Design for Biosecurity

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Lecture 43

Summarizing Glucose Sensing - Part 1

Welcome back to this class. Over the next two to three sessions, I will be summarizing glucose sensing technologies, including those we've already covered and some newer developments. One of the biggest challenges in teaching about biosensors, whether for viral detection, bacterial detection, biomolecule detection, or toxin detection, is the intellectual property (IP) protection surrounding many of these technologies. This limits the availability of open-source literature. Over the past 50 years, most biosensors developed for diagnostics, biosecurity, and toxin detection have been derived from the first sensor, the Clark sensor, followed by the glucose sensor. Modifications of this model have dominated the landscape of biosensor design and manufacturing.

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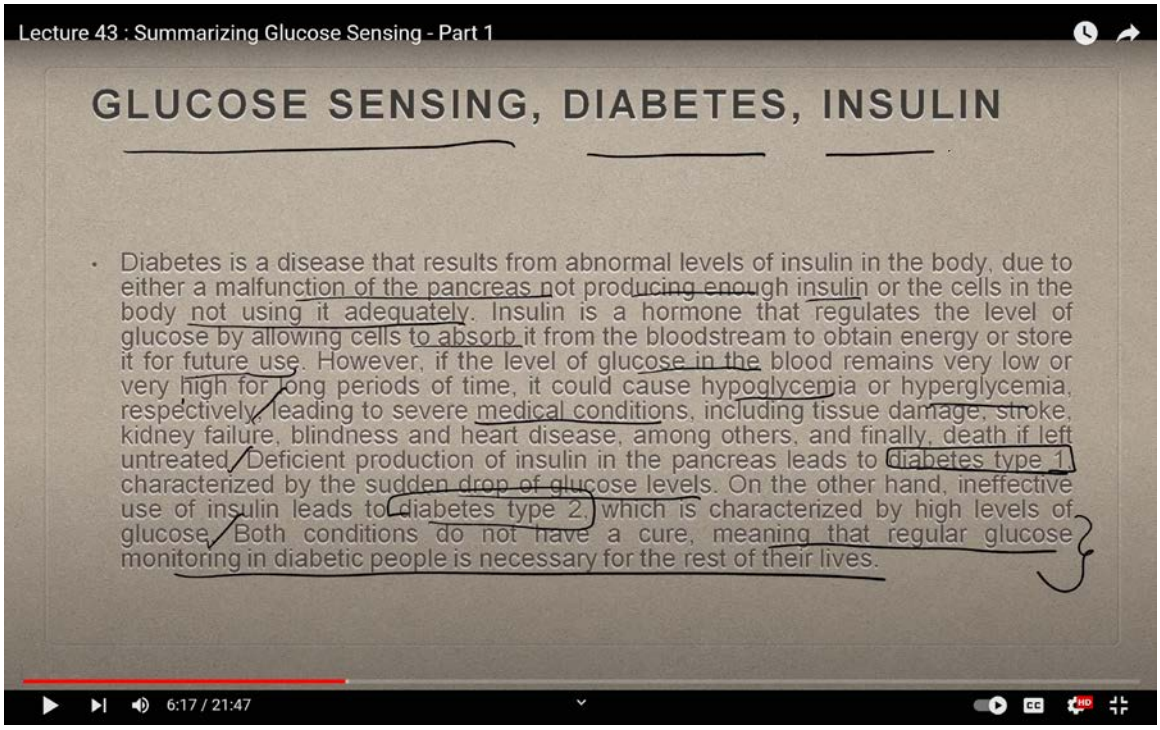

When we look at the progression of biosensor technology, we see that before the advances in non-enzymatic detection, most of the development focused on enzymatic methods. In these next few classes, we will explore both readily available technologies and those still under development. This will give you a clear understanding of what can be achieved, the numerous opportunities in the field, and a deeper appreciation of the basic mechanisms behind various technologies, whether they are optical, non-optical, magnetic, thermal, or spectroscopic. These foundations are critical for developing the next generation of biosensors.

Another key realization through this process will be how miniaturization is shaping the future of biosensor development. Alongside that, we'll discuss the increasing popularity of non-invasive techniques. When we are working with body fluids such as urine or sweat, the procedure is relatively simple. But as soon as we start discussing drawing blood, things become more complicated. Drawing blood requires puncturing the tissue, which means the use of sterile needles, proper handling, and overcoming various detection challenges, such as interference from other molecules and the physiological status of the individual.

Let's begin by recapping how glucose sensors first emerged, and then we will move on to discuss both well-established and cutting-edge technologies. This approach will cover the complete spectrum of biosensors that are currently being explored globally.

Starting with diabetes, we know it is a disease caused by abnormal insulin levels in the body. This happens either because the pancreas fails to produce enough insulin or because the body's cells do not use insulin effectively. Insulin is a hormone responsible for regulating glucose levels in the bloodstream. It enables cells to absorb glucose for energy or to store it for later use. However, when blood glucose levels remain either too low or too high for extended periods, it can lead to conditions like hypoglycemia or hyperglycemia, respectively, which are dangerous and can cause severe health issues.

