Flow-Through Amperometric Enzymatic Biosensors Containing Glucose Oxidase

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Abstract:

Amperometric biosensors are commonly used in clinical diagnostics. In this work, a flow through amperometric biosensor platform for the detection of biologically important species is microfabricated and tested.

The sensor was created by bonding PDMS microchannels to a glass wafer containing platinum electrodes. Using common surface chemistry techniques, enzymes were immobilized onto the platinum electrodes. Varying the enzyme used enables the sensor to be tailored for detection of a particular species. In this case the enzyme localized on the electrode is glucose oxidase, enabling the system to detect glucose concentration. Glucose oxidase catalyzes a reaction that oxidizes glucose and ultimately produces electrons. The electrons are then detected by a platinum electrode and converted into interpretable results. This electron flow is proportional to the number of glucose molecules or the glucose concentration in the sample.

The objective of this project is to examine how different ionic concentrations influence the detection of glucose concentration at a constant voltage.

Introduction:

Biosensors promise low-cost, rapid, and simple-to-operate analytical tools. Amperometric biosensors have found their place in many fields of analytical chemistry and are of great interest in clinical, food, and environmental analysis. Biosensors are commonly linked with blood glucose monitoring and are therefore extremely important for the health of diabetic patients. Other biosensor applications include detection of pathogenic organisms in food supplies, sugar measurement in drink products, and several other uses. Enzymatic amperometric biosensors work well in these areas because of their specificity, speed, simplicity, and reusability. Microfluidic analytical systems are especially helpful because they offer reduced reagent consumption, smaller analysis volumes, and better throughput than macroscopic systems.

There are many factors that influence the performance of the sensor. Some factors that affect electron detection by the electrode include pH, temperature, and ionic concentration. In this project, we chose to concentrate on the effects of increasing ionic concentration in solution. To test the different solutions, we fabricated a device using several techniques including chemical deposition, photolithography, reactive ion etching, and plasma bonding.

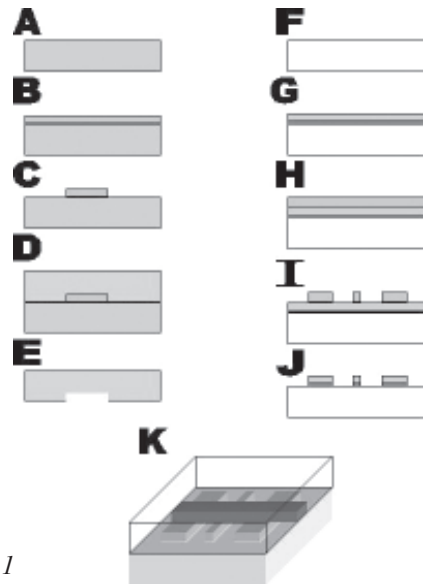

Figure 1

Procedure:

The fabrication of the bioanalytical device consists of two main parts; creating a glass wafer with platinum electrodes and synthesizing a PDMS cast containing microchannels. The PDMS microchannels are made by pouring a solution of PDMS over a patterned silcone wafer. To create the patterned wafer for PDMS casting, we placed a 25 μm layer of SU8-25 onto a silicone wafer (refer to Figure 1A and 1B). To do this, we performed a dehydration bake at 250°C followed by an application of HDMS. The wafer was spun dry at 4,000 rpms. We then static applied SU8-25 and slowly increased the speed to 1500 rpm for 35 seconds. A pre-exposure bake was performed before exposing the wafer to UV light on vacuum contact for 20 seconds. We then allowed a short post-exposure bake and developed the wafer in SU-8 developer for 2 minutes. Figure 1C shows the wafer after the photolithography process.

