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RECHERCH ARTICLE

Decoding Glucose Biosensors: Clinical Significance, Technology, and Evolving Trends

Seema Sharma[1], Satinder Kakar[2],Pawandeep Shukla[1], Sampat Singh Tanwar[1*]

ABSTRACT

It is well known that blood glucose monitoring is an efficient method to regulate diabetes. Given the recommendation to maintain normal blood glucose levels, several types suitable glucose biosensors have been established. During the past half-century, there has been a notable advancement in glucose biosensor technology, encompassing point-of-care instruments, continuous glucose monitoring systems, and noninvasive glucose monitoring systems. Still, there are a number of obstacles standing in the way of achieving precise and trustworthy glucose monitoring. It must be done to make more technical advancements in glucose biosensors, establish the analytical objectives for their performance, and continually evaluate and inform lay users. This paper analyzes the underlying concepts, analytical performance, context, and present situation of glucose biosensors in clinical use.

Keywords: diabetes mellitus; glucose biosensor; performance; self monitoring of blood glucose

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INTRODUCTION

According to the World Health Organization, approximately 422 million individuals, which is equivalent to approximately 5% of the global population, are afflicted with diabetes. Diabetes is projected to become the sixth most common cause of mortality. This condition is perilous due to its associated complications, which include cardiovascular disease, blindness, the potential need for amputation, and renal failure. The glucose concentration in the blood is a crucial indicator for diabetic patients. By keeping this concentration at an optimum level, it is possible to delay the onset of health issues associated with diabetes. Nevertheless, the level of glucose in the blood can vary significantly, making it necessary to often measure it numerous times a day, especially in severe situations. Furthermore, the significant demand for glucose biosensors in clinical analyzers has resulted in the essential advancement of personal glucose testing. A comprehensive overview of this topic may be found in reference [1]. Currently, the market for personal glucose tests surpasses 15 billion USD annually. [2].

Biorecognition elements

The distinguishing feature of biosensors, which sets them apart from chemical sensors, is the Biorecognition aspect. The primary function of this component is typically to ensure the necessary selectivity. For blood glucose detection, this is very crucial. Glucose is a specific isomer of a cyclic hydrocarbon called C6, along with other isomers such as fructose, mannose, and galactose. Furthermore, glucose residues are present in both oligo- and polysaccharides. For instance, glycogen, present in various organs and cells, including blood cells, is indeed a glucose polysaccharide. The detection of blood glucose using physical methods is hindered by the significant background signal caused by the presence of glucose and its isomers in oligo- and polysaccharides. Specifically, near-infrared spectroscopy lacks the necessary sensitivity and selectivity [3-6]. The initial glucose biosensors were developed using the enzyme glucose oxidase (EC 1.1. 3.4)

[1]Department of Pharmacy, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore, M.P, India

[2]Department of Pharmacy, Himachal Institute of Pharmacy , Poanta Sahib. H.P, India

Corresponding Author: Sampat Singh Tanwar, Department of Pharmacy, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore, M.P, India. E-Mail: sampattanwar1999@gmail.com

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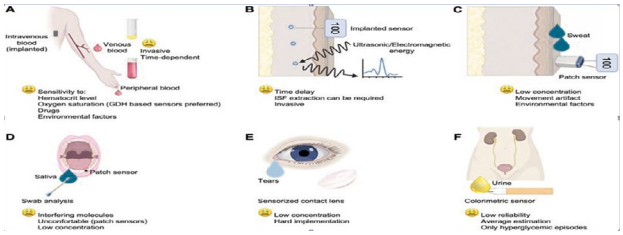

Figure 1 : Technologies and biofluids for glucose monitoring

[7, 8]. This enzyme facilitates the chemical reaction in which glucose is oxidized by molecular oxygen, resulting in the production of hydrogen peroxide as a by-product. β-D-glucose is the most optimal substrate for glucose oxidase, with a relative activity towards other substrates that is less than 1%. The Michaelis constant of this enzyme for glucose is approximately 20 mM, which means that even at the highest level of blood glucose, the enzyme still provides a high level of sensitivity. In recent times, glucose sensors have utilized both FAD (flavin adenine dinucleotide)- and PQQ (pyrroloquinoline quinone)- dependent glucose dehydrogenases. Nevertheless, the enzymes exhibit a substrate selectivity that is far wider in scope compared to glucose oxidase. Enhancing the specificity of FAD-dependent glucose dehydrogenase remains a difficult task [10], whereas PQQ-dependent glucose dehydrogenase is recognized for generating inaccurate glucose measurements in the associated biosensors. [11].

