Nanotechnology Enabled Platforms for Trace Detection of Pharmaceuticals

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Abstract—The current research leverages a nanotechnology enabled sensor platform for highly sensitive detection of pharmaceuticals contaminants in water samples using electrochemical impedance spectroscopy. The sensor surface consists of lithographically patterned gold electrodes and is functionalized with anti-pharmaceutical antibodies. A nanoporous alumina membrane is placed over the sensor surface to achieve enhanced signal amplification. Erythromycin and Ibuprofen were successfully detected on the sensor in amounts as low as parts per quadrillion. The design has potential to be developed into portable diagnostic equipment, offering rapid, inexpensive and highly reliable detection of pharmaceuticals with applications in the assessment of water quality.

I. INTRODUCTION

Recent studies have shown accumulation of trace pharmaceuticals in treated drinking water supplies of many large cities [1], [2]. It is believed that trace pharmaceutical compounds enter the water supply when people ingest drugs, and the excess or metabolite is passed through the body and into the wastewater. These drugs then accumulate in the local water supply because current water treatment techniques do not remove them [2]. These trace pharmaceuticals are believed to have pharmacological effects similar to full dose drugs over a long term of exposure, and their presence in treated drinking water is a potential risk to human health [2].

Current methods of detection for trace pharmaceuticals involving liquid chromatography and mass spectrometry can be time consuming and lack portability. Additionally water testing currently tests a random set of samples, as a result there is a high probability of false negative in detection. This research leverages an established nanotechnology enabled platform [3] to design an inexpensive, single use, portable sensor with fast response time, capable of detecting specific trace pharmaceuticals in water samples with high sensitivity (detection below 50 parts per billion). This sensor platform architecture has been successfully developed for other sensing applications, such as low-dose protein detection [3]. The purpose of this paper is to explore the design of a sensor platform that leverages confinement of pharmaceuticals, which are small molecules, in nanowells to achieve enhanced electrochemical detection. Small molecules tested include the antibiotic Erythromycin, and the anti-inflammatory drug Ibuprofen. In addition to offering enhanced detection of pharmaceutical contaminants in drinking water, this sensor can also be developed for military and defense applications to detect contaminants (such as Botulism and E. coli) deliberately added to drinking water sources as an act of violence.

II. MATERIALS AND METHODS

A. Sensor Design and Assembly

The sensor platform comprises of a polymer printed circuit board (PCB) with a 13 mm diameter circular electrode pattern. The gold counter and working electrodes (CE and WE, respectively) are arranged concentrically. A 13 mm diameter nanoporous alumina membrane (Whatman Scientific, Kent, UK) with 200 nm diameter pores for Erythromycin experiments and 20 nm diameter pores for Ibuprofen tests were integrated on top of the electrode pattern, covering the entire electrode surface on each of the substrates. Addition of the membrane creates nanoscale well structures (nanowells) over the electrodes, where the base of the wells consist of gold electrode, and the sides consist of a membrane nanopore. The surface was encapsulated with a

![Image of PCB chip with Working and Counter Electrodes (WE and CE) along with the alumina membrane and the PDMS manifold with pores to access the solutions](image-url)
transparent Polydimethylsiloxane (PDMS; Dow Corning, Midland, MI) manifold structure that provides a well for containment of fluids. The well diameter was 12.9 mm, which allows its edges to hold the alumina membrane flat against the PCB surface. Pores on the manifold top enable loading of fluids into the well via a pipetter (Fisher Scientific, IL). The volume of space in the well allows loading of 150 µL fluid samples. The manifold was secured to the PCB with Scotch 665 double sided tape (3M Corporation, St. Paul, MN) to complete the sensor assembly, which is shown in Figure 1. Wire leads soldered to the WE and CE terminals on the PCB were used for probing the dose under study.

B. Assay Protocol

1) Ibuprofen

During building of the assay, volumes of all agents were standardized at 150 µL except the antibody which was kept at 100 µL. An immunoassay was built on top of gold electrode by adding 150 µL of 10 mM di-thiobis-succinimydyl propionate (DSP) in DMSO for 30 minutes. Excess DSP was removed by an initial wash cycle of DMSO followed by triple wash cycle of DI Water. DSP consists of amine-reactive esters located on each end of a disulfide link. The disulfide was absorbed into the gold electrode surface, while the terminal esters remained unbound and available for binding reactions with antibodies. Antibody dilutions were made from the as purchased stock solution by adding a 100 fold volume of DI water to the stock volume. Standardized volume was incubated on the DSP activated surface for 15 minutes. Another triple rinse of the DI water removed the unbound protein from the substrate. Super Block T-20 was used straight out of the bottle and the volume was maintained at 150 µL for 15 minutes on the substrate to cover any open areas on the surface of the gold coating. Specific binding control experiment dilutions of small molecules were made with deionized water and with river water samples. River water samples were collected from Arkansas River in downtown Wichita, KS. Deionized water and river water were filtered with a 0.22 µm filter prior to the experiment and were used throughout the experiment for further dilutions and in building the immunoassay. For the small molecule test solutions, a stock concentration of 10 mg/mL was made from the Ibuprofen sodium salt (specific). All the solutions and dilutions were prepared on the day of the specific experiment. A total of eight concentrations (0.001, 0.05, 0.25, 0.5, 1, 250, 1000 and 10000 pg/mL) were prepared from the femto to the nano regime thus covering the new limit of detection along with the existing limits of sensitivity.

