DEVELOPMENT OF AN ELECTROKINETIC SURFACE PLASMON RESONANCE BIOSENSOR

by

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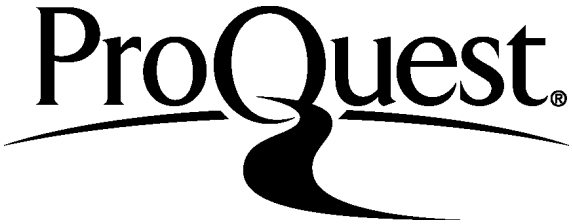

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SENSOR

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ABSTRACT

Work towards the development of a novel electrokinetic surface plasmon resonance (EK-SPR) biosensor is presented. Electrophoretic exclusion is coupled with SPR sensing capabilities to allow for a desired analyte to be separated and preconcentrated prior to detection, eliminating the need for preprocessing of the sample. Protein solutions containing bovine serum albumin are in the process of being studied; however, due to technical difficulties with various instruments in the lab, there is no separation data of proteins to present as of yet. Sucrose solutions were exposed to different electric fields, 600 V cm^{-1} and 1900 V cm^{-1} to observe the effect on the preconcentration. Experimentally, a 0.01833M sucrose solution was preconcentrated to 0.02710M using the EK-SPR biosensing platform.

Chapter 1

INTRODUCTION

While there are point-of-care (POC) devices, such as malaria and typhoid rapid diagnostic tests, available commercially today, there is a growing interest in the development of POC devices to detect other biomarkers, including but not limited to cardiovascular disease biomarkers.[1] POC devices allow for medical diagnoses to be made at the patient's location by meeting the following criteria - being portable, robust, low cost, fast, and require minimal training for use. The capability of making a diagnosis in a shorter time frame at the site of the patient allows for faster, and sometimes more effective, treatment decisions.

According to the Centers for Disease Control (CDC), heart disease is the leading cause of death for most people in the United States, regardless of ethnicity. [2] Of the approximately 610,000 deaths due to heart disease, approximately 42% of sudden cardiac deaths happen outside of a hospital setting.[2] The ability to provide care for a patient suffering from a cardiac event at their location is imperative in lowering the number of deaths occurring outside of a hospital setting, and POC devices present a solution to decreasing these statistics.

Currently, there are not many POC devices being utilized regularly due to a lack of conformity to present standards according to the specifications set forth by the National Academy of Clinical Biochemistry (NACB). NACB states that POC devices are to perform to the same specifications applied to current central laboratory tests.[3]

In light of the need for improved POC biosensors, a novel Electrokinetic Surface Plasmon Resonance (EK-SPR) sensor is in the process of being developed. The aim is to create a POC sensor capable of meeting all of the NACB specifications previously mentioned by coupling a SPR sensor with an electrokinetic separation.

1.1 Surface Plasmon Resonance

SPR is a well explained phenomenon that occurs when surface plasmons are excited due to the total internal reflection of polarized light coming into contact with a conductive layer of gold. The gold is at an interface of a glass slide with a high refractive index, and a medium with a low refractive index, such as water, is used externally. Based on the angle of the incident light, the surface plasmons are excited, resulting in an observed absorbance band in the reflected light.

SPR is a label-free, real-time technique, making it ideal for use as a biosensor.[4] SPR is also sensitive to surface events, allowing for a limit of detection (LOD) in terms of ng/mL, which is biologically relevant.

Consequently, SPR is also prone to non-specific binding (NSB) events. NSB can sometimes be indistinguishable in the data, ultimately skewing the results. Therefore, it is necessary to minimize the effects of sensor fouling and NSB through modification of the sensing surface and preprocessing of the sample to be analyzed.

1.2 Electrokinetic Separation

Electrokinetic separation utilizes an electrophoretic force simultaneously coupled with hydrodynamic flow, allowing selective separation of desired analyte(s) within a sample for preprocessing of the sample to occur. [5] Preprocessing the sample is important, due to the susceptibility of SPR to NSB. Another advantage gained

through preconcentration of the sample is that lesser amounts of the desired analyte have the potential to be detected.

The electrophoretic force that is applied during the electrokinetic separation creates electric fields. The electric fields are dependent on the difference in potentials and the distance between the electrodes. By varying the electric fields coupled with a constant hydrodynamic flow rate, the sensor has the capability to separate and preconcentrate a variety of target analyte(s).

1.3 EK-SPR

The coupling of the electrokinetic separation with a SPR sensing pad leads to the development of a novel EK-SPR biosensor, allowing for the preprocessing of the sample while simultaneously obtaining data. The EK-SPR sensor utilizes a dual sensing pad design previously established, and polydimethylsiloxane (PDMS) to create the microfluidic channels. [6]

Chapter 2

EXPERIMENTAL METHODS

2.1 Experimental Setup

Light from a white light emitting diode source was collimated using a collimating lens, passed through a spatial filter, a polarizer, and a BK7 dove prism where the SPR sensor made contact with the prism through the use of refractive index (RI) matching oil (RI=1.52).

