Design for Biosecurity

Prof. Mainak Das

Department of Design

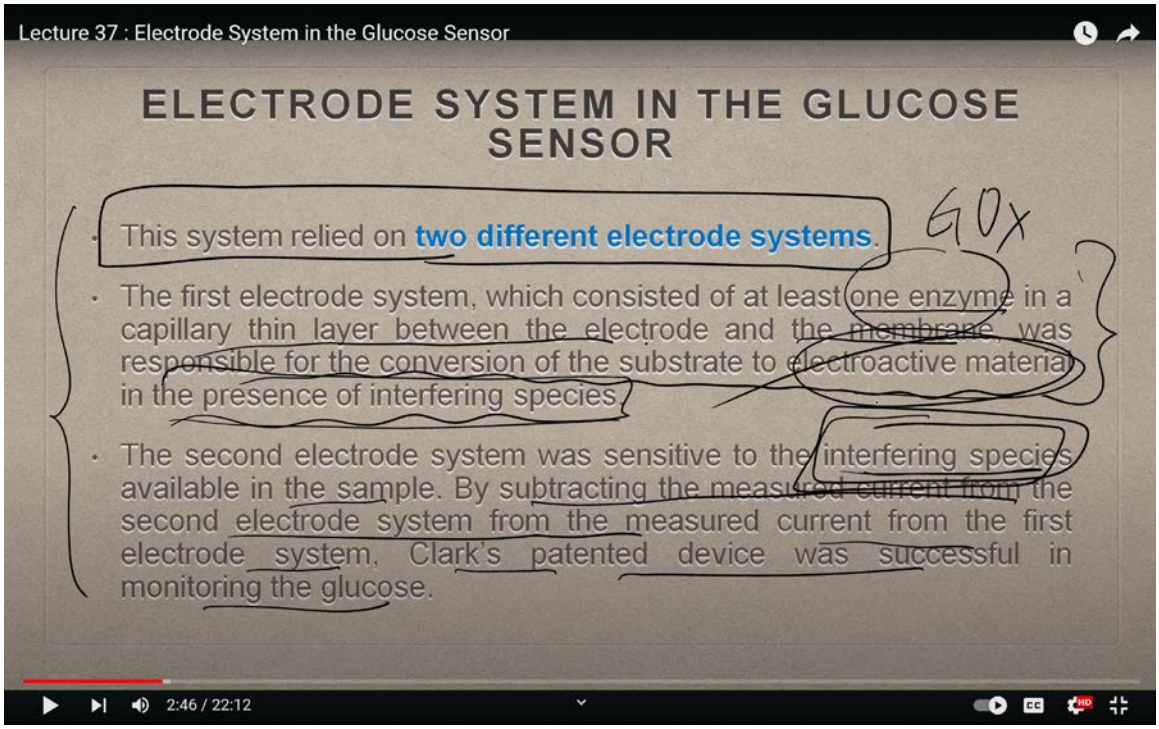
Indian Institute of Technology, Kanpur

Lecture 37

Electrode System in the Glucose Sensor

Let's begin today's session. In the previous class, we discussed enzymatic, catalytic, or electrocatalytic biosensors using the glucose sensor as an example. We talked about how glucose, in the presence of oxygen and the enzyme glucose oxidase, is converted into gluconic acid. Simultaneously, oxygen gets consumed and transformed into peroxide. The key here is that during this process, electrons are liberated, and these liberated electrons are recorded. Essentially, the amount of oxygen consumed serves as a direct or indirect indicator of the glucose concentration in the analyte.

