µL of iron magnetic nanoparticles (NPs), from a 300 mL suspension that was prepared according to a previously described method (Lu et al. 2007), were washed 3 times with 100 mmol/L phosphate buffer (PB), pH 7.0. These were used to produce 3 differently functionalized NPs to study the best conditions to produce analytical curves using electrochemistry. In method 1, NPs were treated with chitosan 1 mg/mL in 2.5% acetic acid and incubated at room temperature (RT) for 50 min. The NPs were washed 3 times in PB and then mixed with monoclonal antibodies (MAB) using 300 µL of a 10 µg/mL solution (Ganganboina & Doong 2019). In method 2, NPs were washed with 100 mmol/L PB, pH 8.0 and were treated with the same buffer containing 1% BSA for 60 min at RT. The functionalized nanoparticles were washed 4 times with 500 µL of PB, and then treated with a 10 µg/mL MAB solution, containing 1% glutaraldehyde in PB, pH 8.0. Incubation was for 30 minutes at RT and 13h at 4°C. After incubation the NPs were washed 5 times with PB, pH 7.0 and resuspended in 100 µL. In method 3, the NPs were directly treated with 300 µL of 10 µg/mL MAB solution producing the DANPs.

To produce a biosensor, 20 µL of the NPs suspension was added to the working electrode's surface and the device was treated with a low flux of air until completely dry. The functionalized electrode was placed within a 3 mL electrochemical cell containing PB, pH 7.0. Electrochemical impedance spectroscopy (EIS) was used to evaluate the effect of the concentration of the S protein on charge transfer resistance. To test the applicability of each of the methods described above, at least 3 independent experiments were carried out using freshly prepared biosensors and increasing concentrations of the S protein, which was added to 3 mL of PB within the electrochemical cell. The additions of antigen were followed by a first EIS determination, allowing the antigen to diffuse within the solution. Only the second measurement was considered valid. Therefore, an interval of approximately 5 min was given for antigen diffusion at the double layer. The value of charge transfer resistance was determined from three points of the double layer region of Nyquist Plots (Mei et al. 2018). EIS experiments were carried out with the potentiostat/galvanostat PGSTAT 204 from AUTOLAB using the impedance mode FRA32M. Working conditions used a sinusoidal wave with an amplitude of 10 mV and variable frequency in the range of 0.1 Hz to 1 MHz.

In method 3, to confirm that antibody was leaking from the DANPs, an ELISA assay was designed to detect trace amounts of MAB. Briefly, an aliquot of 50 µL of the volume within the electrochemical cell was collected and used as an antigen, considering the use of a peroxidase-labeled anti-mouse IgG as a secondary antibody. The samples were collected before and after the addition of increasing amounts of antigen. Immobilization of leaked MAB was carried out by mixing the sample with an equal volume of 100 mM cabonate-bicarbonate buffer, pH 9.6 (Bantroch et al. 1994). The ELISA plate was then developed according to the protocol described by Sigma-Aldrich.

## RESULTS AND DISCUSSION

The pandemic caused by coronavirus SARS-CoV-2 is still a threat to the world due to the appearance of several mutations and increasing infection rates (Akkiz 2022). Electrochemical detection offers low-cost procedures, which provide immediate and accurate results (Suleman et al. 2021). Based on this rational, the aim of the present work was to test which adsorption method would give best results, considering the use of low-cost reagents, to produce an immune biosensor that could be used to detect SARS-CoV-2. Of the three methods described, only the strategy that used direct adsorption of MAB on iron magnetic NPs presented a linear response. The other two methods started with an increase in resistance, as a function of antigen concentration. However, this was followed by a decrease, which was interpreted as desorption of antibodies (method 1) and desorption of nanoparticles from the electrode's surface (method 2), observed as a pellet at the bottom of the electrochemical cell. For a better understanding of the three methods, a scheme is presented in Figure 1.

Figure 2 shows a linear curve for transference charge resistance as a function of the concentration of the RBD of S protein, ranging from 5 to 30 ng/mL. The concentration values are calculated based on a final volume of 3 mL of PB within the electrochemical cell. Considering that very small amounts of the antigen were added at each measurement, we used EIS, which gives errors at individual measurements of approximately 0.1 Ohm (according to equipment features). These characteristics support the

