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GLUCOSE BIOSENSORS - STATE OF THE ART AND PROSPECTS

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БИОСЕНСОРЫ ДЛЯ ОПРЕДЕЛЕНИЯ ГЛЮКОЗЫ - ТЕКУЩЕЕ СОСТОЯНИЕ И РАЗВИТИЕ

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Abstract. Rapid glucose concentration detection in technical and biological systems is an important scientific and technical task of modern chemistry, engineering and technology. The article provides an overview of the last technical solutions in this area. The issues of developing first generation biosensors are considered. However, the main disadvantage of such systems was the significant influence of ascorbic and uric acids on the generated signal, which significantly reduced their selectivity and accuracy. To solve this problem, it is possible to use ion-selective membranes such as Nafion and polycarbonate. The second generation of glucose biosensors uses artificial mediators to facilitate electron transfer between the enzyme and the electrode. These mediators can be immobilized directly by the enzyme or introduced into an enzyme-modified electrode. Suitable mediators include conducting organic salts, ferrocene, quinone compounds, ferricyanide, transition metal complexes, phenothiazine and foxazine compounds. Effective interactions between enzymes and mediators are critical for efficient electron transport. Various approaches have been proposed to tailor mediators, such as the use of Os complexes, noncovalent functionalization of carbon nanotubes, and stabilization of artificial mediators. The third generation of enzyme glucose biosensors uses direct electron transfer to perform electrochemical reduction. Various approaches have been considered, including reassembling apoproteins on cofactor-modified enzymes and electrically coupling enzymes to electrode surfaces using redox polymers or nanomaterials such as gold nanoparticles. Such approaches ensure the formation of an effective enzyme-electrode bond. In addition, the thickness of the enzymatic layer can affect the performance of the biosensor. External factors such as temperature, pH and humidity can have a significant impact on the performance of such electrodes.

Аннотация. Быстрое определение концентрации глюкозы в технических и биологических системах является важной научно-технической задачей современной химии, техники и технологии. В статье проведен обзор последних технических решений в данной области. Рассмотрены вопросы разработки биосенсоров первого поколения. Основным недостатком таких систем было существенное влияние на формируемый сигнал таких веществ как аскорбиновая и мочевая кислоты, что существенно снижало их селективность и точность. Для решения этой проблемы возможно использование ионоселективных мембран таких как как нафион и поликарбонат. Второе поколение биосенсоров глюкозы использует искусственные медиаторы для облегчения переноса электронов между ферментом и

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электродом. Эти медиаторы могут быть иммобилизованы непосредственно с помощью фермента или внесены в модифицированный ферментом электрод. Подходящие медиаторы включают проводящие органические соли, ферроцен, хиноновые соединения, феррицианид, комплексы переходных металлов, фенотиазин и соединения фоксазина. Эффективное взаимодействие между ферментами и медиаторами имеет решающее значение для эффективного транспорта электронов. Для адаптации медиаторов были предложены различные подходы, такие как использование комплексов Os, нековалентная функционализация углеродных нанотрубок и стабилизация искусственных медиаторов. Третье поколение ферментных биосенсоров глюкозы использует прямую передачу электронов для проведения электрохимического восстановления. Были рассмотрены различные подходы, в том числе повторная сборка апобелков на ферментах, модифицированных кофактором, и электрическое соединение ферментов с поверхностями электродов с использованием окислительно-восстановительных полимеров или наноматериалов, таких как наночастицы золота. Такие подходы обеспечивают образование эффективной связи фермент-электрод. Кроме того, толщина ферментативного слоя может влиять на работу биосенсора, температура, pH и влажность могут оказывать существенное влияние на функционирование подобных электродов.

Keywords: D-glucose, biosensors, electrodes, detection.

Ключевые слова: D-глюкоза, биосенсоры, электроды, определение.