(Refer Slide Time: 02:46)

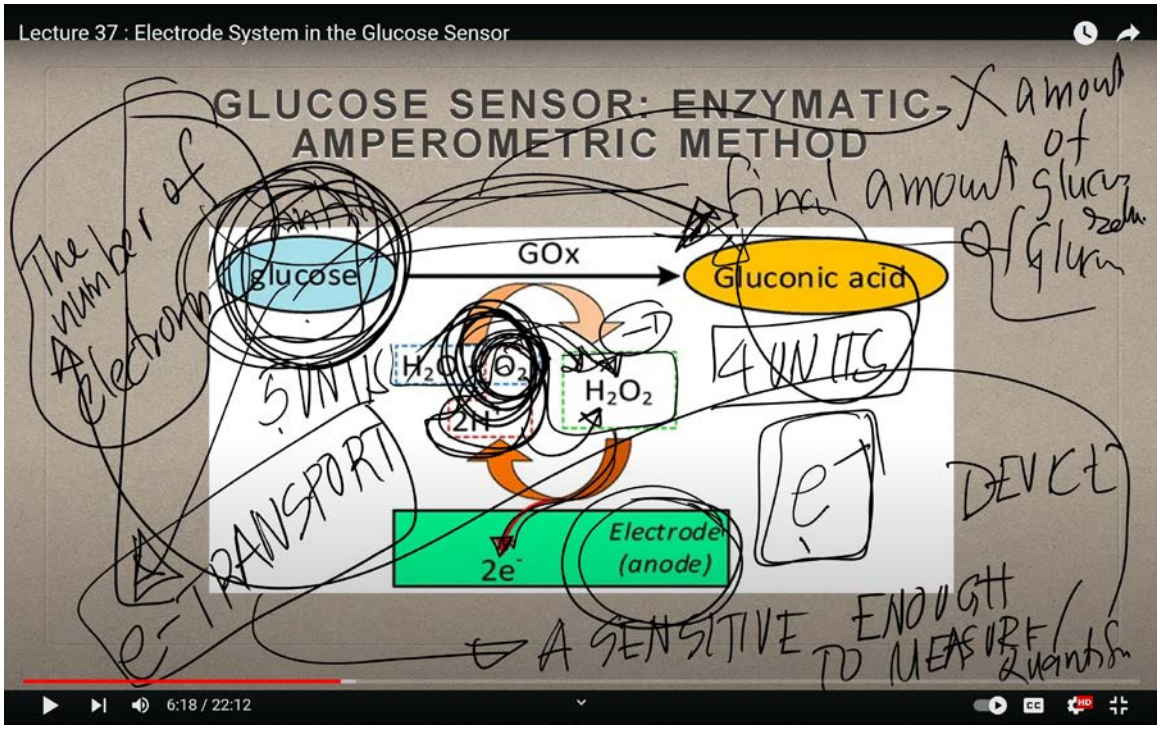


Now, when we explore the electrode systems used in glucose sensors, they primarily rely

on two distinct electrode configurations. The first electrode system involves at least one enzyme, typically glucose oxidase, which is positioned in a capillary-thin layer between the electrode and the membrane. This system is responsible for converting the substrate (glucose) into an electroactive material, even in the presence of interfering species in the sample.

The second electrode system, on the other hand, is specifically sensitive to these interfering species present in the sample. Here's how the device works: the current measured from the second electrode system (which detects the interfering species) is subtracted from the current measured from the first electrode system (which detects the glucose conversion). By doing so, Clark's patented device was able to accurately monitor glucose levels. So, you can see that these two electrode systems are working in tandem to make Clark's electrode functional.

(Refer Slide Time: 06:18)

