

Nanoporous Surfaces: Bioelectric Interfaces for Pathogen Detection

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Abstract:

The goal of this project was to build a biosensor to detect low concentrations of pathogens [1]. The purity of a liquid depends on the pathogens that contaminate it. We used a label free technique, which uses electrochemical impedance spectroscopy (EIS). By keeping our methods label free, detecting pathogens is cheaper and less time consuming, as compared to conventional methods. We used a printed circuit board (PCB) based-device and an alumina membrane to generate a nanoporous surface. By overlaying the membrane on top of the interdigitated electrodes of the PCB, a high-density array of nanowells was formed, which facilitated nano-confinement and allowed for size based trapping of the pathogens. We used layer-by-layer chemistry. The membrane was functionalized such that the cationic polymer attached to the membrane. The endotoxin, being anionic, bound to the cationic polymer, forming an electrical double layer. The variations in the impedance of the electrical double layer due to the changes in the concentrations of the pathogen were characterized using EIS. We have identified the performance parameters of the biosensor for pathogen detection.

Introduction:

Testing the quality of drinking water for bacterial contamination is important because by testing for low concentrations of pathogens, many illnesses that are contracted via polluted water, such as cholera, can be avoided [2].

This summer we embarked on the first steps of building the said biosensor that would be portable, fast, and cost efficient. This was accomplished by using a label free technique, electrochemical impedance spectroscopy (EIS). In order to use EIS to characterize the impedance changes that occurred for different concentrations of endotoxins, layer-by-layer chemistry was used to modulate the charge in the electrical double layer at the solid/liquid interface, which allowed us to detect specific stepwise changes in impedance, that occur for the different dose concentrations of the endotoxin.

Materials/Methods:

The materials used include an alumina membrane with a nanoporous surface, polyacrylic acid (PAA), a cationic polymer, and an endotoxin (Lipopolysaccharide, LPS). Having an alumina membrane with pores that have an upper diameter of 200 nm and a lower diameter is 20 nm was crucial because when the membrane is placed on top of the interdigitated electrodes, it forms a high-density array of nanowells, which facilitate nano-confinement of the cationic polymer and allow for size based trapping of the endotoxin. Size matching is important because it allows for an increase in the binding efficacy of the endotoxin, which amplifies the signal. Layer-by-layer chemistry [3], which was used to create an electrical double layer, was accomplished by

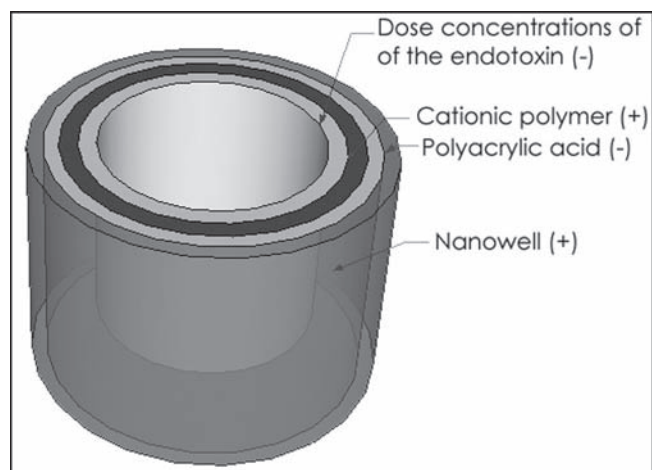


Figure 1: Layer-by-layer chemistry.

first functionalizing the nanoporous membrane with PAA. PAA was used as an adhesion layer between the alumina membrane and the cationic polymer. The endotoxin was the final layer added. This is illustrated in Figure 1.