The gold films used for the SPR sensors were deposited in-house using a Cressington 308 thermal evaporator and sputter coater. A layer of 5nm chromium was sputter coated as an adhesion layer between the clean slide and gold, and a 50nm gold layer was sputter coated on top of the chromium to be used as the SPR sensor pads and the electrokinetic electrodes.

To create the dual-pad sensor, a piece of tape was used to create a ~1mm wide clear space between SPR pads until a copper mask was fabricated for uniformity. [6]

2.2 Sensor Modifications

The SPR sensing pads were modified using diazonium salts which were prepared using an established method. [7] The precursor salt was 4-aminophenylalanine (4-APhe). Once the diazonium salt was prepared, a pulsed deposition procedure was used to create a diazonium salt layer on the SPR pad(s). [6] A solution consisting of 5mM N-hydroxysuccinimide (NHS, 98%, Fisher Scientific) and 20mM N-(3-dimethylaminopropyl)-N’-ethylcabodiimide (EDC, 99%, Sigma-

Aldrich) was then passed over the SPR pads, preparing the pads for a treatment of antibodies if necessary. [6]

2.3 Separation Channel Fabrication and Assembly

2.3.1 Wafer Fabrication

A silicon wafer was coated with negative photoresist (SU-8, Microchem) and developed to create the pattern used for the PDMS. Briefly, the wafer was cleaned and coated with photoresist using a spin coater. The wafer was then baked at 65C for 20 min followed by 50 min at 95C. A photolithography mask and a UV lamp were used for exposure, and the wafer was baked again at 65C for 1 min and 95C for 12 min. Finally, the wafer was immersed in SU-8 Developer (Microchem) for several hours.

One separation channel consisted of three reservoirs linearly connected by channels. The reservoirs measured 5 mm x5 mm.

2.3.2 PDMS Preparation

PDMS was prepared by mixing a 10:1 mass ratio of polymer to curing agent (Sylgard184, Dow Corning) and pouring the mixture over a wafer. 3mm diameter tubing was placed into the PDMS before curing to create the hole necessary for the reservoirs. After the PDMS was cured for 60 min at 100C, it was removed from the wafer. The final product was 5mm thick, with reservoirs and channels 10μm deep.

2.3.3 Assembly

The cured PDMS and the prepared SPR sensor were placed in an oxygen plasma etcher (Harrick Plasma Cleaner PDC-32G) and exposed to plasma for 5 min., followed by the PDMS being placed on the sensor and allowed to self-adhere. The seal

was tested by placing water into the reservoir and inspecting for leaks. After successful adhesion, plexiglass blocks were attached to act as supports for tubing, and leads were attached to the gold electrodes using a conductive silver epoxy. The finished EK-SPR sensor is shown in Figure 1.

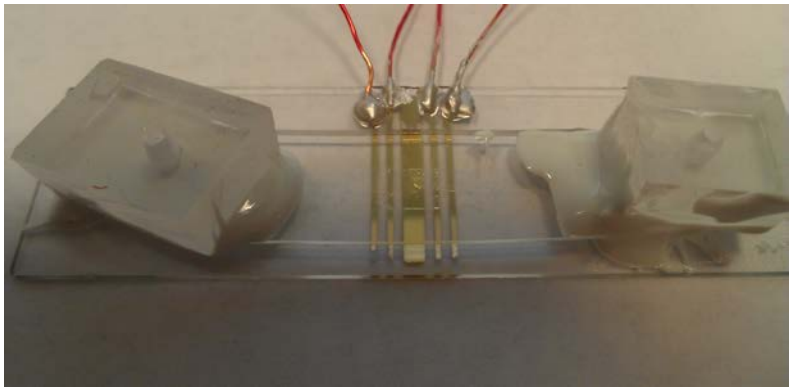

Figure 1. A picture of the EK-SPR sensor fully assembled. The first and last electrodes have independent voltages applied, while the middle two electrodes have the same potential applied to create appropriate electric fields.

Chapter 3

RESULTS AND DISCUSSION

3.1 Bulk Sensitivity

The SPR wavelength was observed with the bare gold SPR pads using sucrose solutions to determine how the pads responded to changes in the bulk RI. The sucrose solutions had varying RIs which are shown in Table 1.

Table 1. Refractive indices and corresponding concentrations by mass percent used to determine the sensitivity of the SPR setup to bulk refractive index changes.

