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Mini Review

Screen Printed Electrodes in Biosensors and Bioassays. A Review

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Screen-printed electrodes and screen-printed electrochemical sensors (devices containing more electrodes on the same printed platform) are a promising tool in for the construction of portable analytical devices. Various biosensors and bioassays are connected with the screen-printed electrodes respective electrochemical sensors outcoming from the electrodes. The glucose biosensors containing glucose oxidase, devices also called personal glucometers, are the most commercially relevant biosensors based on screen printed electrodes. Nevertheless, the research on this issue is resuming and new devices having practical impact can be expected in the future. This review summarizes basic data about biosensors and similar bioanalytical devices, provides overview of actual literature and disses practical relevance of the methods. Recent papers are cited in this review article.

Keywords: 3D printing; amperometry; assay; bioassay; biosensor; carbon; electrode; gold; print; voltammetry

1. INTRODUCTION

In the current analytical chemistry, progress of materials is a necessary condition for further improvement of measuring methods, lowing of costs and making analysis more available to current praxis. Small and cheap detectors are generally suitable for outside laboratory use and making the devices affordable for unskilled workers. The transfer of technologies into construction of sensors and small analytical devices is wide and various materials like magnetic particles and nanoparticles [1-4], quantum dots [5,6], metal nanoparticles [7-10], and piezoelectric detectors [11-13] can be mentioned. The methods like lateral flow chromatography [14-17], digital camera colorimetry [18-20] and electrochemistry [21-24] may serve as examples readily to be used for routine analyses outside laboratories.

Screen-printed electrodes are one of the emerging technologies that are readily for practical applications in the field of biosensors and bioassays. They are a commercially available platform serving for multiple analytical applications where a part of biological origin plays a substantial role. This review summarizes the basic facts and recent progress in the field of screen-printed electrodes and their application in the current analytical chemistry.

2. SCREEN-PRINTED ELECTRODES: GENERAL PURPOSE AND MANUFACTURING

Screen-printed electrodes gained high popularity in the voltammetric analytical techniques due to simple design allowing integration them into a small measuring devices or flow through cells, low price, easy mass production and acceptable sensitivity to redox active substances. The screen-printed electrodes can be directly used for measuring of redox active compounds. The detection of formaldehyde by screen-printed carbon electrodes and square-wave voltammetry technique can be mentioned [25]. In a paper by Li and coworkers, there were identified three major directions in the use of screen-printed electrodes in environmental analyses: assay of phenolic compounds, heavy metal ions assay, and enzymatic detection of pesticides [26]. Analytical devices based on screen-printed electrodes can be easily integrated into wearable electronic and be used for instance in personalized medicine [27,28] and be a part of an affordable point of care testing [29]. The screen-printed electrodes can be also used outside standard chemical analysis and chemical processes because they are a part of electrotechnology. Manufacturing of solar cells [30-34] or supercapacitors [35] can be mentioned.

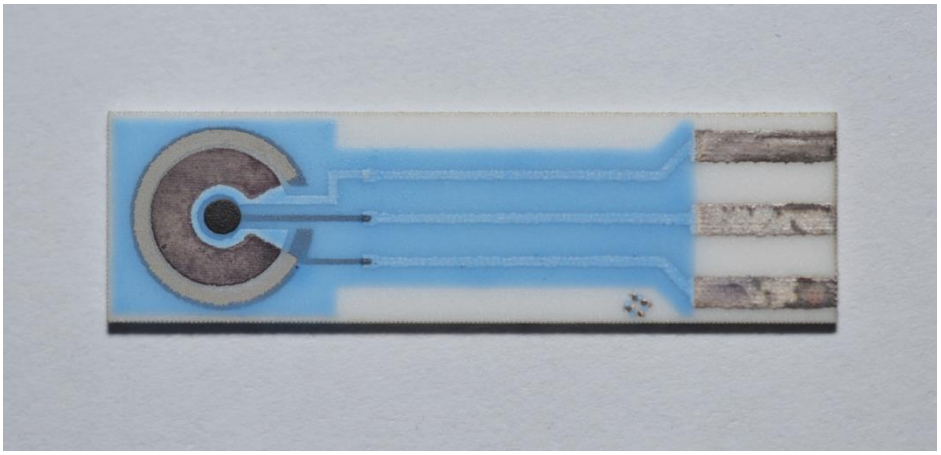

Figure 1. A screen-printed electrochemical sensor containing graphite working, silver chloride reference and silver auxiliary electrode (right side), and silver outputting contacts (left side).