These fluctuations in blood glucose can alter the blood flow parameters, either slowing it down when glucose levels increase due to a higher particle count or decreasing it when glucose levels fall. Prolonged imbalances in glucose levels can result in tissue damage, strokes, kidney failure, blindness, heart disease, and even death if left untreated. Type 1

diabetes is caused by insufficient insulin production in the pancreas, leading to sudden drops in glucose levels. Type 2 diabetes, on the other hand, occurs when the body becomes inefficient at using insulin, resulting in consistently high glucose levels.

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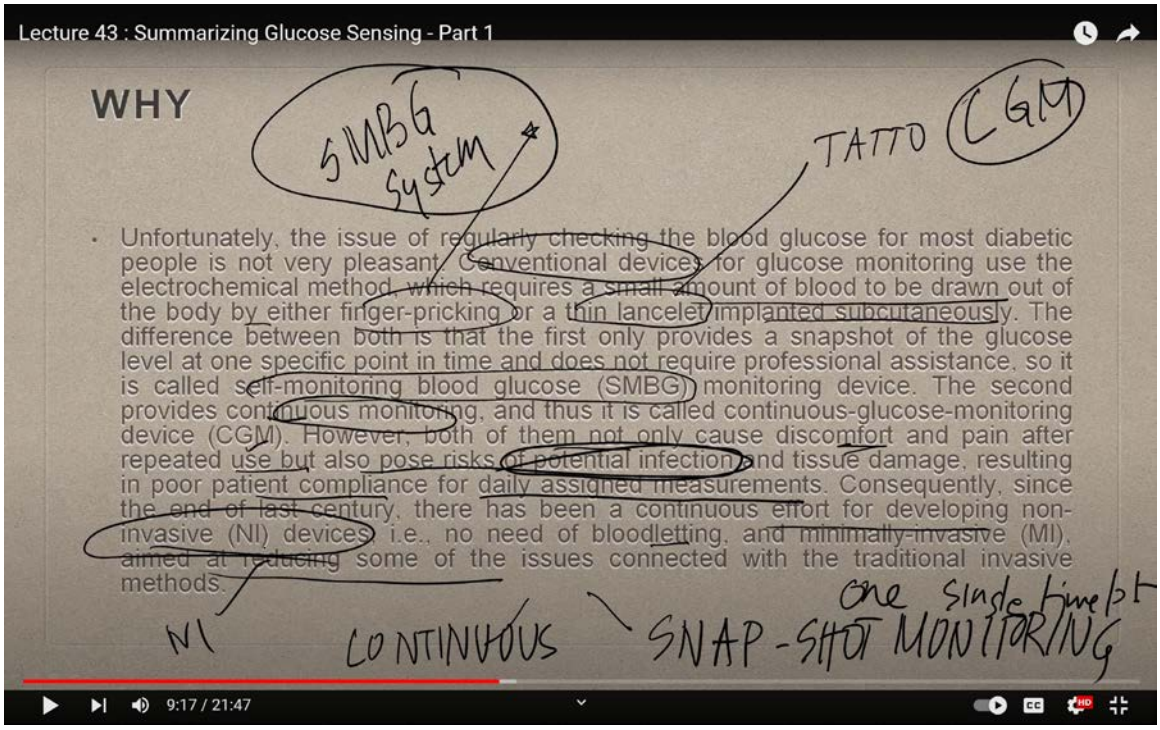

Unfortunately, both conditions are incurable, and people with diabetes must regularly monitor their blood glucose levels throughout their lives. This is the central link between glucose sensing, diabetes, and insulin, as well as type 1 and type 2 diabetes. The reality is that for most diabetic individuals, the process of routinely checking their blood glucose is unpleasant.

This brings us to conventional glucose monitoring devices, which predominantly use electrochemical methods. These methods typically require a small amount of blood to be drawn, either through finger pricking or by using a thin lancet implanted subcutaneously.

If you recall our discussion on tattoos, this is a pertinent topic, particularly in the context of glucose monitoring. I've already covered the concept of fingerprinting as well. The primary distinction between these two methods is that a fingerprinting system only

provides a snapshot of the glucose level at a single point in time, and it does not require professional assistance. This is known as a self-monitoring blood glucose (SMBG) system. So, this represents one of the systems available for glucose monitoring.

In contrast, the second type provides continuous monitoring of glucose levels. Thus, we can classify these systems based on their functionality: one offers snapshot monitoring, while the other provides continuous glucose monitoring, or CGM. To clarify, SMBG allows you to monitor your blood glucose levels independently, while CGM represents a continuous glucose monitoring device that provides ongoing insights.