When all the species bind together, they create an electrical double layer. The electrical double layer acts like capacitance. As the capacitance changes, so does the impedance of the system. Electrochemical impedance spectroscopy (EIS) [4] was used to characterize the system by measuring the change in impedance that occurred at different frequencies. This was accomplished by applying a voltage across a range of frequencies, measuring current, and calculating

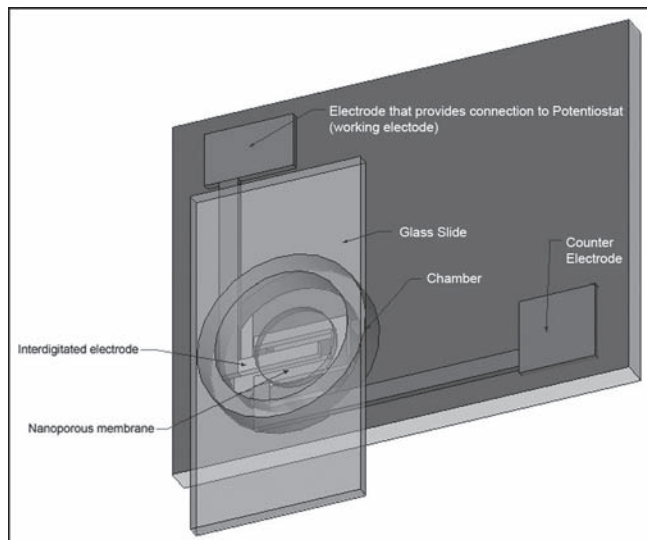


Figure 2: Experimental set-up.

the impedance at each frequency. This is called frequency response. When the frequency response is obtained for different doses, it is called the dose response. Figure 2 shows the experimental setup.

Results:

There were two experiments conducted, one with PEI-25, and the other with NPGDE 1,5 BIS. PEI-25 is a commercially available polymer, which was used to establish the concept. NPGDE is a polymer that was synthesized specifically for our experiment.

Experiment 1: As seen in Figure 3 (dose response), the impedance normalized to a phosphate buffered saline (PBS) baseline was plotted across dose concentrations in micrograms per milliliter ($\mu\text{g/ml}$). We took measurements for seven concentrations from 1-100 $\mu\text{g/ml}$. Higher concentrations are not applicable for future applications as they are not clinically relevant.

Experiment 2: After the establishment of concept with PEI-25, NPGDE was used. Data was plotted in the same way as PEI-25, as shown in Figure 4. This time there were 11 LPS concentrations from 1-500 $\mu\text{g/ml}$. Higher concentrations were tested for this run because less information is known about how this polymer interacts, and thus, performance parameters could be determined.

Conclusion:

The device was shown to be capable of detecting endotoxins in the lower $\mu\text{g/ml}$ regime. An increase in the measured impedance was observed for increasing concentrations of the endotoxin. There also appeared to be a significant increase in the impedance changes associated with endotoxin binding with the polymer NPGDE which indicated that NPGDE was more effective than PEI-25 at detecting endotoxins.

Future Work:

Future plans involve screening a library of polymers to identify the polymer that will be the best match for endotoxin detection. Eventually this will lead to the development of a portable water quality monitoring device.

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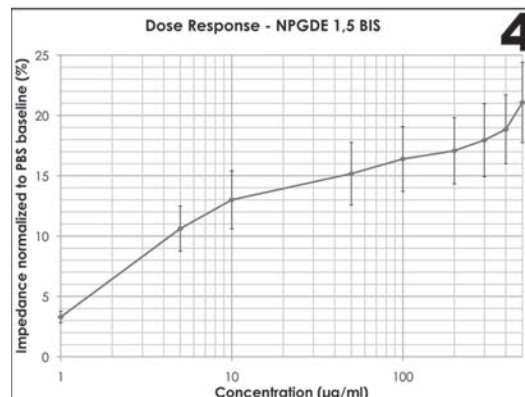
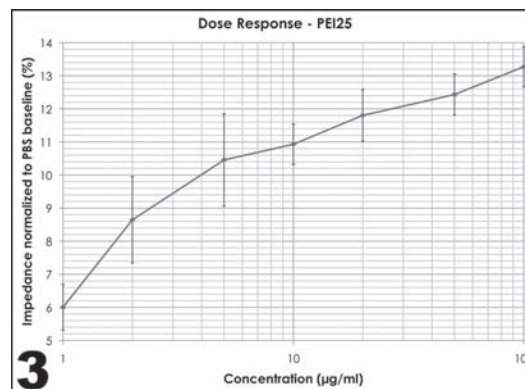


Figure 3: Dose response of PEI-25.
Figure 4: Dose response of NPGDE 1,5 BIS.