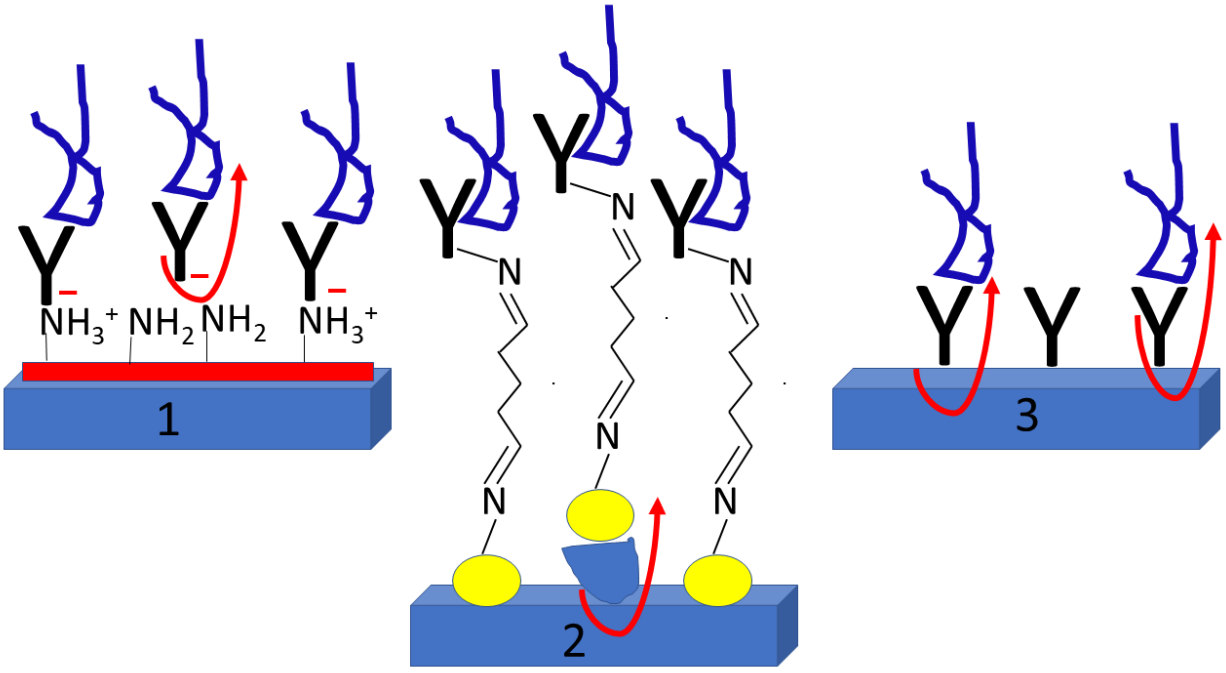


**Figure 1.** A scheme showing methods 1, 2, and 3, where nanoparticles attached to the electrode are blue; red is chitosan (where the amino groups are indicated); "Y " is an antibody, and the blue structure is RDB of S protein. In 1, the possible cause of non-linearity is that antigen may remove antibodies that are weakly adsorbed, for instance bearing no electrostatic interaction with chitosan. In 2, another possible cause of non-linearity is that the strong interaction of antigen with the covalently bound antibodies causes some nanoparticles to desorb from the electrode. In 3, antibodies adsorbed to DANPs are removed from nanoparticles in a linear fashion, according to the antigen's concentration.

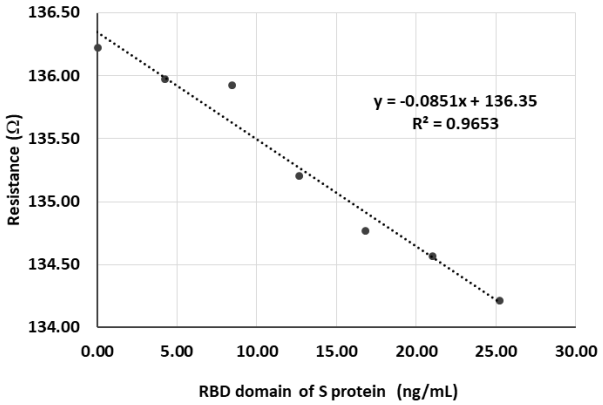


**Figure 2. A decrease in charge transference resistance was observed in method 3, in which antibodies were directly adsorbed to nanoparticles. When plotted as a function of antigen concentration, a linear response was observed.**

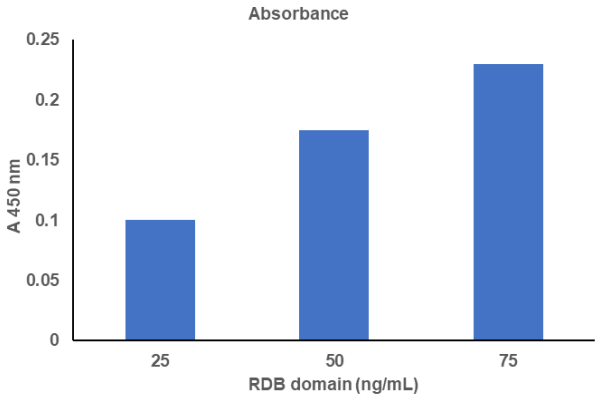


**Figure 3. Results from ELISA using anti-mouse IgG confirm that monoclonal antibodies against the RBD domain of protein S were desorbed from the electrode's surface.**

results that show reliable minor variations on the transference charge resistance.

In electrochemical measurements, the interaction of molecules at electrode's surface, resulting in adsorption of ligands, normally causes an increase in resistance. However, if a decrease in resistance is observed, there may be multiple causes, for instance, a rearrangement at the surface, or a leakage of biomolecules (Soto & Orozco 2022). To confirm that MAB are leaking from the electrode, aliquots were collected before and after the addition of increasing concentrations of antigen, using method 3. These were coated onto ELISA plates using qualitative analysis, as shown in Figure 3. The results from ELISA confirmed that MABs were desorbed from the electrode, suggesting that the decrease of charge transfer resistance that occurs upon antigen interaction, is due to MAB desorption from nanoparticles.

## CONCLUSION

In the present work we show that a simple and low-cost device can be produced based on the direct adsorption of antibodies onto iron NPs. The principle uses immune detection and the non-orthodox specific desorption of antibodies. Further experiments can be designed using, for instance cyclic voltammetry, differential pulse analysis, ELISA, and quartz crystal microbalance, for COVID detection and additional antibody/antigen detection systems, using appropriate controls.

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## Author contributions

AE designed the work and wrote the manuscript. RKM contributed with expertise in analytical chemistry, biosensor development and characterization. WEG contributed with expertise in physics, experimental design, and execution. The Master's students JJTJP and SLCM and undergraduate student LOC were supervised by AE.