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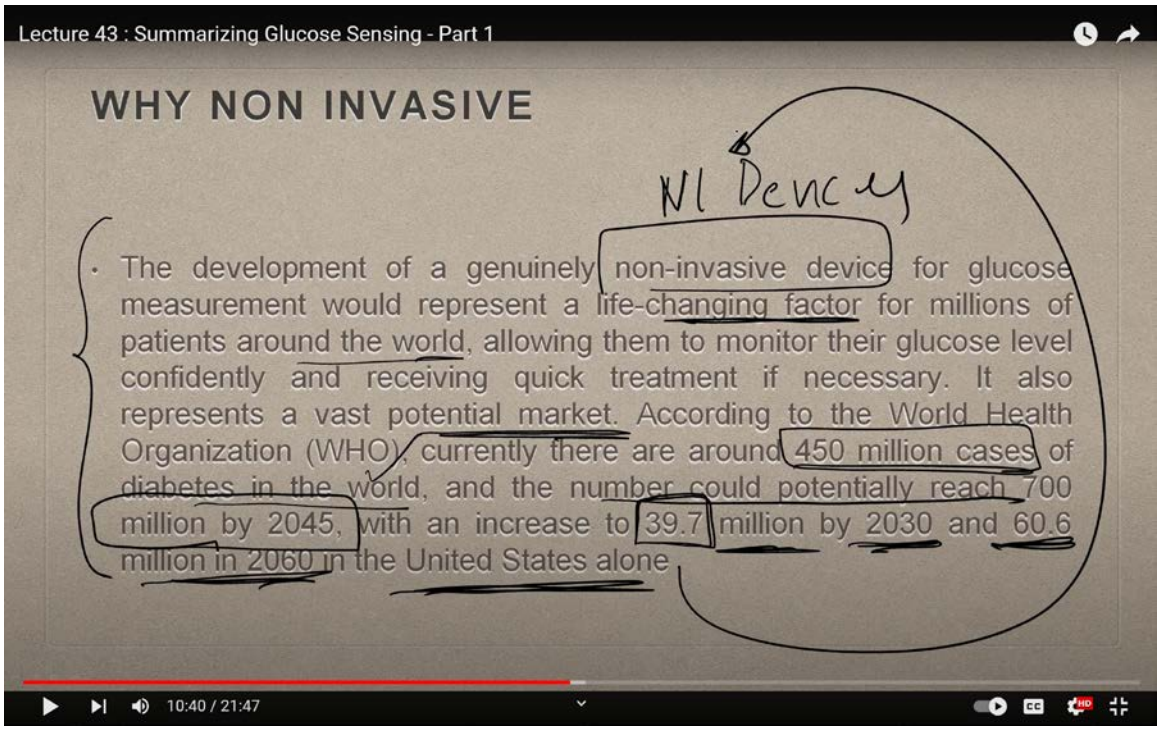

However, it is essential to note that both types of systems can cause discomfort after repeated use and pose a risk of potential infection. This aspect is extremely important to consider. When I mention the need for a sterile needle for a finger-pricking device or for an implanted tattoo, you must understand that these devices are not typical tattoos. They involve a complex set of instrumentation, including electrodes and transponders. This complexity increases the risk of infection and can lead to poor patient compliance regarding

daily measurements.

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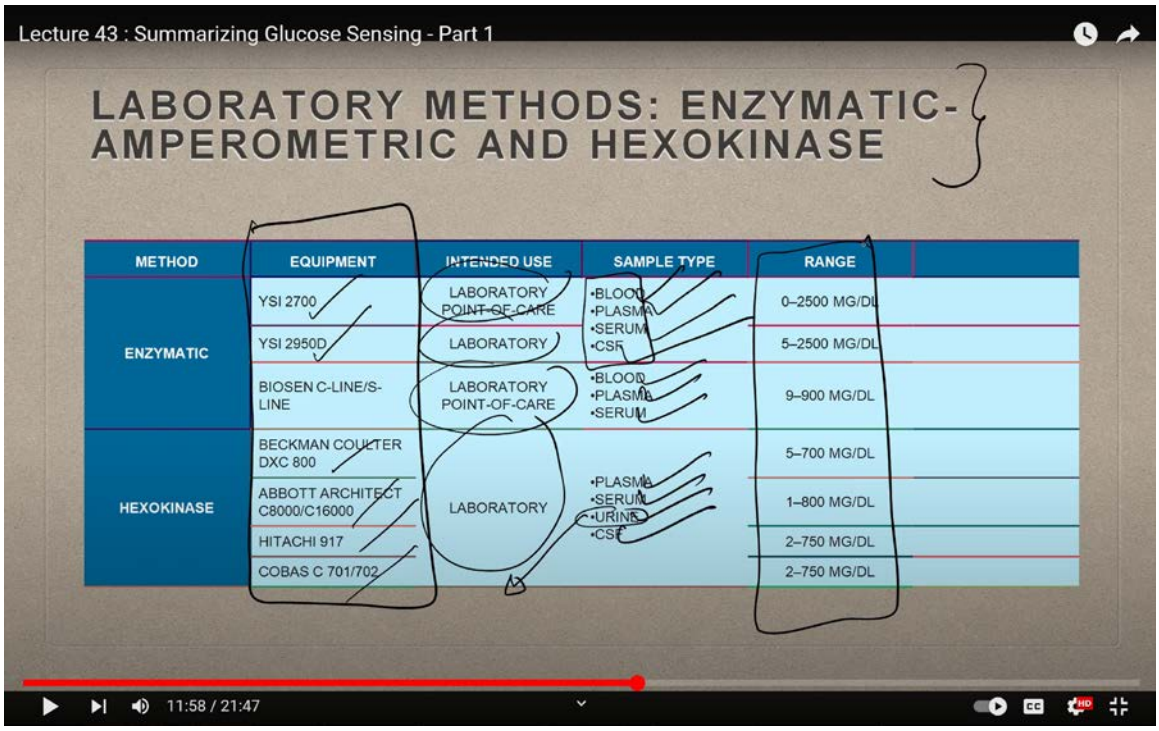