For over 50 years, biosensor technology has been advancing to support diabetic patients in routine disease screening, diagnosis, and long-term management [4, 9–11]. Glucose biosensors are specifically designed to measure glucose concentration in biological samples such as blood or urine. These devices typically consist of a glucose-specific enzyme, like glucose oxidase, immobilized on a transducer surface that converts the biochemical reaction into an electrical signal [11]. This signal is measured and correlated to the glucose concentration in the sample. Despite significant progress in the development of point-of-care devices, continuous analyses, and non-invasive systems, only a few biosensors for glucose detection have achieved commercial maturity due to several drawbacks Research has focused on the development of cutting-edge technologies in the fields of material science, rational design, microfluidics, sensor printing, and nanotechnology to improve the stability, specificity, reproducibility, and reliability of biosensor technology. Nanoscale materials offer unique properties and functionality that can increase the analytical performance of biosensors in terms of sensitivity, selectivity, and robustness. These advancements allow for the miniaturization and integration of biocomponents, transduction systems, electronics, and microfluidics in complex nanobiosensor architectures capable of performing both continuous glucoses monitoring as implantable devices and high throughput analyses as lab-on-chip devices for rapid and low-cost screening of glucose and other physiological metabolites using small samples of patient material [11].

There are various types of biosensors available, including enzyme-based, tissue-based, immunosensors, DNA biosensors, thermal biosensors, piezoelectric biosensors, magnetic biosensors, and optical biosensors. Enzyme-based biosensors are the most common and use immobilization methods to measure glucose concentration in biological samples such as blood or urine. Other types of biosensors use different methods to detect specific analytes, such as antibodies for immunosensors or single-strand nucleic acid molecules for DNA biosensors. Recent

Т. 9. №12. 2023
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advancements in nanotechnology have allowed for the miniaturization and integration of biocomponents, transduction systems, electronics, and microfluidics in complex nanobiosensor architectures capable of performing both continuous glucose monitoring as implantable devices and high throughput analyses as lab-on-chip devices for rapid and low-cost screening of glucose and other physiological metabolites using small samples of patient material [6]. Additionally, genetically encoded biosensors have been developed that are user-friendly and easy to engineer, manipulate and transfer into cells [6].

The development of enzymatic glucose sensors has gone through three generations. The first-generation sensor, proposed by Clark and Lyon [6], monitored oxygen consumption during the enzyme-catalyzed reaction. However, it faced interference from background oxygen. To overcome this, the second-generation sensor, developed by Updike and Hicks [6], utilized two oxygen working electrodes to measure the current differential and remove the noise caused by background oxygen. Another approach by Guilbault and Lubrano involved monitoring the released hydrogen peroxide (H_2O_2) as an indication of glucose concentration [1, 2].

The catalytic reaction in these sensors involved the reduction of the enzyme's flavin group (GOx(FAD)) to its reduced form (GOx(FADH2)). To maintain the enzymatic cycle, an electron acceptor and oxidation mediator (Medox) were used to reoxidize the enzyme and regenerate the oxidized form (GOx(FAD)). This regeneration step was crucial for the sensor's continuous operation.

The three generations of glucose biosensors are categorized based on the type of oxidation mediator used. The first generation utilized oxygen (O_2) as a physiological mediator. The second generation employed an artificial electron acceptor, typically synthetic in nature. The third generation of biosensors achieved direct electrical communication with the electrode, eliminating the need for any mediators. These advancements in enzymatic glucose sensors have contributed to the development of more efficient and reliable glucose monitoring technologies.

Types of biosensors

First-Generation Enzymatic Glucose Biosensors

The first generation of enzymatic glucose biosensors used oxygen as the oxidation mediator to regenerate the enzyme and detect glucose by monitoring oxygen consumption or the generation of H_2O_2. The detection process involved the anodic oxidation and cathodic reduction of H_2O_2. These biosensors were stable, simple, and suitable for miniaturized applications. However, a major drawback was the interference caused by electroactive species such as ascorbic acid and uric acid, which reduced the selectivity and accuracy of the biosensor. To address this, permselective membranes like Nafion and polycarbonate were used to limit the access of interferents to the biosensor surface. Other approaches included metallized carbon and metal-hexacyanoferrate transducers to reduce the operational potential and avoid interference. Wang and Wu developed a glucose biosensor with high selectivity by incorporating rhodium particles in an Nafion film [6].

Various approaches were explored to improve selectivity and sensitivity, including the use of Prussian blue (PB) as a catalyst for H_2O_2 reduction. Nanomaterials such as carbon nanotubes and Pt nanoparticles were also utilized to enhance selectivity. However, these biosensors faced challenges such as electroactive interference from species like ascorbic acid and uric acid. Permselective membranes like Nafion were employed to reduce interference. Another limitation was their dependence on oxygen, leading to errors due to oxygen tension fluctuations. Researchers also investigated alternative materials such as Os complexes (Tables 1, 2) and PB to address these issues [6].