A sensor for an electrochemical assay can be based on a single screen-printed electrode but combination of more electrodes and integration them into a single screen-printed sensor is more common. General appearance of a screen-printed sensor containing three electrodes is depicted in figure 1. The screen-printed electrodes respective sensors are fabricated by a technique called screen printing that is a variant of 3D printing in which the individual layers are consequently deposited and then hardened [36]. Organic polymers or ceramics can serve as a basic substrate for screen printed devices. Material is typically applied in form of an ink (e.g. carbon ink, silver ink), the pattern can be reached by covering of the substrate by a perforated adhesive tape, a template from a solid material etc., the

electrodes are then polished, excess of material is removed and the ink can be hardened by an physical process (desiccation, sintering etc.) [37-40]. The process of an ink application can be repeated. Surfaces of the electrodes can be further improved. For instance, McCormick and coworkers described electrodeposition of a nanoporous platinum film on the surface of carbon electrode on a screen-printed electrochemical sensor [41]. Chloroplatinic acid/copper sulphate solutions and electrodeposition cycle in the voltage range -0.6 up to 1.4 V vs argent chloride electrode were the substrate for modification respective reaction conditions.

3. COMMERCIAL BIOSENSOR AND BIOSENSOR-LIKE DEVICES BASED ON SCREEN-PRINTED ELECTRODES

The screen-printed electrodes respective the whole electrochemical screen-printed devices containing more electrodes together (sensors) serve as a substrate for fabrication of several types of marketed types of biosensors. The screen-printed electrodes are offered by many companies and the current market is quite competitive. Companies like PalmSens (Houten, Netherlands), Metrohm AG (Herisau, Switzerland), BVT Technologies (Brno, Czech Republic), Nano Research Elements (Kurukshetra, Harana, India), Zimmer and Peacock (Horten, Norway). Technologies for screen-printed electrodes and similar devices are not expensive and small workshop is sufficient for accomplish the production process. This is the reason why the sensors and electrodes can be produced by a high number of small companies. Many of the companies (including some of the aforementioned) get offer of biosensors for glucose or pesticides assay beside pure screen-printed electrodes and screen-printed electrochemical sensors.

Biosensor for glucose assay belongs to the top important bioassay devices based on the screen-printed electrodes that were successfully commercialized. The devices serve as personal glucometers in diabetes diagnosis and distinguishing between normal glycemia and hyperglycemia. Appearance of a glucose biosensor can be learned from figure 2.

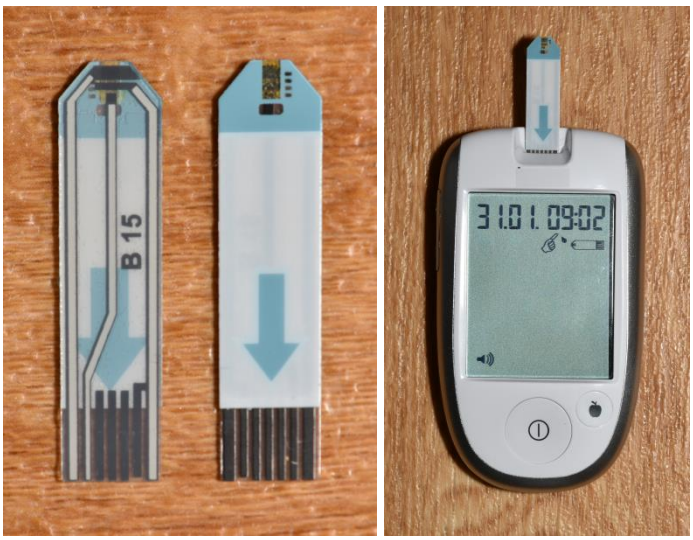

Figure 2. A commercial biosensor – personal glucometer. Left part of figure – the biosensor from top and rear sides. Right part of figure – a biosensor inserted into measuring device.

The glucose biosensor was the first biosensor at all. It was derived from the original oxygen sensor invented by Leland Clark and Champ Lyons [42]. The original sensor measured concentration of diluted oxygen and was suitable for the determination of partial oxygen pressure pO_2 in the blood as a parameter in clinical biochemistry. The further evolution led to immobilization of glucose oxidase and measurement of glucose. The biosensor works on the principle of glucose oxidation to gluconolactone and consequently to gluconic acid. Oxygen solved in the solution is reduced to hydrogen peroxide followed by electrochemical reduction to water or oxidation to oxygen [43]. The common principle of glucose biosensor is depicted in figure 3.

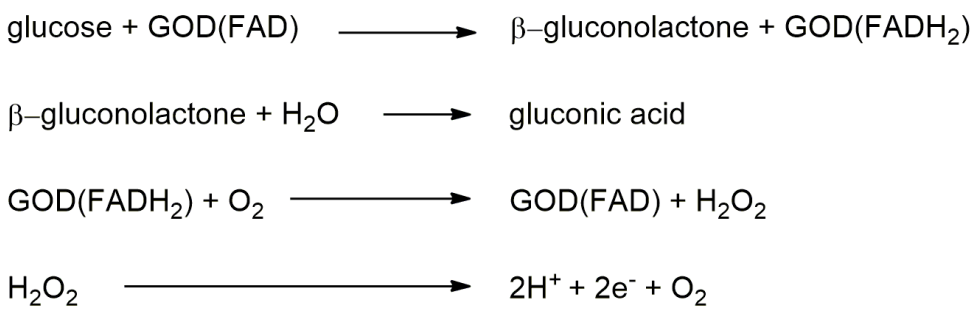

Figure 3. Chemical principle of a glucose biosensor.