As a result, since the end of the last century, there has been a continuous effort to develop non-invasive (NI) devices that eliminate the need for bloodletting and minimize the issues associated with traditional invasive methods. You can classify glucose monitoring techniques into two main categories: invasive and non-invasive methods. Additionally, you can differentiate them based on whether they are SMBG or CGM systems. These classifications help to simplify our understanding of the various available technologies.

Now, why is there such a strong push for non-invasive solutions? The development of genuinely non-invasive devices for glucose measurement would be a life-changing advancement for millions of patients worldwide. It would allow them to monitor their glucose levels confidently and receive prompt treatment when necessary. This shift also represents a vast potential market. According to the World Health Organization, there are currently around 450 million cases of diabetes globally, with projections suggesting this number could rise to 700 million by 2045. In the United States alone, we anticipate an

increase of 39.7 million cases by 2030 and 60.6 million by 2060.

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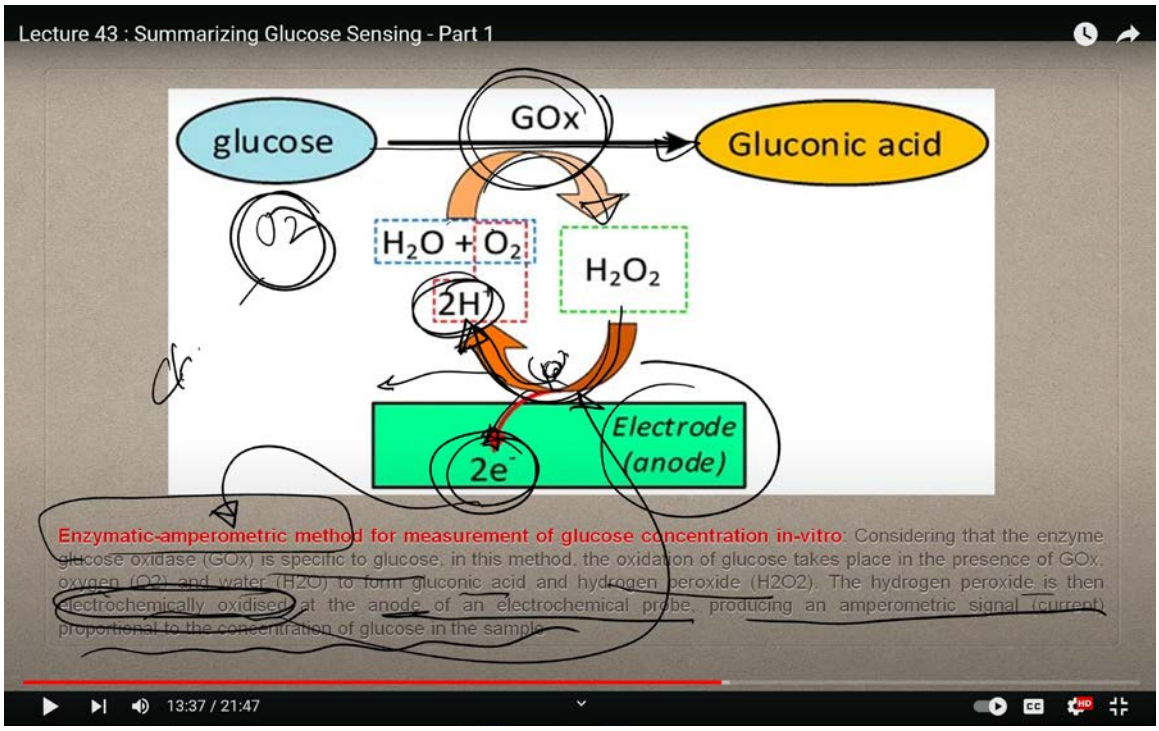

This staggering data underscores why non-invasive monitoring methods are destined to become immensely popular; this is where the future of glucose monitoring lies.

Now, let’s revisit some of the laboratory methods we've discussed. Among these are the enzymatic amperometric method and the hexokinase method. Although I have not yet developed the hexokinase method in our discussions, I will provide a brief overview of it. I have already covered the enzymatic amperometric method, but for the sake of completeness, I’ll touch on that again. Additionally, I’ll introduce you to some of the equipment available in the market for your general knowledge. Notable examples include the YSI 2700 and other products from the same company, such as the Biosense C and Line S.

