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Rapid electrochemical impedance spectroscopy for protein detection in Lab-on-a-Chip devices

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Abstract

Lab-on-a-Chip devices form an ever-growing segment of the IVD market, and there is a pronounced need for reliable, rapid detection methods for various biomarkers, especially label-free methods. Electrochemical impedance spectroscopy (EIS) in liquids means the determination of passive electrical properties of ingredients in a continuous or segmented flow of fluids. More specifically, it means label-free discovery, counting and characterization of particles, mostly concentrations of various ionized molecules in chemical solutions or biological particles in fluids. We present results in rapid solution impedance spectroscopy to detect protein interactions (antibody-antigen) in human serum. The experimental setup was based on screen-printed electrodes (Dropsens DRP-C220AT) and cuvettes (Brandtech 7592 00), and the serum was buffered in PBS. Two different serum-antibody mixtures were created and impedance spectra recorded over 15 minutes to determine whether the system was capable of discerning solution compositions. Significant shifts in impedance magnitude and phase were detected in the 10 Hz-100 kHz range, with a clear difference between peaks for both solutions. Although in the described experiment, the solution was static, this setup could be adapted to be part of a flow cell.

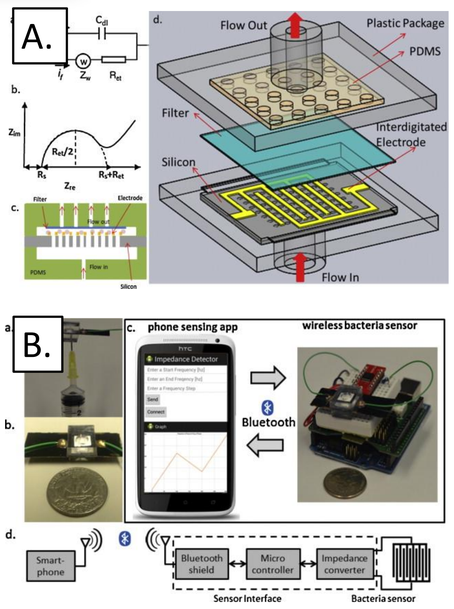

Fig. 1: Impedance spectroscopy for pathogen detection is a widely researched field, especially as detectors in Lab-on-a-Chip applications, due to the inherent advantages of compactness, low cost of manufacturing and automation possibilities. The sum of these advantages makes these sensors favorable for portable and/or disposable rapid tests. A good example is the work of Jiang et al. [1], demonstrating a smartphone-based sensing application for the detection of bacteria in field water. Their setup contained micromachined electrodes in a porous silicon-PDMS microfluidic chip (A.). Bacteria would pass through the system and the impedance change recorded. Results were to be displayed on a smartphone, communicating wirelessly with the Lab-on-a-Chip device.

Pilot

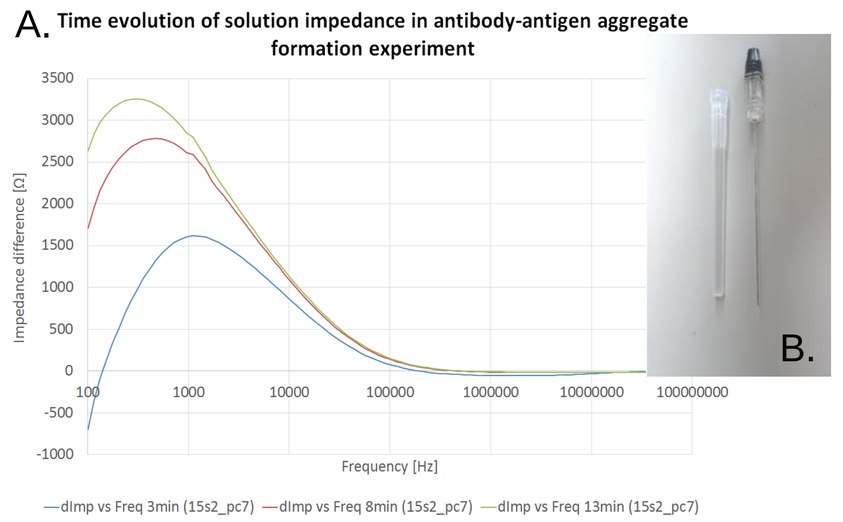

Fig. 2: A. The resulting impedance spectra recorded, showing antibody-antigen aggregate formation, B. the proprietary syringe probe from Injeq

A mixture of human serum and proprietary phage-bound antibodies (from ProtoBios OÜ) was diluted in PBS and a proprietary syringe probe (from Injeq) inserted in the solution for recording impedance spectra. The antibody-antigene aggregation process was observed during 13 minutes and the impedance spectra recorded at preset times. Results indicate a significant change at lower frequencies as the reaction takes place.

Experimental setup

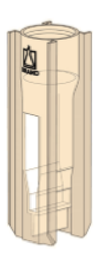

Fig. 3: Dropsense electrode DRP-C220AT (left), DRP-C220AT electrode with adapter (middle) and Brandtech 7592 00 micro-cuvette (right)

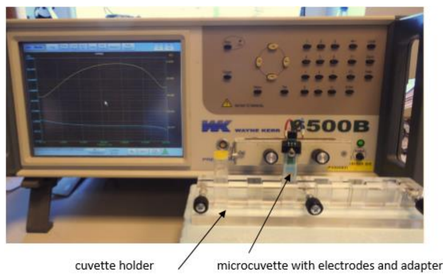

Fig. 4: Wayne-Kerr 6500B Impedance Analyser

Solution components: Protobios proprietary phage-bound antigen solutions (P1,P2), human blood serum samples (S1,S2), PBS buffer in the same concentration for experiment 1&2

Electrodes and Electrochemical cells: Dropsense electrode DRP-C220AT purchased from Dropsens with ceramic substrate, gold working (WE) and counter electrode (CE), and silver reference electrode (RE). For the experiment only WE and CE have been used.