2) Erythromycin

In the case of erythromycin, similar experimental steps were implemented until the small molecule detection stage. Here, a stock solution of erythromycin small molecules was prepared at 1 mg/mL from which the dilutions ranging from 1 fg/mL to 10 ng/mL were prepared. These dilutions were injected into the assay, and the following measurement was called the dose response.

C. Electrochemical Impedance Spectroscopy (EIS)

EIS is the technique used for interrogating specific biomolecule binding onto inorganic surfaces. When biomolecules are placed in an ionic solution, such as phosphate buffered saline, there is an ion exchange as well as modulation to the surface charge at the solid-liquid interface i.e. at the electrode and buffer interface. The equilibrium condition, associated with biomolecule binding results in creating a double layer capacitance. The Helmholz – Gouy – Chapman model with Sterns correction is an acceptable model for representing the charge distribution dynamics at the metal – solution interface [4]. Since the antibody – antigen interactions have not been mediated using a redox probe for obtaining charge transfer it can be assumed that all the conduction occurring is non-faradic in nature, and the simplified model of a serial resistive capacitive (RC) circuit may be employed to characterize the biomolecular interactions occurring at the surface. Due to the binding of the bio molecules inside the nanowells, the double layer capacitance changes because of the change in the surface charge concentrations. Thus, the double layer capacitance directly correlates to the amount of binding taking place at the solid – liquid interface. The amount of binding is directly proportional to the concentration of the target species. Therefore, by characterizing the double layer capacitance, we get an accurate estimate of the concentration of the target species. We used EIS to characterize the double layer capacitance.

The changes to the electrical double layer due to biomolecule binding are best characterized at low frequencies i.e. below 1 kHz. At these low frequencies the resistive component of the RC circuit can be assumed to be constant hence the impedance changes measured using EIS is a measure of the change to the double layer capacitance. Hence in this study we have represented the sensor response an impedance change. In order to provide a better understanding on the magnitude of capacitance changes in the electrical double layer associated with biomolecule binding we have represented one set of results as capacitance.
changes. This sensor achieves detection of pharmaceutical molecules through confinement in nanoscale wells, and measurement of the electrical double layer (EDL) capacitance, using electrochemical impedance spectroscopy (EIS) [7 – 9]. Briefly, small molecule binding in each pore of the assay perturbs the EDL at the solid/liquid interface, producing a capacitance change in the EDL. Each nanowell contains a biomolecule complex modeled by a capacitor. These capacitors are arranged parallel to each other, and orthogonal to the surface, which results in capacitance measurement over the WE and CE that provides the sum of capacitance in each nanowell. Using a potentiostat (Gamry Reference 600, Gamry Instruments, PA) a 10 mV AC bias voltage was applied across the sensing electrodes. EIS is used to measure the impedance at discrete frequencies ranging from 50 Hz to 1 kHz. Research has determined that a frequency less than 1 kHz results in an impedance measurement where the EDL capacitance, as opposed to the solution resistance, dominates the measurement [7 – 9]. Impedance at 100 Hz were analyzed and provide a sufficiently large impedance change to detect Ibuprofen and Erythromycin concentrations in parts per quadrillion.

III. RESULTS AND DISCUSSION

A. Antibody Incubation

Baseline impedance levels for the assay were collected by EIS measurement after the antibody incubation step during the surface functionalization process. A study was performed to test the effects of antibody incubation time. Ibuprofen and Erythromycin antibodies were studied separately on individual chips. Diluted stock solutions were injected into the well after the DSP incubation and impedance levels were measured every 15 minutes for the first one hour and then for every 30 minutes for the next one hour. Figure 3 shows the average impedance levels on the two chips. It is seen that the average change in impedance over the time course is less than 1 kΩ, and changes between each 15 minute measurement are minimal. It was determined that 15 minute incubation provides sufficient time to ensure adequate antibody binding to the surface.