Next, let's discuss some key players in the glucose analysis market. We have the Beckman Coulter, which utilizes the hexokinase method, along with the Abbott Architect from Abbott Laboratories, Hitachi, and Cobas. These instruments are primarily intended for

laboratory use and point-of-care testing. Thus, when classifying these systems, it’s crucial to consider their intended use and functionality.

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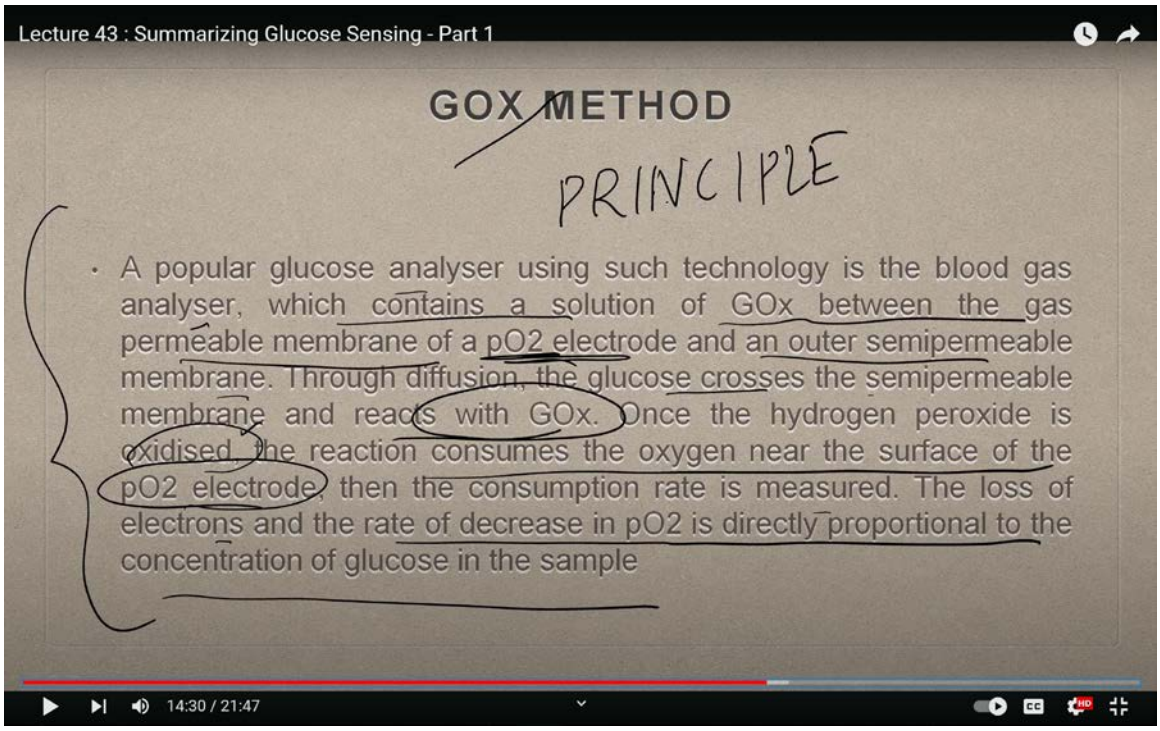

Now, let's examine the types of samples these devices analyze. They can handle blood, plasma, serum, cerebrospinal fluid, and urine. Notably, urine is the only sample that is excreted from the body, while the other fluids are typically obtained invasively. The detection range for these devices is generally measured in milligrams per deciliter (mg/dL). This overview provides you with general knowledge about the various instruments available for glucose measurement today.

We previously discussed the enzymatic amperometric method. This method is termed "enzymatic" because it involves an enzyme, specifically, glucose oxidase, that facilitates the conversion of glucose into gluconic acid. During this process, oxygen, the other substrate, is reduced to hydrogen peroxide. The hydrogen peroxide is then present at the electrode, where the anode undergoes oxidation. This oxidation-reduction reaction results in the release of two protons and two electrons, which are measured amperometrically.

Given that glucose oxidase is specific to glucose, the oxidation of glucose occurs in the presence of glucose oxidase, oxygen, and water, yielding gluconic acid and hydrogen peroxide. The hydrogen peroxide is subsequently electrochemically oxidized at the anode of the electrochemical probe, producing an amperometric signal or current that is directly proportional to the glucose concentration in the sample.

The earliest glucose sensors relied on measuring the amount of oxygen consumed to determine the glucose content in the sample, utilizing Clark's oxygen detection electrode. One notable application of the glucose oxidase method is in blood glucose analyzers. These analyzers typically feature a glucose oxidase solution situated between a gas-permeable membrane and an outer semipermeable membrane.