Electrochemical equipment and measurements: All electrochemical measurements were performed using Wayne-Kerr 6500B Impedance Analyser. The measurements were performed in a frequency range from 100 Hz to 120 MHz with excitation signal amplitude of 1V. Impedance measurement data was recorded in 1 min intervals.

Results

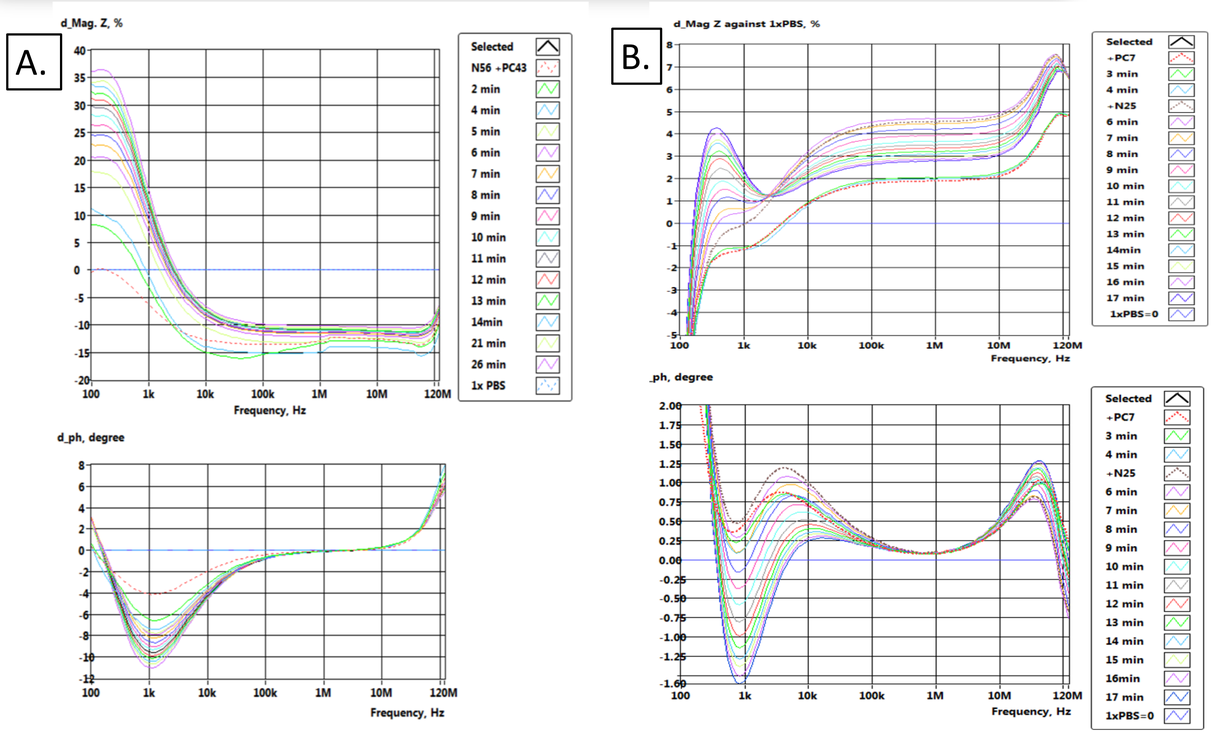

Fig. 5: Results from experiment #1 (A.) with serum S1 and phages P1, as compared to PBS solution. Results from experiment #2 (B.) with serum S2 and phages P2, as compared to PBS solution. Signal deflections at high frequencies (above 20MHz) are largely influenced by the stray capacitance of electrodes and connection wires, and therefore only changes in the lower frequency range (10-100 kHz) are to be considered substantial.

Relative changes of the impedance against the baseline (PBS). The dotted red curves represent the first change in the impedance of the mixture of human blood serum samples with phage-bound antigens, buffered in PBS. The rest of the curves show impedance changes in time (as marked in the legend). In both experiments, impedance magnitude changes are observed at lower frequencies (<10 kHz), and phase angle changes at 1 kHz, indicating a significant change of the ionic composition of the solution as protein binding and antibody-antigen aggregate formation takes place. However, to confirm the diagnostic significance of this observation, experiments must be repeated.

Conclusion & outlook

In the work presented, two different mixtures of human serum and proprietary phage-bound antibodies were diluted in PBS and impedance spectra have been recorded. Significant shifts in impedance magnitude and phase were detected in the 10 Hz-100 kHz range with a clear difference between peaks for both solutions. It has to be noted that the results in two experiments are not directly comparable. In the first experiment, the mixture of phages and serum was prepared before adding it to PBS solution while in the second experiment the solution with phages and serum were added consequently. Since the changes of the impedance are emphasized in the low-frequency area, a further experiment with the same solutions and lower excitation level (≤100 mV) is required.

References

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