Transduction principles

The utilization of glucose oxidase as the foundation for the initial glucose-sensing devices is to be expected [7, 8]. An efficient method of combining enzymatic and electrochemical processes involves the detection of a co-substrate or by-product produced during the enzyme reaction. Thus, the initial biosensors [7, 8] relied on the measurement of oxygen levels. Subsequently, it was discovered that measuring the by-product hydrogen peroxide (H2O2) instead of oxygen [12] could yield lower detection limits. Electroanalytical chemists are continually grappling with the subject of the most effective method for detecting H2O2. The technique given in [12] for oxidizing hydrogen peroxide on platinum has a drawback, which is explained as follows. Since the 1980s, it has been established that all biological liquids, such as blood, include various chemicals that can be readily oxidized on platinum at similar potentials, resulting in the generation of inaccurate positive signals [13]. While the importance of testing blood after an overnight fast is not significant for clinical usage, several "low-invasive" monitors available in the market operate based on this idea. These monitors have the ability to react to anti-inflammatory medicines that contain ascorbate or paracetamol. This reaction can lead to a falsely elevated glucose level, which can be very harmful if the person decides to inject insulin. It is important to note that low blood glucose is far more hazardous than high blood glucose. The enzyme peroxidase, which was discovered in the 1970s [14], enabled the direct bioelectrocatalysis and subsequent creation of reagentless biosensors for hydrogen peroxide. Soon after, the concept of bi-enzyme glucose electrodes was introduced [15]. The process of converting oxidase-catalyzed processes through peroxidases has been examined in [16]. Enzymes, by their very nature, are fundamentally unstable and so cannot offer long-term stability. Furthermore, the idea of bi-enzyme electrodes involves the immobilization of an oxidase enzyme alongside a peroxidase enzyme, allowing for direct electron transfer to occur between the active site of the enzymes and the mediator. This method has primarily been utilized in combination with carbon paste electrodes [17], but it has not yet been widely implemented in practical settings. Ferric hexacyanoferrate, also known as Prussian Blue, is the most effective electrocatalyst for detecting hydrogen peroxide by its reduction in the presence of oxygen. This kind of catalyst helps to avoid issues with reductants. [18] The utilization of Prussian Blue in electrochemical biosensors was initially documented in 1994 [19]. In the subsequent year, S. Dong documented a biosensor that employed a comparable transduction mechanism [20]. When compared to the commonly used platinum detection method, Prussian Blue is significantly more effective in a neutral media suitable for biosensor operation. It is 1000 times more active, resulting in a three-order-of-magnitude increase in the electrochemical rate constant. This improved activity leads to enhanced sensitivity. Additionally, Prussian Blue is 1000 times more selective towards H2O2 reduction, enabling the detection of low potentials. These findings were reported in a study [18]. Furthermore, the process of reducing H2O2 through electrocatalysis is a unique characteristic of Prussian Blue. The limited electrocatalytic capability of non-iron hexacyanoferrates can be attributed to structural imperfections [21]. Instead of using electrosynthesis, Prussian-Blue-based electrocatalysts can be deposited using a specifically developed open-circuit approach that does not require any current. This method enables the mass manufacture of improved H2O2 sensors. The second generation of biosensors was responsible for the commercial success of personal glucose tests. The identification of ferrocene as a mediator for glucose oxidase [23] resulted in the creation of the initial electrochemical glucose test strips