RI	Mass %
1.3326	0.00
1.3339	0.80
1.3340	1.12
1.3341	1.21
1.3344	1.50
1.3358	2.19
1.3364	2.41
1.3371	2.77
1.3385	3.63
1.3394	4.59
1.3412	5.53

The bulk sensitivity of the SPR setup was determined to be 1573.89 ± .03nm RIU^{-1}. Sensitivity in this instance is defined as the change in wavelength over the change in RI. The sensitivity of a similar setup using the same spectrometer was reported to be 1.93 (±0.06) × 103 nm RIU^{-1}. [6] The R^2 value for triplicate measurements was 0.9882 for the SPR pads before any functionalization was implemented.

3.2 Diazonium Salt Deposition

To help reduce the occurrence of NSB during SPR measurements, the SPR sensing pad was modified using diazonium salts. While the diazonium salt creates a monolayer on the gold pad, it is not a true self-assembled monolayer (SAM) due to the fact that the monolayer only forms during the pulsed deposition. This characteristic allows for the SPR pads to be functionalized independently, a key step in using the dual-pad sensor design.

The diazonium salt was electrografted by a pulsed deposition to create a uniform coverage of the gold sensor using a previously published method. [6] [7] Figure 2 shows the SPR pads before and after electrografting the sensing pad, which showed an observable wavelength shift of 17nm. While this is lower than the expected shift of 25.8nm, the 17nm shift does indicate some coverage of diazonium salt on the sensing pad. [6] Also, it is observed that the sensing pad, which is depicted as the top pad in Figure 2, shows the largest shift, as anticipated.

The reference pad – the bottom pad in Figure 2 – also shows a slight shift of 4nm after the functionalization of the sensing pad. The shifting of the reference pad suggests that some diazonium salt was electrografted onto the reference pad. One explanation for this observation is that the potential applied to the sensing pad is travelling to the reference pad, allowing the diazonium salt to electrograft onto the gold. This suggests that the deposition of the gold used for the SPR sensor may not be completely separated, and is currently under further investigation.

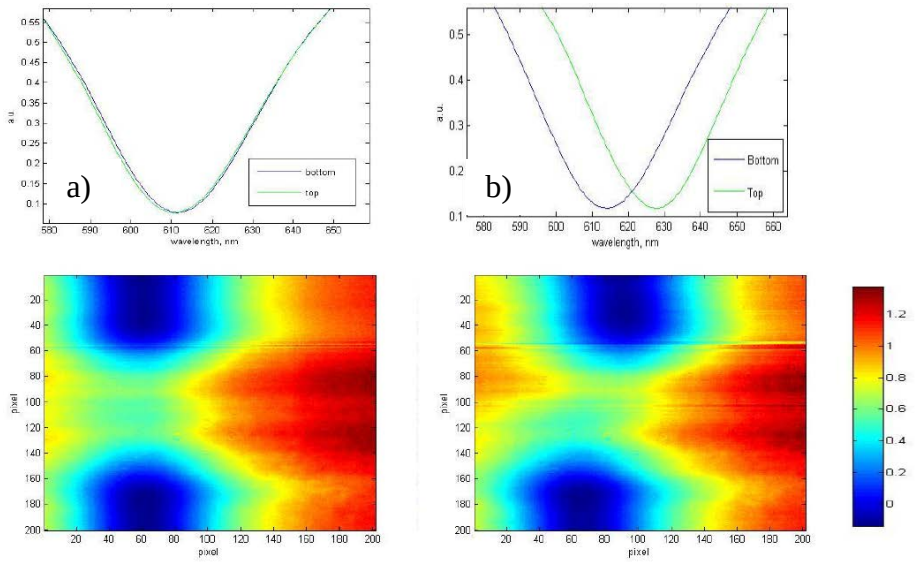

Figure 2. SPR data showing the functionalization of dual-pad sensor where a) is pre-electrografting and b) is post electrografting of diazonium salt onto the top pad. The top pad is the sensing pad while the bottom pad is the reference pad.

3.3 Preliminary Preconcentration

Sucrose solutions were first used to demonstrate the successful combination of electrokinetic separation with SPR sensing capabilities. A solution of 0.01833M sucrose was flowed through the separation channels at a rate of 0.0500 mL min^{-1}. Initial separation data showed a 1.5x increase in concentration to 0.02710M after 8 min of applied trapping voltages. The voltages used for the separation of the solid diamonds in Figure 3 were 0.09, 0.03, and 0.03 kV respectively on the electrodes pictured in Figure 1, creating a 600 V cm^{-1} electric field. Figure 3 shows the linear trend observed in the wavelength shift as a function of time, with an R^2 value of 0.9975. This linear trend shows that the 600 V cm^{-1} electric field is more suitable for the trapping of sucrose molecules when compared to a 1900 V cm^{-1} electric field.