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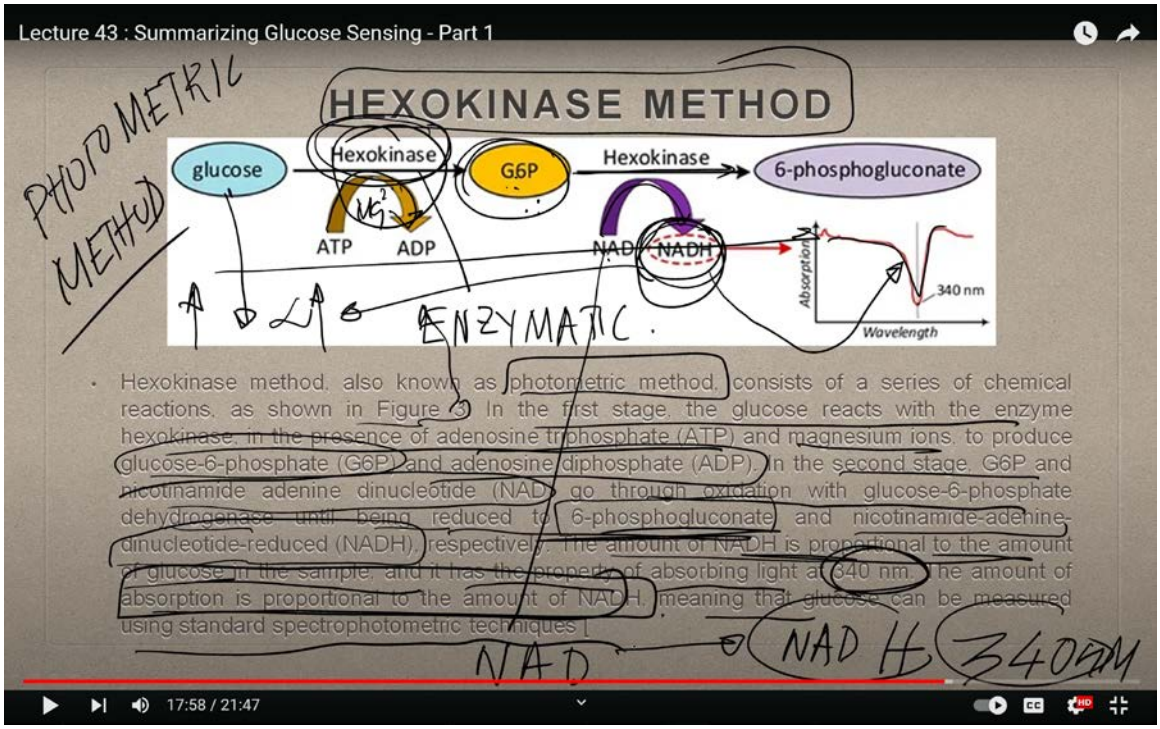

As glucose diffuses across the semipermeable membrane, it reacts with glucose oxidase. The oxidation of hydrogen peroxide consumes oxygen near the surface of the membrane, and the oxygen electrode measures the rate of this consumption. The loss of electrons and the decrease in partial pressure of oxygen (P O_2) are directly proportional to the glucose

concentration in the sample. This succinctly summarizes the basic principle behind this method.

Now, let's move on to another popular method that I haven't discussed yet: the hexokinase method. This method also employs an enzymatic approach, where glucose is processed in the presence of hexokinase, an enzyme that converts ATP to ADP while generating glucose-6-phosphate.

In the presence of hexokinase and NAD, glucose-6-phosphate is transformed into NADH. The reduction of NAD to NADH produces a characteristic signal associated with the formation of NADH. The concentration of NADH generated in this process is directly proportional to the amount of glucose present in the sample. Essentially, higher glucose levels result in higher NADH concentrations, leading to a more intense signal.

The hexokinase method, also known as the photometric method, involves a series of chemical reactions, which are illustrated in the accompanying figure.

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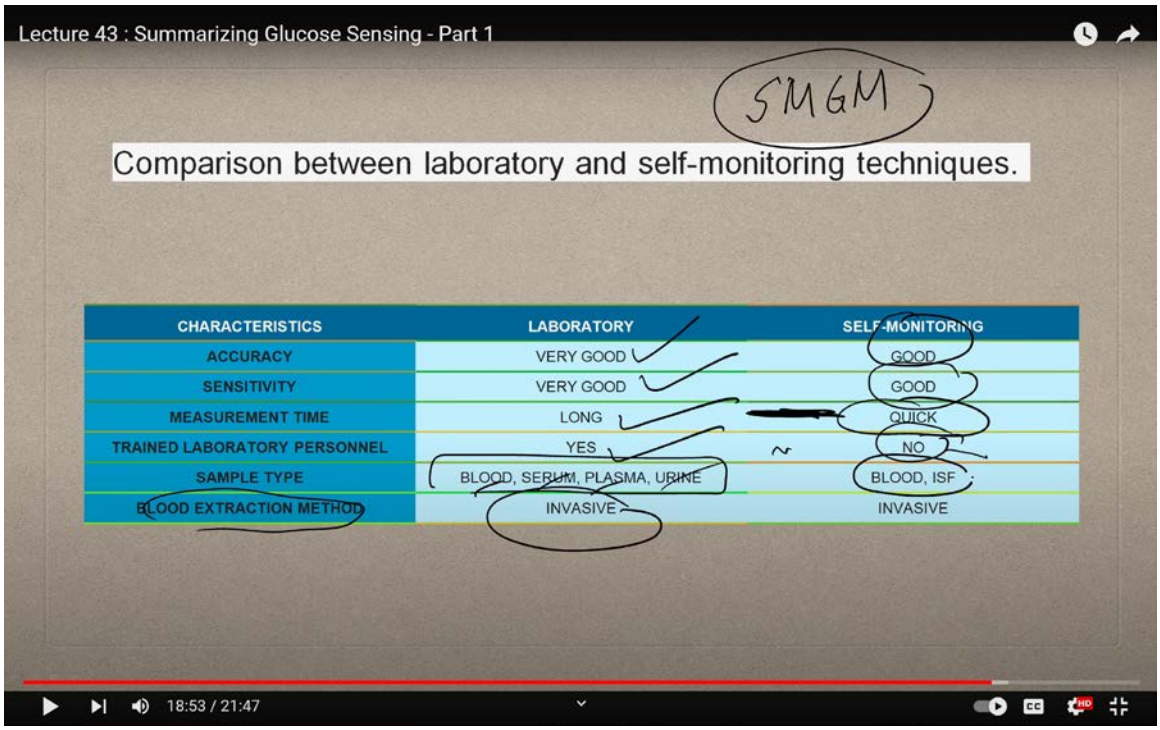