The PDMS solution consists of a 10:1 ratio of PDMS to activator Sylgred184. The solution was degassed in a vacuum chamber at 30 mtorr for 1 hour and then poured over the patterned silicone wafer and baked overnight (Figure 1D). The PDMS solution hardened and was then peeled off of the patterned wafer leaving an impression of

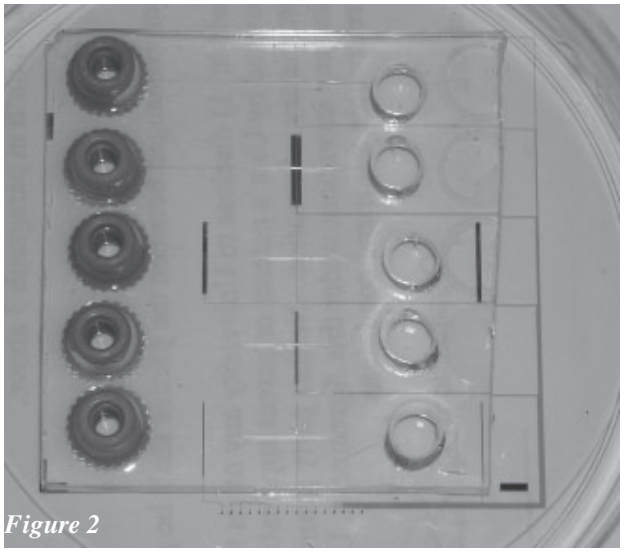

Figure 2

microchannels on the surface as shown in Figure 1E.

To create the electrodes, we evaporated 10 nm Cr and 100 nm Pt onto a 3" glass wafer in the Kurt J Lesker Evaporator (see Figure 1F and 1G). We then applied a layer of photoresist (Figure 1H) by doing an evaporation bake at 110°C for 5 minutes. We then static applied NFR-012 and spun at 300 rpm/s until we reached 4000 rpms. A pre-exposure bake followed by a 4 second exposure on vacuum contact was performed. We then completed a post exposure bake and developed the wafer in a 1:1 ratio of MF-312 developer and water for 60 seconds. Figure 1I shows the results after development.

The glass wafer was then placed in a PlasmaTherm and a 10 minute Platinum and 30 second Chrome etch was applied. We then performed a wet etch with a 2:1 solution of H_2O and Cr etch. Afterwards, a 4 minute photoresist etch was applied to clear all photoresist off the wafer. Figure 1J diagrams the finished glass wafer with electrodes.

To bond the PDMS cast to the glass wafer with electrodes, we placed both in an oxygen plasma at 120 mtorr for 20 seconds. The device was baked at 90°C for 24 hours to assure adhesion (see Figure 1K). Figure 2 shows a photo of the finished microfluidic amperometric biosensor.

After the biosensor is completely fabricated, we immobilized glucose oxidase onto the platinum electrode using common surface chemistry techniques. The sensor was then tested using phosphate buffer solutions containing different ionic concentrations. A constant voltage was applied and the resulting current was monitored.

Results and Conclusions:

We were able to successfully fabricate a microfludic amperometric enzymatic biosensor containing five microchannels and platinum electrodes. When testing the device using increasing substrate concentrations, we found that the chemical detection at the electrode also increased (see Figure 3). Therefore, increasing the amount of substrate in solution causes more reactions to take place on the electrode.

We also tested the device by flowing a 3 mM and 30 mM phosphate buffer solution containing 100 μM glucose through the microchannels. As shown in Figure 4, an increase in ionic concentration results in an increase in current and therefore better detection at the electrode.

Future Goals:

Future work includes experimenting with different pH solutions, and also varying temperatures to examine how they influence the reaction and the detection of the electrode.

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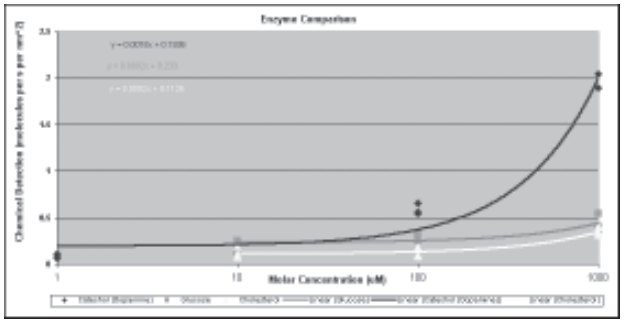

Figure 3, above. Figure 4, below.