Т. 9. №12. 2023
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Second Generation Enzymatic Glucose Biosensors

The second generation of enzymatic glucose biosensors incorporates artificial mediators to facilitate electron transfer between the enzyme and the electrode. These mediators can be immobilized directly with the enzyme or entrapped in an enzyme-modified electrode. Suitable mediators include conducting organic salts, ferrocene, quinone compounds, ferricyanide, transition-metal complexes, phenothiazine, and phoxazine compounds. The catalytic process involves the reduction of the enzyme's flavin adenine dinucleotide (FAD) centers, electron transfer to the artificial mediator, and subsequent electron transport to the electrode. The effective interaction between enzymes and mediators is crucial for efficient electron transportation. Various approaches have been proposed to tailor the mediators, such as using Os complexes (Tables 1, 2), non-covalent functionalization of carbon nanotubes, and stabilizing artificial mediators.

Table 1

COMPARISON OF ANALYTICAL PERFORMANCE
OF THE GLUCOSE BIOSENSORS BASED ON MEDIATORS, REFERENCE [3]

Mediator	*Operating potential, V*	*Liner range, mm*	*LOD, mm*	*Sensitivity, cm^{-1} mm^{-1}*
Os-complex	0.00	0-0.7	0.0003	28.24
Os(2,2′ bpy)2 -RP	0.25	0-10.0	-	16.5
1,1′ -Di-methylferrocene	0.205	1.0-30	-	6.63
Ferrocene	0.35	0.1- 10.0	0.13	12.42
$[Ru(NH_3)_6]^{3+}$	0.00	0-27.7	-	-
Ru3 (ų3 -O)$(AcO)_6(Py)_3(ClO_4)$	0.00	0.01-0.5	-	15.4
$[Ru(trpy)(phen)(OH_2)]^{2+}$	0.52	-	-	-
trans-$[Ru(2,2′ bpy)_2 (OH_2)(OH)]^{2+}$	0.50	0-24.0	-	0.4
$[Ru(4,4′ bpy)(NH_3)_5]^{2+}$	0.24	0-5.6	-	7.2
Ru-RP	−0.15	0-10.0	0.29	24.3

Table 2

DETERMINATION OF GLUCOSE CONCENTRATION IN FRUIT JUICE [3]

Juice sample	*Measured (g/L)*	*Relative error (%n = 3)*	*Reference (g/L)*	*Recovery (%)*
Apple	83.4	5.2	85.0	97.2
Blackcurrant	33.7	12.5	25.9	108.7
Guava	29.6	7.8	31.0	114.3
Mango	46.8	7.5	45.8	102.2
Orange	38.4	10.1	39.6	97.0

Immobilized mediator-based biosensors require proper immobilization near the enzyme's redox center and the electrode surface to ensure efficient electron exchange. However, immobilized mediators have limited range of motion compared to solution-based mediators [6].

Third Generation Enzymatic Glucose Biosensors

The third generation of enzymatic glucose biosensors utilizes direct energy transmission (DET) to achieve direct electrochemistry of enzymes. Various approaches have been explored, including reassembling apo-proteins on cofactor-modified enzymes and electrically wiring enzymes to electrode surfaces using redox polymers or nanomaterials such as gold nanoparticles (AuNPs) and carbon nanotubes (CNTs). These methods enable efficient enzyme-electrode connectivity but

Т. 9. №12. 2023
https://doi.org/10.33619/2414-2948/97

can be complex. DET was initially proposed by Heller and Degani, demonstrating the covalent connection of the enzyme active site to the electrode surface. Researchers have successfully achieved GOx electrochemistry using nanomaterial-coated electrodes, modified enzyme surfaces, or bio-mediated nanoparticles. These advancements have led to the development of mediator-free glucose biosensors with improved performance. However, enzyme activity in these biosensors can still be affected by external factors like temperature, pH, and humidity. Additionally, the thickness of the enzymatic layer can impact biosensor performance. Due to the limitations of enzymatic biosensors, non-enzymatic glucose detection systems have been developed as the fourth generation of glucose sensors, focusing on direct glucose oxidation on the electrode surface.

In a study conducted by Deng et al. [3] an enzyme-based biosensor, Nafion/PPMP–GOx-BSA/Au, that is cost-effective, simple to use, and highly sensitive, selective, and stable. The biosensor was able to detect a wide range of glucose concentrations and exhibited excellent selectivity towards glucose monitoring. The recombinant enzyme from the corn kernel production system used in this biosensor was found to be a low-cost, reproducible, sensitive, and selective alternative to conventional enzymes like HRP for glucose detection. Future work involves incorporating nanomaterials and conductive polymers to improve the LOD of the PPMP-based biosensor, making it more effective in measuring various biofluids in real sample use [6].