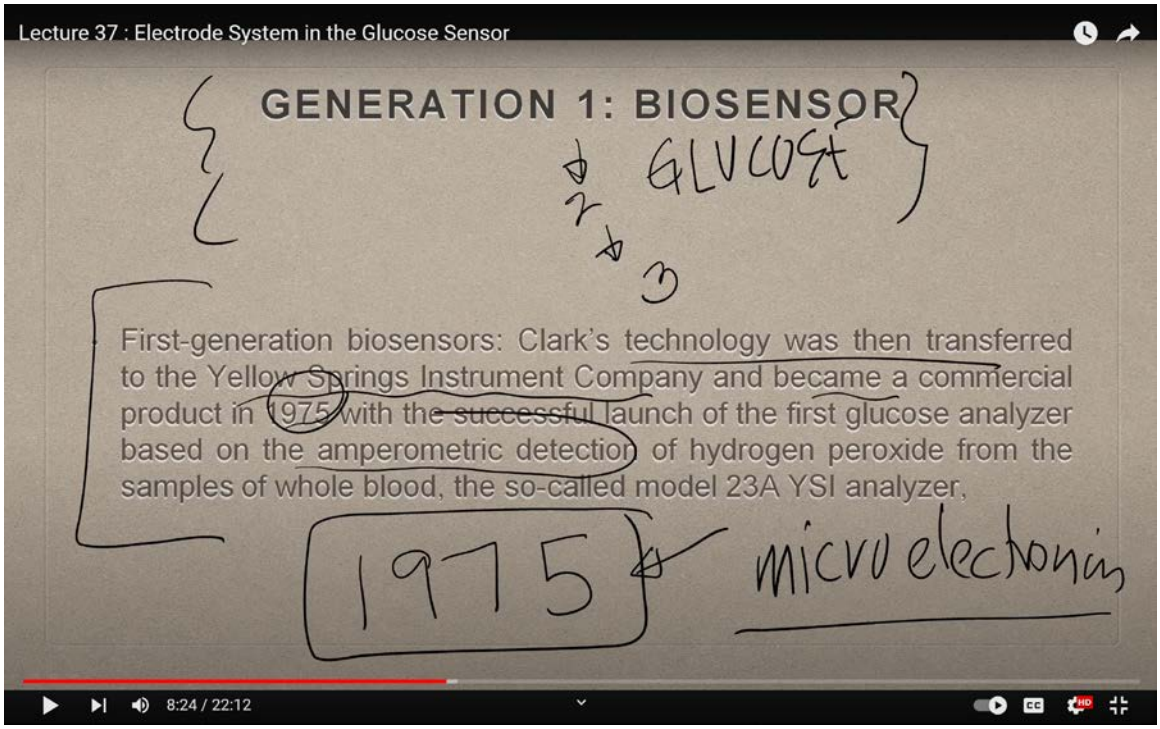


To reiterate, the first electrode system contains at least one enzyme, glucose oxidase, in this case, which is located in a thin layer between the electrode and the membrane. This

layer converts the substrate (glucose) into an electroactive material, even in the presence of interfering species. The second electrode system is sensitive to the interfering substances generated during the process. By subtracting the current measured from the second system from that of the first system, you can calculate the amount of glucose consumed.

Let's break this down further with an example. Imagine you start with five units of oxygen. During the reaction, this oxygen is consumed and drops to four units, indicating a reduction by one unit. Now, this decrease of one unit of oxygen is assumed to have fully transformed into peroxide. If that one unit of oxygen has indeed become peroxide, there must have been some electron transfer involved. If we can quantify that electron transfer, we can accurately track the reduction in oxygen from five units to four units.

(Refer Slide Time: 08:24)