![Figure 3: Antibody incubation time studies of two antibodies (Ery – Erythromycin and Ibu – Ibuprofen). A saturation point of 15 minutes can be understood from the figure.](image)

B. Small Molecule Dose Response

1) Erythromycin

Impedance levels were collected for concentrations over 3 orders, with the lowest in the parts per quadrillion range, determined using the mass ratio of drug and molecular weight of DI water. Erythromycin was diluted in both DI water and river water samples, and concentrations from 1 fg/mL to 1000 pg/mL were tested on the sensor. Measurements of these concentrations on a unique control sensor were used to normalize the entire concentration range, because the doses were implemented in separate assays. In DI water trials, individual assays were used to measure a range of low concentrations (1 fg/mL, 10 fg/mL, 50 fg/mL and 150 fg/mL). The same sensor components were then cleaned and reused to build new assays to measure a range of

![Figure 4a: Dose response of erythromycin small molecule. Dosage ranged from 1 fg/mL to 10 pg/mL. The limit of detection with a reliable signal response was determined to be 50 fg/mL for both DI water and river water samples. Figure 4b: Dose response of ibuprofen small molecules Dosage ranged from 1 fg/mL to 10 ng/mL. Linear trendlines were plotted with a regression value of 0.96 for DI water and 0.98 for River water. The limit of detection in this case was 250 higher concentrations 250 fg/mL, 500 fg/mL, 1 pg/mL and 10 pg/mL. Impedance measurements were collected during each step of the assay protocol, and were found to be similar for the original and rebuilt assays. It was determined that the inter-assay variation is minimal, and the measurements can be compared. Measurements taken at antibody incubation, and target incubation on each assay were used to calculate percent change of impedance, which is referred to as sensor response. Sensor response of nearly 10% and 50% is observed over the erythromycin dilutions 50 fg/mL and 10](image)
ng/mL tested on a control sensor. The linear regression on this control sensor was used to normalize the entire concentration profile, and the results are shown in Figure 4a. This trend of decreasing impedance and rising percent change due to increased erythromycin concentration is consistent with the expected response according to EIS theory. It also indicates that more target material is binding to the surface. In river water trials, a 50% mixture of river and DI water was spiked with 1 μg/mL erythromycin concentration. The sample was further diluted to obtain the same erythromycin concentrations used in the DI water trials. The measurements were normalized similar to the method used for the DI water trials. Sensor response of 5% - 65% was found over 1 fg/mL, 10 fg/mL, 250 fg/mL, 10 pg/mL and 10 ng/mL. The linear regression was used to normalize the entire concentration profile, and the results are also shown in Figure 4a. The sensor is found to have a dynamic range in the parts per quadrillion range, enabling ultra-sensitive detection of pharmaceutical small molecules. The limit of detection is nearly 50 fg/mL, and lower than the range of conventional liquid chromatography-mass spectrometry methods, which are useful for detection as low as 1 pg/mL [10].

2) Ibuprofen

Dose response for Ibuprofen were collected similar to the method used in Erythromycin. Percentage change of the impedance from the antibody’s response was considered as the base line and the response was plotted against the concentrations. The impedance values were also normalized to the lowest concentration, 1 fg/mL. Figure 4b indicates the results of the experiments that were conducted with DI water as the buffer solution. The limit of detection was 250 fg/mL with the percentage change in impedance more than 10%. Despite the lowest concentration that was detected was 1 fg/mL, the most discernible signal came from the 250 fg/mL and hence the limit of detection. Similar experiments were also conducted with river water as the buffer solution which was plotted in red in Figure 4b. In this case as well, the least possible dose that was detected turned out to be 250 fg/mL with the detection sensitivity at around 10%. These results verify binding of ibuprofen molecules to the complete assay, suggesting diffusivity of the water based solution deep enough into the 20 nm diameter nanowells to allow attachment of drug to the top of the immobilized antibody. Computational studies also suggested the possible diffusivity as in reference [11].

C. Cross Reactivity Data Analysis

Cross-reactivity of the ibuprofen antibody was tested against small molecules of erythromycin to establish the reliability in the signal response. Similar experimental setup was conducted for a range of concentrations that is higher than the limit of detection. A very minimal binding can be noticed from Figure 5. Considering one outlier (0.5 pg/mL) for erythromycin, we achieved a regression value of 0.4817. This difference may be addressed to the inherent chemistry between ibuprofen antibody and the erythromycin small molecule.

IV. CONCLUSIONS

An experimental model system was designed to investigate the sensitivity of the device in analyzing the pharmaceutical small molecules. We achieved a new limit of detection (250 fg/mL) and increased the sensitivity of the device for an anti-inflammatory drug (ibuprofen) and an antibiotic drug (erythromycin). The new limit of detection is in the femto gram regime and is a very well distinguished signal from its baseline. Distinguished signal responses were observed when both drugs were tested in DI water and in river water. Cross reactivity of the erythromycin small molecules were tested with the ibuprofen experiment. Minimal cross-reactivity was observed in these sets of experiments. With these sets of data, we hope to increase the device sensitivity by applying dielectrophoresis and similar amplification techniques. The design has potential to be developed into a portable, single use sensor platform which offers rapid detection of pharmaceuticals and other biological contaminants in drinking water, with a lower limit of detection than current methods. Experiments establishing proper packaging and shelf life of the prototype device have not yet been investigated. A final design would likely be functionalized in a laboratory, and packaged for delivery to the test site. Specialized measurement equipment would be developed to offer automatic measurement and data analysis for the user.

REFERENCES