In the initial stage of the hexokinase method, glucose reacts with the enzyme hexokinase in the presence of adenosine triphosphate (ATP), which is the energy molecule, and magnesium ions. Understanding the conditions under which this reaction occurs is crucial when developing a sensor. If these native conditions are not thoroughly understood, the sensor design will inevitably be flawed. It's essential to include both hexokinase and magnesium in this reaction because magnesium significantly enhances the efficiency of the process.

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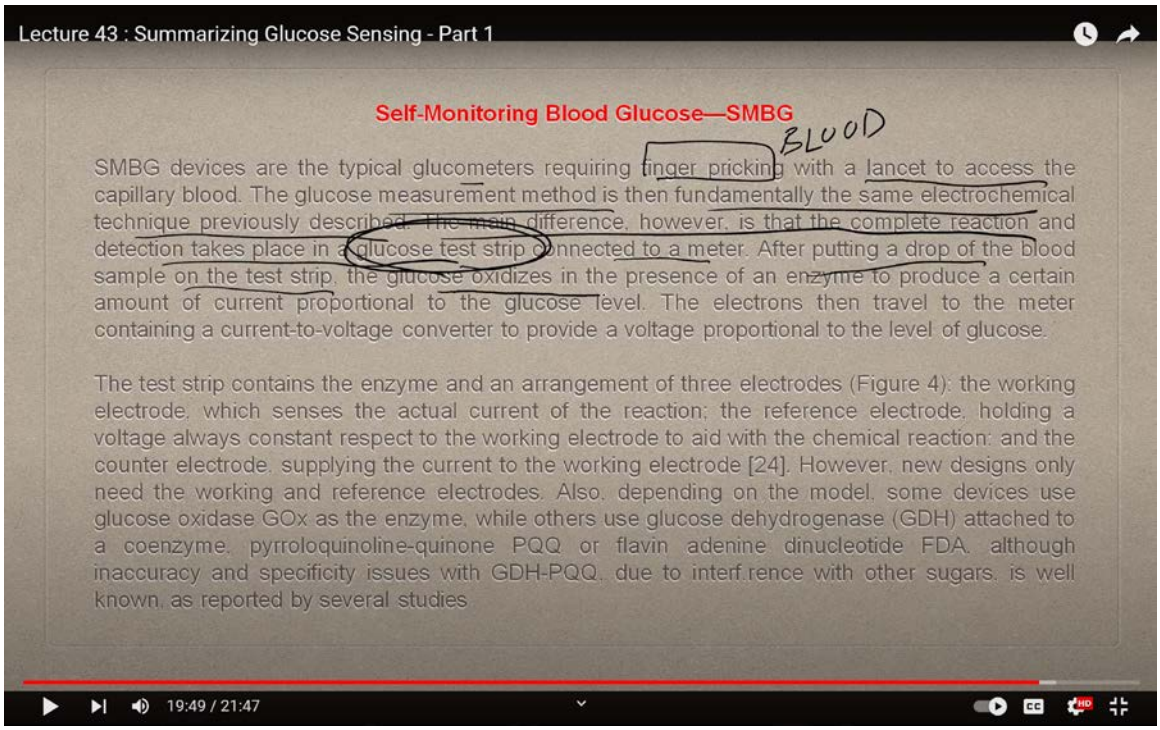

As a result of this reaction, glucose-6-phosphate is produced alongside adenosine diphosphate (ADP). In the second stage, glucose-6-phosphate and nicotinamide adenine dinucleotide (NAD) undergo oxidation facilitated by glucose-6-phosphate dehydrogenase, resulting in the formation of 6-phosphogluconate and reduced NAD, or NADH. The amount of NADH generated is directly proportional to the amount of glucose in the sample, and this compound exhibits the unique property of absorbing light at 340 nanometers. This is a critical point! The degree of light absorption correlates directly to the concentration of NADH present.