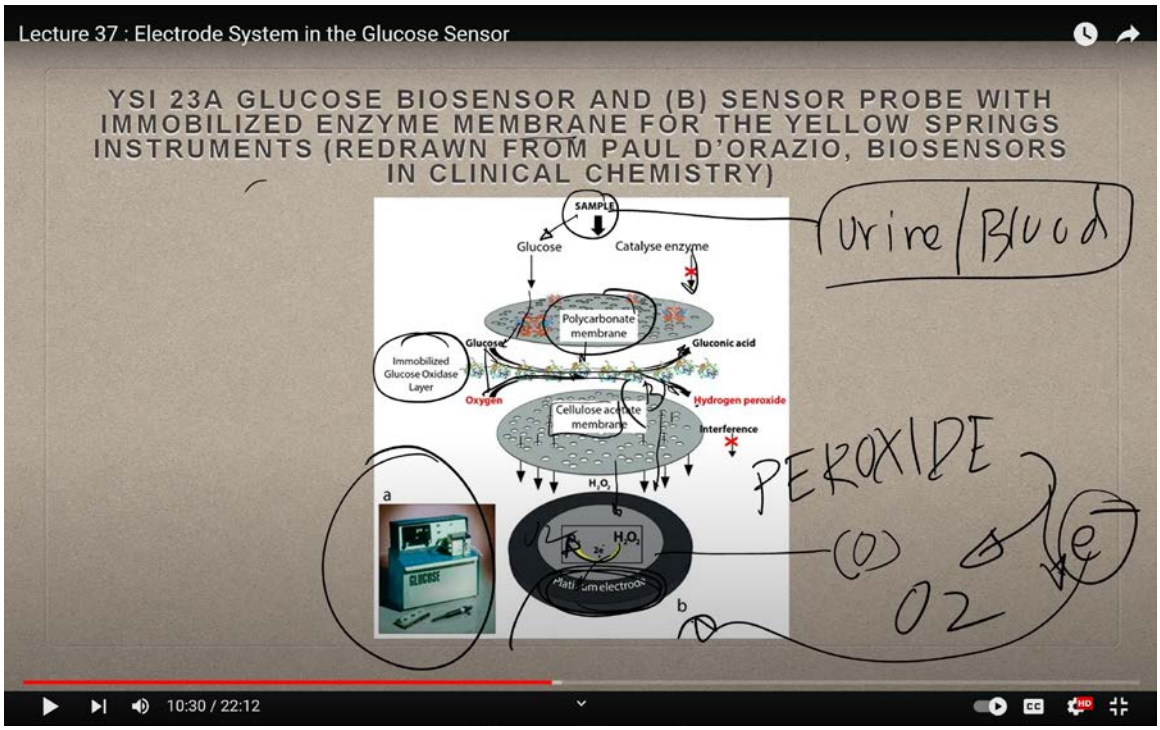
Therefore, if you know the initial and final amounts of glucose in the system, you can determine how many electrons were transferred during the process. This gives you a direct measurement of how much glucose was consumed.

What this means is that the reduction in glucose concentration during the process can be

directly quantified by measuring the electron transport. The main challenge you'll face, however, is finding a device sensitive enough to accurately measure or quantify the number of electrons being transferred. For example, if this process leads to a certain reduction in glucose, say, an amount we'll call "X," and you can measure that current, then you can back-calculate based on this electron transfer to determine how much glucose is present. You can even apply this to any unknown sample by analyzing the electron transfer to measure glucose content.

There's another way to approach this measurement: oxygen consumption. In the early stages of developing Clark's electrode, they measured oxygen consumption as a direct indicator of how much glucose was being consumed because glucose gets oxidized during this process. Electrochemically, Clark was able to quantify oxygen consumption, and a reduction in oxygen content directly correlates with the electroactive species they were measuring, indicating either an increase or decrease. This, in turn, tells us how much glucose is being consumed and converted.