While this data does show that sucrose was isolated and preconcentrated, the increase in concentration is low compared to what was anticipated. In the literature, a 1200x increase in concentration has been reported when concentrating protein using electrokinetic methods. [8] According to Fick's Law, smaller molecules are more susceptible to diffusion than larger molecules. Smaller molecules are prone to experience diffusion at rates on the order of 10^{-6} cm^2 s^{-1}, while larger molecules are on the order of 10^{-8} cm^2 s^{-1}. Work with preconcentrating protein samples is ongoing.

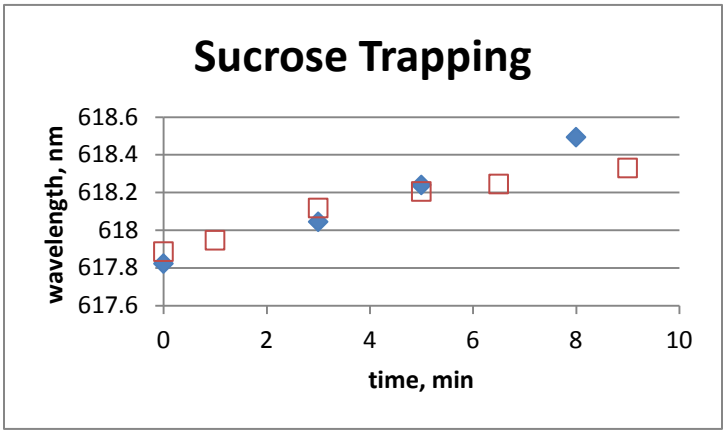

Figure 3. Data showing the capture and increase in concentration of a sucrose solution in varying electric fields. The solid diamonds represent an electric fields of 600 V cm^{-1} and the open squares represent an electric field of 1900 V cm^{-1}. A 1.5x increase of concentration over 8 min was observed in the 600 V cm^{-1}electric field. Both trials had a flow rate of 0.0500 mL min^{-1}.

Chapter 4

CONCLUSION

The development of an EK-SPR sensor for use as a POC device is well underway. An increase in concentration has been shown for a sucrose solution using an EK-SPR sensor; however there is still more work necessary in order to have the sensor meet POC guidelines. Protein solutions containing bovine serum albumin are in the process of being studied; however, due to technical difficulties with various instruments in the lab, there is no separation data of proteins to present as of yet. The successful concentration of a protein within the sensor platform while obtaining SPR data is crucial. Demonstrating the separation and preconcentration of a desired analyte from a simple mixture, followed by separation and preconcentration from a complex mixture, will lead to a sensor able to separate and preconcentrate a sample of whole blood and ultimately to a POC sensor capable of detecting cardiac biomarkers.

REFERENCES

[1] M. Pedrero, S. Campuzano and J. Pingarron, "Electrochemical Biosensors for the Determination ofCardiovascular Markers: a Review," *Electroanalysis,* vol. 26, no. 6, pp. 1132-1153, 2014.

[2] "Heart Disease Facts," Centers for Disease Control and Prevention, 19 February 2015. [Online]. Available: http://www.cdc.gov/heartdisease/facts.htm. [Accessed 25 April 2015].

[3] R. H. Christenson and H. M. and Azzazy, "Cardiac point of care testing: A focused review of current National Academy of Clinical Biochemistry guidelines and measurement platforms," *Clinical Biochemistry,* vol. 42, pp. 150-157, 2009.

[4] M. Cooper, "Optical biosensors in drug discovery," *Nature reviews. Drug discovery,* vol. 1, no. 7, pp. 515-528, 2002.

[5] S. Kenyon, N. Weiss and M. Hayes, "Using electrophoretic exclusion to manipulate small molecules and particles on a microdevice," *Electrophoresis,* vol. 33, pp. 1227-1235, 2012.

[6] Q. Zou, N. Menegazzo and K. Booksh, "Development and investigation of a dual-pad in-channel referencing surface plasmon resonance sensor," *Analytical Chemistry,* no. 84, pp. 7891-7898, 2012.

[7] N. Menegazzo, Q. Zou and K. Booksh, "Characterization of electrografted 4-aminophenylalanine layers for low non-specific binding of proteins," *New J. Chem.,* no. 36, pp. 963-970, 2012.

[8] M. Meighan, S. Staton and M. Hayes, "Bioanalytical separaions using electric field gradient techniques," *Epectrophoresis,* vol. 30, pp. 852-865, 2009.

[9] J. F. Masson, L. Obando, S. Beaudoin and K. Booksh, "Sensitive and real-time fiber-optic-based surface plasmon resonance sensors for myoglobin and cardiac troponin I," *Talanta,* vol. 62, no. 5, pp. 865-870, 2004.