The GOx catalyzed glucose oxidation in phosphate-buffered solution (PBS, pH = 7.4) during chronoamperometry. Both PDTP derivatives afforded wide linear detection ranges, and polymer 2 gave an especially low LOD of 0.0986 μM

GOx was immobilized by covalently bonding to polymer amine groups, assisted by glutaraldehyde (GA) crosslinking. Glucose was detected in PBS (pH = 7.0) with a low LOD of 0.348 μM, superior to many electrochemical glucose biosensors (Table 1).

Graphene biosensors

Graphene has been dubbed a “miracle material” due to its superior properties, including its large surface area, thermal conductivity, charge carrier mobility, and mechanical strength [11]. It can be produced easily and cost-effectively in the laboratory and quickly functionalized with different chemical groups. Graphene has been widely used as a functional nanomaterial for designing glucose biosensors due to its ability to operate as a label/loading agent for biomolecules and nano-materials, as well as an electron transfer enhancer for electrochemical transduction and a smart customized fluorescence-quencher for optical transduction. Numerous graphene sensors and biosensors have been reported in literature, including those designed to detect main bioclinical markers such as glucose [11].

Graphene-based optical biosensors have gained attention as an alternative to electrochemical methods for glucose detection. These biosensors utilize the unique properties of graphene derivatives, such as their ability to quench fluorescence through energy transfer, depending on factors like the number of graphene layers and oxidation degree. Oxidized graphene derivatives, in particular, have been extensively studied due to their photoluminescent properties resulting from the recombination of electron-hole pairs within a carbon domain embedded in a matrix. By tuning the oxygen-containing groups, lateral size, and oxidation degree, the fluorescence emission wavelength of these derivatives can be modulated. This has enabled the development of customized DNA sensors that interact effectively with graphene derivatives, allowing for the detection of target analytes. The sensing mechanism involves the detachment of the single-strand DNA/graphene complex in the presence of the analyte, leading to the recovery of fluorescence. Different graphene derivatives, such as graphene quantum dots (GQDs) and graphene oxide (GO), have been employed

Т. 9. №12. 2023
https://doi.org/10.33619/2414-2948/97

in these biosensors. For instance, a nanobiosensor based on a graphene oxide nanocomposite achieved glucose detection using surface-enhanced Raman spectroscopy, demonstrating a linear response range and good recovery values in blood samples. Quantum dots, when combined with graphene, have also shown promising performance in optical glucose biosensors, offering low toxicity, high photoluminescence, water solubility, and excellent photochemical properties. Additionally, bimetallic CuPd nanoparticles decorated on reduced graphene oxide have been utilized for glucose detection based on peroxidase-like activity [11]. These nanoparticles, in combination with glucose oxidase, facilitated the colorimetric detection of glucose. Another approach involved using reduced graphene oxide/MnO_2 combined with tetramethylbenzidine and horseradish peroxidase-mimic for glucose analysis in whole blood without sample pre-treatment. Reduced graphene oxide has also been used in fluorescence quenching-based sensors, where its proximity to fluorescence probes allows for the detection of glucose in aqueous solutions. These graphene-based optical biosensors offer promising capabilities for glucose detection, with potential applications in clinical settings.

One study by the Henry group from Colorado State University developed an ePAD for quantifying glucose, lactate, and uric acid in human serum samples. They utilized microfluidic channels built on filter paper and screen-printing technology to create an electrochemical cell. The ePAD demonstrated good reproducibility and accuracy for glucose and lactate detection.

Other researchers have explored simpler configurations for ePADs. For example, Kong et al. [6] used commercially available sensors printed on alumina and Whatman chromatography paper to detect glucose. The paper disk with the blood sample was laminated onto the electrode surface, and electrochemical measurements were conducted. This approach offered a linear range of detection within the physiological range.