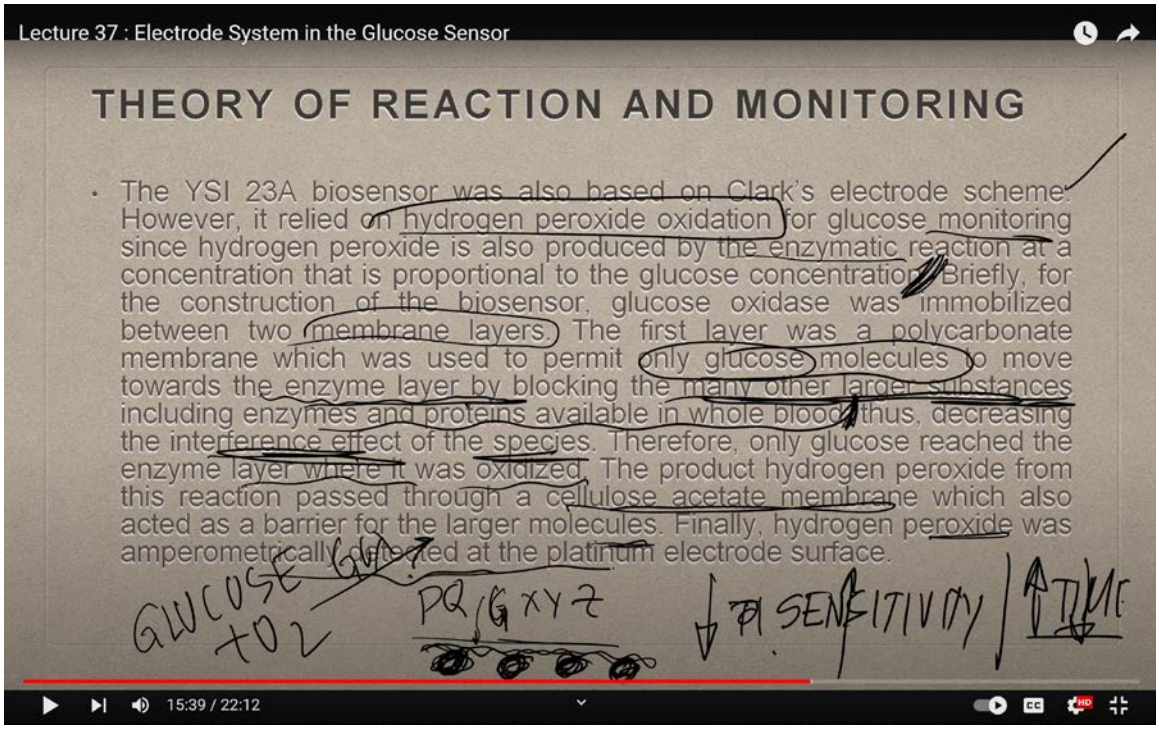
(Refer Slide Time: 10:30)



At first glance, this concept seems simple, but there are complexities that make it more challenging than it appears. We will dive into why that is later. Now, moving on to the history of glucose biosensors, specifically focusing on glucose, these devices have come a long way since their inception in the 1960s. It has been nearly 60 years of progress, and we are currently in the third generation of biosensor technology. But it’s important to understand the driving factors behind each generation of improvements.

The first generation of glucose biosensors was based on Clark's technology, which was eventually transferred to the Yellow Springs Instrument Company and became a commercial product in 1975. This coincided with the rise of microelectronics. The first commercial glucose analyzer, based on amperometric detection, where "ampere" refers to current, measured the hydrogen peroxide generated from glucose oxidation in blood samples. This model was called the 23A-YSI analyzer, and it became quite iconic. The 23A-YSI glucose biosensor had a distinct structure, which we'll discuss.

(Refer Slide Time: 15:39)



In this device, the sensor probe was made of an immobilized enzyme membrane. Yellow

Springs Instruments developed this based on the work of Paolo D'Orzio. So, here’s how it worked: you would have a sample, which could be blood, urine, or any other bodily fluid. Of course, there would be various interfering species present in these samples, but let's focus on the glucose content.

The glucose would pass through a polycarbonate membrane and encounter an immobilized glucose oxidase layer. Here, glucose was transformed into gluconic acid in the presence of the enzyme. This reaction involved the orchestration of glucose and oxygen, with glucose oxidase catalyzing the process to produce gluconic acid and hydrogen peroxide. A cellulose acetate membrane allowed the hydrogen peroxide to pass through to a platinum electrode, where it would release two electrons and become oxygen. This process represents oxidation, while the conversion of oxygen to hydrogen peroxide represents reduction. This flow of electrons, measured as current, forms the basis of glucose detection in these early biosensors.