(Exatech, 1987). To achieve reagentless operation, it is necessary to immobilize the mediator. Osmium-containing redox hydrogels are the most commonly utilized systems for glucose oxidizing enzymes [24]. By altering the ligands that surround the Os2+/+ ions, it is feasible to modify the redox potential of the redox center [25], so adjusting the mediator to suit a particular enzyme. Reducing the mediator redox potential allows for a reduction in the operational potential of the biosensor, hence reducing the impact of reductants (as mentioned above). Due to these factors, PQQ-glucose dehydrogenase is frequently substituted with FAD-glucose dehydrogenase in commercial devices [26]. The primary objectives in the advancement of personal glucometers were to reduce both the latency period and the amount of blood needed. Due to the need for pre-electrolysis in ferrocene, as its oxidized state (the ferricinium ion) serves as an electron acceptor for the enzyme, a mediator with significantly lesser effectiveness [27], known as ferricyanide ([Fe(CN)6] 3-), which was found earlier [28], is now commonly employed in personal glucometers. The blood sample volume was reduced to 1 µL or less by creating a capillary channel on the biosensor. The creation of consistent capillary tubes with a height of 50 µm enabled the use of coulometric measurements with personal glucometers [26]. Coulometric measurements offer two notable advantages compared to amperometric measurements. Coulometry is not affected by sensor sensitivity, making it suitable for personal test strips that cannot be calibrated. This eliminates the issues of reproducibility in manufacturing and the impact of storage on enzyme activity. These issues rank as some of the most significant challenges for personal glucometers. Furthermore, the utilization of coulometric measurements significantly enhances the selectivity of individual glucometers. The amperometric response is equal to the product of the concentration and the sensitivity. For instance, the first-generation platinum-based biosensor exhibits a sensitivity to paracetamol that is ten times greater than its sensitivity to glucose [13]. Under these circumstances, interfering chemicals, even when present in significantly smaller amounts, might produce a response that is similar to that of the target analyte. In contrast, when using coulometric readings with a set and reproducible volume, the reaction is directly proportional to the concentration. This means that the impact of interfering substances, which are present in blood at much lower quantities, becomes insignificant. Undoubtedly, glucose oxidase is the most valuable biorecognition element in second-generation biosensors because to its exceptional selectivity. Nevertheless, the utilization of distinct transduction principles has facilitated the employment of PQQ-glucose dehydrogenase and, more recently, FAD-glucose dehydrogenase as final enzymes in Abbott glucometers [26]. Despite numerous attempts, the utilization of glucose oxidase for direct bioelectrocatalysis must be deemed failed. Glucose dehydrogenases, which possess a wider range of specificity, can participate in electrocatalysis by directly exchanging electrons between the active site of the enzyme and the electrode [10, 29]. The bioelectrocatalysis of glucose dehydrogenase on carbon surfaces, as reported, is not highly efficient. Introducing a mediator that can transfer electrons between the electrode and the enzyme typically results in a significant increase in the catalytic current, often by several orders of magnitude. Consequently, the fraction of the active enzyme participating in direct bioelectrocatalysis is quite small. The reported exceptionally high limiting current for direct bioelectrocatalysis by PQQ-dehydrogenase on carbon cryogel [30] raises two questions. Firstly, the addition of a mediator enhances the limiting current by a factor of 20. Secondly, the reported current density of 4.5 mA cm-2 with the mediator is 2.5 times greater than the value we reported for the diffusion-controlled limiting performance characteristics of a hydrogen enzyme electrode (1.7 mA cm-2) [31], considering that H2 clearly diffuses at a faster rate than glucose.

Non-invasive monitoring of diabetes

Non-invasive diagnostic approaches, which not only prevent harm to blood vessels but also minimize damage to the skin, are the preferred choice. These methods are painless and eliminate the risk of infection and trauma to patients. Nevertheless, despite persistent endeavors, the issue of accurately determining blood glucose levels without intrusive methods has not been resolved to a satisfactory extent. Conventional techniques for measuring blood glucose levels, such as near-infrared spectroscopy, have not been effective in providing the necessary level of sensitivity [3-6]. Efforts to quantify glucose levels in voided fluids, namely using glucose iontophoresis [32, 33], have been implemented in commercial devices such as the "Glucowatch" by Cygnus Corp. However, this product was quickly withdrawn from the market following its release. The "tattoo-based non-invasive glucose monitor" mentioned in [34] is essentially a redesigned version of the iontophoresis-based "Glucowatch". Sweat collected non-invasively has already been utilized in the clinical diagnosis of mucoviscidosis (cystic fibrosis) through the measurement of its conductivity [35-37]. In order to stimulate sweating, the generally employed method of activating sweat glands is