This means that glucose can be effectively measured using standard spectrophotometric techniques. Thus, we add the hexokinase method to your growing repertoire of technologies used for glucose monitoring.

Now, let's compare laboratory techniques with self-monitoring techniques. Laboratory methods are exceptionally sensitive and provide highly accurate measurements, although they require a longer measurement time and trained laboratory personnel. The types of samples used include blood, plasma, serum, and urine, and the blood extraction method is invasive.

In contrast, self-monitoring techniques, which we will explore shortly, offer decent accuracy but are not as reliable as laboratory methods. They have the advantage of being quick; this is the key differentiator, the measurement time is significantly shorter. Self-monitoring devices do not require laboratory personnel, making them more personalized. They can utilize blood as well as interstitial fluid (ISF), but like laboratory methods, they are invasive.

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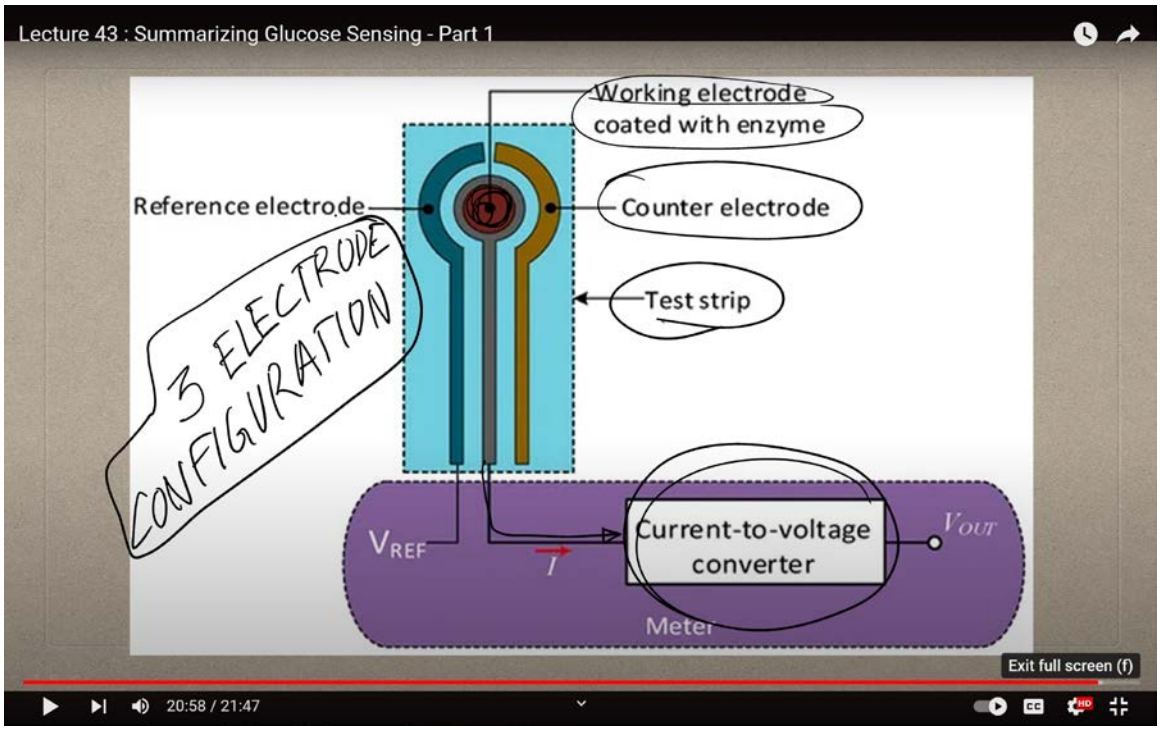

Now, let’s delve into the basics of how self-monitoring blood glucose devices function. Typically, self-monitoring blood glucose (SMBG) devices are glucometers that require finger pricking to draw blood using a lancet, accessing capillary blood. The glucose measurement method fundamentally employs the same electrochemical technique described earlier. However, the main difference lies in the fact that the entire reaction and detection occur on a glucose test strip connected to a meter.

Once a drop of blood is placed on the test strip, glucose oxidizes in the presence of the enzyme, producing a specific amount of current that is proportional to the glucose level. The electrons generated during this process travel to the meter, which contains a current-to-voltage converter that provides a voltage output proportional to the glucose concentration.

Now, let’s take a look at the device. This is what it looks like! This section is where current-to-voltage conversion occurs, and this is where the actual calculations take place. The test strip, known as the glucose test strip, features a counter electrode and a working electrode coated with an enzyme. Here, you can see the enzyme represented in red and the reference electrode.

Practically speaking, this setup consists of a three-electrode configuration. It’s important to remember this because we will revisit the concept later. The three-electrode configuration is primarily used in most electrochemical sensors, and glucose sensors have adopted this configuration as well. In our next class, we will briefly discuss the three-electrode configuration before moving on to the next set of sensors. Thank you!