We are essentially reversing the process on the platinum electrode. This marks the first generation of biosensors. In this process, if you know the amount of hydrogen peroxide being converted back to oxygen through electron ejection, you can quantify the number of electrons being generated. This principle of reaction monitoring is the foundation of the YSI 23A biosensor, which also followed Clark's electrode design. However, it relied specifically on the oxidation of hydrogen peroxide to monitor glucose levels.

Since hydrogen peroxide is produced by an enzymatic reaction at a concentration directly proportional to the glucose concentration, it becomes a key indicator. If you examine the reaction closely, you'll notice that peroxide and glucose production are proportional. In constructing this biosensor, glucose oxidase was immobilized between two membrane layers. The enzyme was essentially trapped between these two layers to form the biosensor. The first layer was a polycarbonate membrane designed to permit only glucose molecules to reach the enzyme layer while blocking larger substances, such as enzymes and proteins, found in whole blood.

This introduces the first major challenge in biosensor design: creating a membrane that selectively allows only glucose molecules to pass. In biosensor research, membranes play

a crucial role. What type of membrane can we use that is selectively permeable? This leads us to consider the biology of cell membranes, which are also selectively permeable, allowing only specific chemical entities to cross the barrier. The challenge here is identifying those filtration elements. The polycarbonate membrane in this device effectively blocks nearly everything except glucose.

Think about the chemistry involved for a moment. You have the polycarbonate membrane on one side, and the glucose oxidase on the other. There is a fixed amount of glucose oxidase, and similarly, a finite number of glucose molecules. In addition to glucose (let's call it G), you also have other molecules such as X, Y, Z, P, and Q. If these other molecules come near the enzyme, they can obstruct glucose from binding to or approaching the enzyme, which would reduce the sensitivity of the device. If glucose doesn’t interact with the enzyme in a timely manner, it also increases the detection time because of the interference caused by these other species.

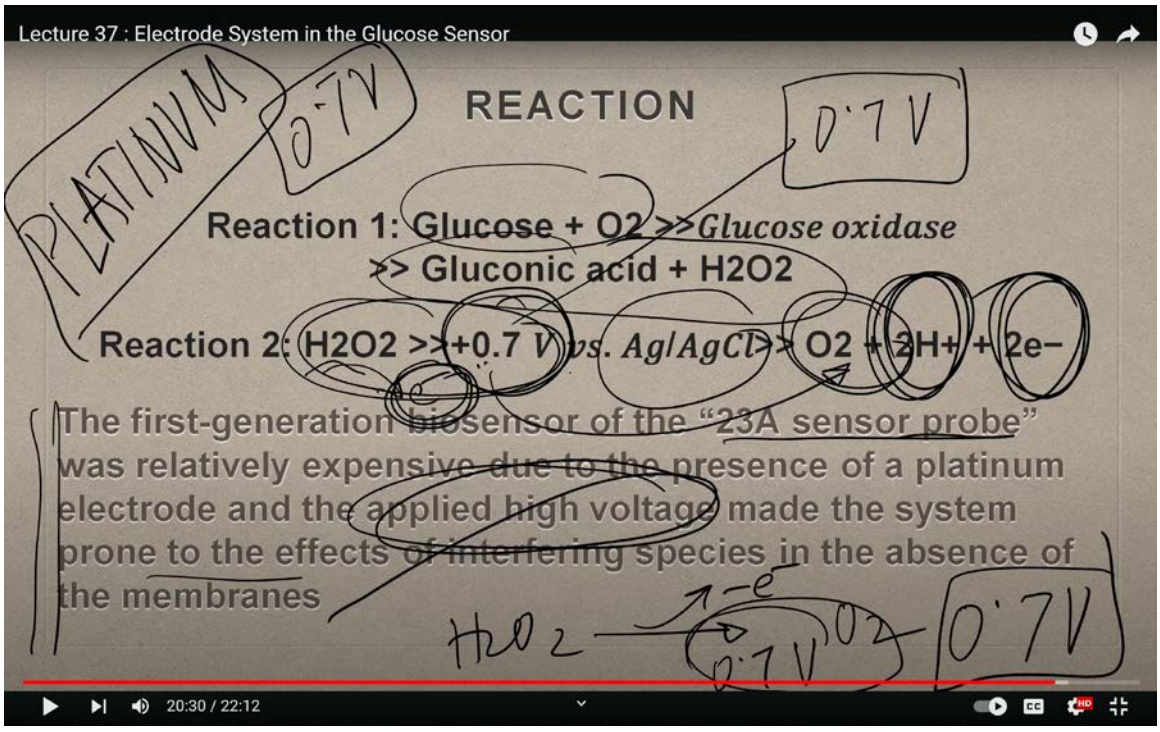
So, as we previously discussed, many species can interfere with the detection process. The design of this biosensor, using the polycarbonate membrane, strategically reduces this interference. The glucose oxidase is immobilized between two membranes, and the polycarbonate layer allows mostly glucose molecules to pass through. Of course, there may be some leakage of molecules with a similar molecular weight, size, or shape, but the goal is to minimize this as much as possible.