Table 1: Classification of enzymatic glucose sensors

Classification	*Characteristics*
1st Generation	The formation of hydrogen peroxide occurs through the utilization of oxygen as an electron acceptor, as per the sensor developed by Clark and Lyons. Interference caused by other electroactive species can lead to errors.
2nd Generation	Substitution of oxygen as a recipient of electrons Introduction of the non-physiological mediator Constraints in the transmission of molecules from the enzyme active site to the electrode
3rd Generation	Lack of intermediary Enzyme-electrode direct transfer Reduced operating potential, increased selectivity, less interference

through pilocarpine electrophoresis [38, 39]. It is worth noting that the concentration of metabolites remains consistent regardless of the rate of sweating[40]. Sweat has also been explored as a potential method for non-invasive monitoring of diabetes. Nevertheless, despite efforts to establish a correlation between sweat and blood glucose levels [41, 42], it was unfeasible to forecast blood glucose levels based on sweat glucose concentration. Non-invasive monitoring by chemical analysis is typically deemed unreliable since none of the voided fluids accurately reflect the metabolite composition of blood. A necessary condition for non-invasive diagnostics would be a correlation in the rates of change between metabolite concentrations in the voided liquid and their corresponding values in blood [43-47]. We have demonstrated that the Pearson correlation coefficient for the variation rates of glucose content is 0.75 [45]. Additionally, the regression line has an intercept close to zero and a slope close to one. When comparing the variation rates of lactate concentration in capillary blood and venous blood, both show a comparable Pearson correlation coefficient (r = 0.8). However, the regression line is far from the origin [48]. Therefore, the connection in metabolite content between sweat and blood is no less significant than the correlation between blood samples from various artery systems. J. Wang [49] has produced wearable gadgets that utilize sweat analysis. Other researchers have also suggested wearable devices for measuring glucose [42, 50-55] and/or lactate [54, 56-59], following the idea of the proposed 'tattoo' biosensor. Nevertheless, the concept of using tattoos for continuous monitoring of blood metabolites is impractical due to the limitation that only the initial fraction of sweat may effectively reach the sensor surface. In this particular configuration, the sweat that is produced later gets mixed with the sweat that was secreted earlier. As a result, instead of obtaining the current concentration of sweat metabolites, we end up with an average concentration. This leads to a noticeable delay in the reaction. The sole method for measuring sweat metabolites in real-time is the flow-through biosensor, which was initially introduced by our team [47]. The concept is founded on the utilization of a Macroduct-type sweat collector, wherein the capillary on its outer surface is substituted with a channel enzyme electrode. In this configuration, the sweat that is released is directed towards the biosensor surface and then exits through an unobstructed outlet. The biosensor's readout is consistent with the sweat metabolite content obtained separately by sampling it from the monitor output. The Pearson correlation coefficient r is greater than 0.98, as indicated by the values 45 and 47. This sort of biosensor is anticipated to have a comparable operational lifespan to other commercially available low-invasive devices. A study has shown that it is feasible to use a flow-through biosensor to continuously analyze sweat for monitoring diabetes [45]. The blood glucose concentration dynamics align well with the biosensor reading. This example illustrates that non-invasive methods can effectively monitor humans, including diabetes patients and individuals undergoing glucose tolerance tests.

CONCLUSION

Undoubtedly, electrochemistry has had a profound influence on medical diagnostics, encompassing both clinical diagnostics and personal medical examinations. The electrochemical technique offers several benefits for the advancement of sensors and biosensors. Electrochemical sensors are very adaptable, possessing the broadest range of detection and being unaffected by the color and turbidity of the sample. Additionally, they are the most cost-effective choice. Unsurprisingly, personal glucometers that use biosensors with electrochemical readouts are the most common in the market. When it comes to the current problem in medicine, electrochemical biosensors appear to

be the sole analytical tool that can be effectively integrated into wearable devices for non-invasive diagnostics. Our findings indicate that blood glucose levels can be accurately assessed by detecting glucose in sweat. This means that individuals, such as diabetes patients or those undergoing glucose tolerances testing can be effectively monitored using a non-invasive method.

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