Different techniques have been employed to create hydrophobic regions in ePADs. Initially, photolithography was used, but wax printing techniques have been adopted to improve sustainability. For instance, Cinti et al. [6] fabricated a paper-based strip for glucose detection using a printer to create a hydrophobic zone. The ePAD demonstrated filtering properties, porosity for reagent loading, and in situ synthesis of Prussian Blue nanoparticles. Glucose detection in blood was achieved with good accuracy [17]. Origami approach, where sheets loaded with biocomponents, buffer, and electrochemical mediators are folded [7]. Li et al. developed an origami paper-based device for glucose detection, which showed promising results when compared to commercial blood glucometers [16].

Energy sustainability is another important consideration. Fisher et al. developed [7] a low-cost and self-powered paper-based biosensor for glucose monitoring. This biosensor utilized a paper reservoir for enzyme immobilization and operated as an enzymatic fuel cell. It demonstrated a linear range of detection and showed suitability for clinical and social settings in resource-limited environments.

Table 3

SUMMARY OF BIOSENSORS CONFIGURATION, MECHANISM, AND PERFORMANCE [7]

№	*Additional specie*	*Analyte*	*Detection method*	*Sensitivity*	*Linear range*
1	GOx, AuNP	Glucose	CA	NR	50/100-2500
2	GOx, AuNP	Glucose	CA	NR	0.0986/50-1000
3	GOx	Glucose	CV	NR	0.348/50-900
4	GOx	Glucose	AM	NR	22/45- 50,000
4	G. oxydan	Glucose	AM	NR	81/190-50,000

№	Additional specie	Analyte	Detection method	Sensitivity	Linear range
5	GOx	Glucose	AM	105.12	2.88/25-1,000
6	GOx	Glucose	AM	65.44	12.8/5-700
7	—	Dopamine	EIS	NR	0.3/7.8-125
7	—	Sialic acid	EIS	NR	1p/10-10^6
8	Acetylcholinesterase - choline oxidase, AuNP	Acetylcholine	CA	NR	0.6/0.7-1,500
9	Anti-IL-1β antibodie	Interleukin 1ß	EIS	NR	3×10^{-6} /$1\times0.003\times10^{-5}$
10	GOx	Glucose	CA/EIS	NR	10/10-10000
10	Lactate oxidas	Lactate	CA	NR	10/10-1000
11a	—	Sodium ions	CA/CV/SS	37	20/10-10^6
11b	—	Potassium ions	CA/CV/SS	49	100/100-10^6
12	GDH, NAD+ 12	Glucose	AM	NR	4.0/10-1000
12	—	Creatine	DPV	0.133	0.2/0.5-900

Optical glucose sensor

An optical glucose sensor is comprised of light source(s), a detector, and an optical transducer that converts the detected light into a measurable electrical signal. There are two modes of operation for an optical sensor, reflection and transmission. For reflection mode, both the light source and the photodetector are located on the same side. In transmission mode, the photodetector is located on one side of the sample and the light source is on the opposite side [11]. An example of optical biosensor is real-time colorimetric biosensor using affordable devices such as LDR, TCS230, and webcam, real-time color detectors have been successfully created [7].

These detectors were able to accurately detect changes in color depending on the concentration of hydrogen peroxide and titanium oxysulfate in test solutions. The TCS230-based detector proved to be the most sensitive, measuring test solutions of 0.1-1.0 mM this color sensor was used to construct a real-time colorimetric glucose biosensor with good linearity and high selectivity to detect glucose in blood samples, The glucose biosensor's performance was comparable to that of commercial glucose biosensors in a study conducted by Maryamsadat shokrekhodaei et al. [13].

A highly sensitive electrochemical biosensor was developed using a Prussian blue-enzyme cascade catalytic system for glucose detection. Coral-like gold micro/nanostructures were formed on carbon cloth electrodes, followed by Prussian blue deposition to enhance electrochemical activity. The biosensor showed a sensitivity of 454.97 μA mM^{-1} cm^{-2} for hydrogen peroxide (H_2O_2) detection, with a linear range of 0.002 mM to 13.97 mM and a detection limit of 0.5 μM. Glucose biosensor was constructed by immobilizing glucose oxidase on the modified electrode, exhibiting a linear response to glucose from 0.05 mM to 3.15 mM. The sensitivity was 70.76 μA mM^{-1} cm^{-2} and the detection limit was 10 μM. The biosensor demonstrated strong resistance to interference from other electroactive materials and showed good reproducibility and long-term stability. It also successfully detected glucose in human serum samples. This biosensor holds promise for various biosensor applications [14].