Thus, the first layer, this polycarbonate membrane, serves to allow glucose molecules to pass while blocking larger entities. This reduces interference, improves the sensitivity of the biosensor, and significantly shortens the detection time.

Therefore, only glucose reaches the enzyme layer, where it undergoes oxidation. To reiterate for clarity: glucose, in the presence of oxygen, gets oxidized within the glucose oxidase (GOX) layer. This reaction produces hydrogen peroxide as a byproduct. The hydrogen peroxide then passes through the cellulose acetate membrane, which serves as a selective barrier, preventing larger molecules from passing through. Finally, the hydrogen peroxide is detected amperometrically at the platinum electrode surface.

This is the fundamental theory of how the entire process works. From an electrode's perspective, it's important to note that hydrogen peroxide oxidizes at a specific voltage when compared to an Ag-AgCl reference electrode. By applying a specific potential, you can oxidize the peroxide by removing electrons from it. For instance, when hydrogen peroxide (H_2O_2) loses an electron at a potential of around 0.7 volts, the byproducts are oxygen, protons, and two electrons.

(Refer Slide Time: 20:30)



However, in the first generation of biosensors, this setup, though functional, was relatively expensive due to the presence of platinum electrodes. Platinum, being a costly element, contributed to the overall high cost of the sensor probe. Furthermore, the system was prone to interference due to the high applied voltage, specifically, 0.7 volts. When such a high voltage is applied, not only does it oxidize the peroxide, but it also generates other interfering species from the sample.

This is particularly problematic when working with complex biological samples like urine, blood, or even soil, which are heterogeneous mixtures containing numerous compounds.

At such a high voltage, you cannot easily predict what will happen to each individual entity in the mixture. While the goal is to oxidize the peroxide and generate oxygen, electrons, and protons, the process is frequently accompanied by the generation of unwanted interfering elements.

The membrane used in the first-generation biosensor is not a biologically active, semi-permeable membrane. It is difficult to prevent some interfering substances from passing through, which makes interference from natural samples a significant challenge. This issue arises with every new sample since the exact composition of each mixture can vary widely.

There are two major challenges with this approach. First, platinum electrodes are too expensive for widespread, point-of-care use. You can't build cost-effective devices using such expensive materials. Second, the high voltage used in the process introduces interference, reducing the system's sensitivity and accuracy. These challenges spurred further research and development to create biosensors with higher sensitivity, better recognition, and faster detection.

To summarize, when glucose is present in a sample, there are often many interfering species. A selectively permeable membrane is needed to allow mostly glucose to reach the enzyme layer, where it interacts with glucose oxidase. Oxygen consumption can be used as a detection method, or the reaction that reduces oxygen to peroxide can be monitored. In turn, the peroxide is oxidized to liberate electrons and protons, completing the detection process. This forms the basis of the first generation of glucose sensors. So, in the next class, we will move to the second and try to move to the third generation. Thank you.