Electrochemical biosensors

Electrochemical sensors use electrochemical cells to detect analytes through binding or electrochemical reactions at the surface under an applied potential. This potential can be applied using various methods, including amperometry, potentiometry, impedometry, and cyclic

Т. 9. №12. 2023
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voltammetry. Transistor configurations, such as organic electrochemical transistors (OECTs), can amplify signals and improve biosensor sensitivity. Organic electrochemical biosensors can operate in two modes of detection mechanism: chemically modified organic semiconductors and functionalized semiconductors with complex moieties. Chemical synthesis is crucial for accessing novel material properties and functionalities tailored to detecting specific biomolecules. Recent research has focused on synthesizing organic polymers with semiconducting backbones for electrochemical biosensors, particularly conjugated polymers and polymers of semiconducting organic dyes.

In a study conducted by Wang et al. [5] the immobilization of GOx onto a glassy carbon electrode modified with natural sulfide minerals using glutaraldehyde is shown. The resulting biosensor displayed effective HQ-mediated bioelectrocatalytic activities in an air atmosphere. The biosensors exhibited a broad linear range for all four modified electrodes and showed exceptional repeatability, reproducibility, and applicability. This GOx/GA/SFMs/GCE system has immense potential for understanding the bio-interfaces between GOx and SFMs and expanding the application platform for sulfide minerals [15].

Electrochemical paper-based glucose biosensors have gained popularity due to their advantages such as low cost, simplicity of operation, portability, and disposability. While colorimetric mPADs offer qualitative and semi-quantitative analyte determination using simple imaging tools, they have limitations such as low sensitivity and unsuitability for colored samples. In contrast, electrochemical paper-based analytical devices (ePADs) provide quantitative detection with improved sensitivity, selectivity, and the ability to analyze complex matrices. The pioneering work in colorimetric mPADs was done by the Whiteside group from Harvard University, while the Henry group from Colorado State University [11] developed the first ePAD for quantifying glucose, lactate, and uric acid in human serum samples. The ePAD consisted of microfluidic channels created on filter paper using photolithography and an electrochemical cell printed with screen-printing technology. Glucose oxidase, lactate oxidase, and uricase enzymes were selectively entrapped in different zones on the paper to detect their respective analytes. The detection was achieved by measuring the enzymatic by-product H_2O_2 at the working electrode modified with the electrochemical mediator Prussian Blue. The ePAD showed good performance in terms of reproducibility and accuracy compared to control levels and spectrophotochemical methods.

Other ePAD configurations have been developed for point-of-care glucose detection. Some ePADs allow for the direct detection of glucose in untreated whole blood samples without the need for plasma separation protocols. For example, the Henry group designed an ePAD [11] with a commercially available electrochemical sensor and blood separation zones on paper to achieve glucose detection.

The accuracy of this ePAD was evaluated and compared to spectrophotochemical methods, showing negligible variance. Kong et al. [6] simplified the configuration by using commercially available sensors printed on alumina and Whatman chromatography paper. The blood sample solution was loaded onto the paper and allowed to air dry before conducting electrochemical measurements. To improve sustainability, wax printing techniques have been employed to create hydrophobic and hydrophilic zones on ePADs without the use of organic solvents. Cinti et al. [6] utilized a Xerox ColorQube 8570 printer to create a green hydrophobic zone on the paper-based strip for glucose detection. The paper-based strip offered filtering properties, porosity for reagent loading, and the ability for in situ synthesis of Prussian Blue nanoparticles. Glucose detection in blood samples using this ePAD showed good linearity and correlation with commercial glucose strips.

Т. 9. №12. 2023
https://doi.org/10.33619/2414-2948/97

Amperometric biosensors

Amperometry is a method used by biosensors to measure the current flow between electrodes during a redox reaction. One of the most extensively studied amperometric biosensor systems is the glucose biosensor. Glucose oxidase (GOx) catalyzes the reaction of glucose with oxygen to form gluconolactone and hydrogen peroxide in this system. The signal is typically represented as current (ampere) versus glucose concentration, induced by a redox reaction of a mediator or hydrogen peroxide at the working electrode. It is worth noting that most conjugated polymer (CP)-based amperometric biosensors belong to the third generation of biosensors, where the enzyme and mediator are directly immobilized on the transducer. Therefore, signal detection does not rely on the diffusion of reaction products or mediators. Singh et al. and Habermüller et al. [8] have published excellent reviews on PPY-based biosensors and general electron transfer principles, respectively.

A study conducted by Izadyar in 2021 [8]: the research involved analyzing a Nafion/PPMP–GOx-BSA/Au biosensor that uses an amperometric enzyme-based approach. This biosensor is not only cost-effective and simple to use, but it is also highly sensitive, selective, and stable. It can detect a wide range of glucose concentrations, as demonstrated by successful LSV and amperometric measurements. The biosensor responded well to glucose concentrations ranging from 3.1 μM to 13.2 mM [8]. To test the selectivity of the biosensor, they used an experimental solution containing ascorbic acid and citric acid, which are common interferents in physiological samples. Our results showed that the biosensor exhibited excellent selectivity towards glucose monitoring, detecting concentrations ranging from 3.3 μM to 13.0 mM. The studies have also shown that the recombinant enzyme from the corn kernel production system used in this biosensor is a low-cost, reproducible, sensitive, and selective alternative to conventional enzymes like HRP for glucose detection. In the future, we plan to improve the LOD of the PPMP-based biosensor by incorporating nanomaterials and conductive polymers. This will make the biosensor even more effective in measuring various biofluids in real sample use [8].

Wearable biosensors

Wearable sensors have emerged as a significant advancement in sensor technology for diabetic patients. These sensors are designed to monitor glucose levels and provide clinically relevant data for diabetes management [11]. Wearable sensors involve the integration of devices into wearable objects or directly with the body. Initially, rigid electronic devices were developed in the semiconductor electronics industry, but there has been a shift towards the design of stretchable and flexible electronics. These flexible sensors address the need for cost-effective, non-invasive, and self-testing analysis, eliminating the discomfort associated with finger pricks and skin irritation caused by devices like GlucoWatch.

One notable example of an easy-to-wear flexible sensor is a tattoo-based epidermal device described by Wang's group. This device enables interstitial glucose monitoring through reverse iontophoresis, with glucose detection achieved using glucose oxidase and a printed electrode with Prussian Blue-modified ink. However, this method requires external instrumentation for electrochemical signal transduction, which involves various technological challenges such as signal generation, amplification, calibration, and compensation. An alternative approach involves fully integrated wearable flexible systems where all components are consolidated on a flexible printed circuit board. These systems enable the monitoring of various clinical biomarkers, including glucose, lactate, sodium, potassium ions, and skin temperature, using amperometric glucose detection and Prussian blue as an electrochemical mediator.

While sweat sensors face challenges related to sweat collection and extraction of glucose

Т. 9. №12. 2023
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through intact skin using electro-osmotic flow, microneedle sensors offer a solution by enabling analysis in the transdermal compartment with minimal pain. These sensors can be mass-produced at a lower cost and are designed for short-term use before replacement. Commercially available subcutaneous microneedle-based sensors for continuous glucose monitoring include Enlite, G5, and the FreeStyle Libre. Next-generation microneedle sensors provide advantages such as easy insertion and replacement, reduced biofouling effects, larger electrode surface area for improved sensitivity, and the ability to perform multiplexing analyses [17].

In addition to sensors integrated into the skin, another appliable sensor option is contact lenses for continuous glucose monitoring in tear fluid. Contact lens biosensors have utilized fluorescent indicators absorbed into the lens material or embedded photonic crystals in a hydrogel patch for glucose monitoring. Electrochemical sensors on flexible plastic supports have also been developed for attachment onto the eye or insertion into the tear canal. However, accuracy and interference from electroactive species in tear fluid remain challenges. A miniaturized integrated amperometric glucose biosensor integrated into a contact lens was developed to address these issues. This contact lens biosensor demonstrated fast response, high sensitivity, good linearity, and low detection limit for glucose in the tear film [11].

Although the development of contact lens sensors dates back several years, there is still significant interest from major industrial entities, including Novartis in collaboration with Google, who aim to build revolutionary devices that measure glucose levels and assist individuals with diabetes and eye problems.

Conclusion

This review discusses the importance of glucose biosensors in monitoring blood glucose levels for individuals with diabetes. The working principles, advantages, limitations, and future prospects of glucose biosensors are explored. Glucose biosensors offer convenience, rapid results, portability, and continuous monitoring, but accuracy, calibration and maintenance, cost, and signal interference can pose challenges. Ongoing research is focused on improving accuracy, developing non-invasive approaches, integrating with other technologies, and advanced data analytics. With further developments, glucose biosensors have the potential to revolutionize personalized diabetes care and foster better glucose control.

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