Three generations of the biosensors are distinguished in some works. The first electrode places redox reaction through hydrogen peroxide reduction. Voltammetric methods with electrochemically active mediators (e.g. ferrocene) transferring electrons are can be designated as the second generation of biosensors. The third generation enables direct transmission from an glucose oxidase cofactor (FAD, NAD, NADP, PQQ) to the electrode [44]. The research is further focused on development on biosensors that contains chemically made structures like nanoparticles that can replace enzymes and thus make manufacturing of an analytical device better reproducible and simple [45-47]. On the other hand, the abiotic components replacing the biorecognition parts have not been widely adapted into commercial praxis and the glucose oxidases are still a crucial part of personal glucometers.

The original works by Clark and coworkers were not based on screen printed electrodes. Standard metal, graphite, or carbon paste electrodes were recognized as suitable for the glucose biosensor construction. In the further years, screen-printed electrodes and sensors were distinguished as a good platform for the biosensors manufacturing [48,49]. Chemical modification of electrodes by e.g. nanoparticles improving easiness of electron transfer to electrode bare surface is a relevant issue in the current research on glucose biosensors [50-54]. Currently, screen-printed electrodes are the most common platform for glucose biosensors manufacturing.

The glucose biosensors are the major part of overall biosensor market and most of the glucose biosensors are manufactured just on screen printed sensor platform. The overall biosensor market reached 19.2 billion USD in year 2019 and is expected to grow to 31.5 billions USD in 2024, glucose biosensors are the dominant part of the budget [55]. In another prediction, glucose biosensor market size is expected to constitute 31 billions USD in 2022 [56].

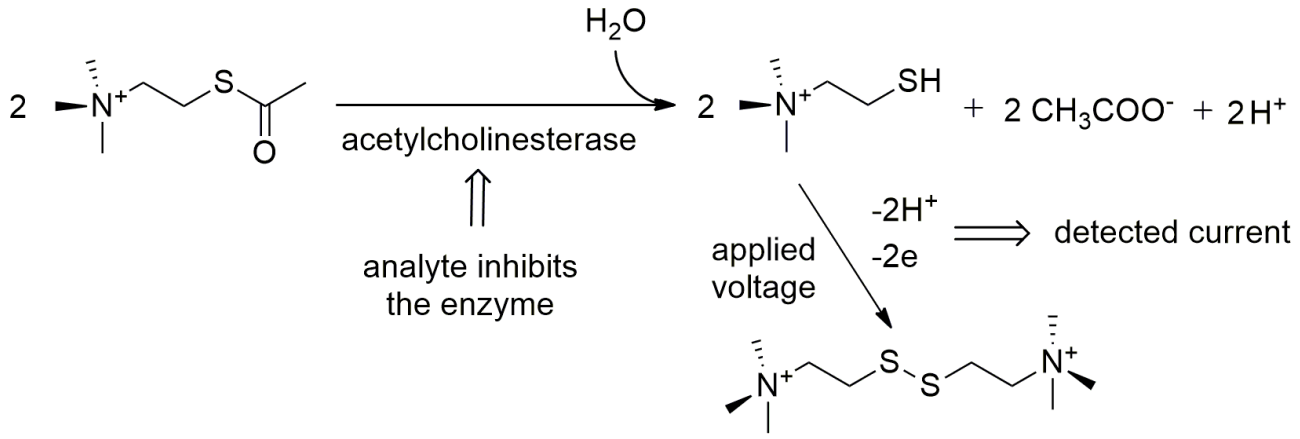

Figure 4. Principle of an acetylcholinesterase voltammetric biosensor.

The other types of biosensor than the personal glucometers (glucose biosensors) are marginal in the current market. On the other hand, there is an application potential of the other types of biosensors and some of them are offered by small producers. The potency to successfully introduce a new type of biosensor can change in the future due to specific demands by laboratories making ecological supervising, change in providing of home care new types of military, fire-fighters and law enforcing institutions and/or due to changes in legislative relating to the aforementioned areas. Biosensors for the detection of pesticides and neurotoxic substances are quite close to general use and they are even marketed by some small companies. Acetylcholinesterase biosensors belongs between devices that are quite reliable and types of these biosensors manufactured on the screen-printed electrodes are available. The acetylcholinesterase biosensors works on the principle of the enzyme inhibition by a wide number of compounds like military nerve agents (sarin, soman, tabun, VX), pesticides (paraoxon, malaoxon), heavy metals (copper, aluminum), drugs (donepezil, rivastigmin, huperzine) and secondary metabolites and natural toxins (caffeine, aflatoxin) and the decrease of enzyme activity is proportional to the inhibitor concentration [20,29,57-60]. The acetylcholinesterase biosensors can work on several types of signal generation, the hydrolysis of redox intact acetylthiocholine to acetic acid and choline by enzyme followed by voltammetric oxidation of thiocholine do the dithiolic form is the major way for the biosensors on screen-printed electrodes. Principle of the assay can be learned from figure 4.

4. RECENT RESEARCH ON THE SCREEN-PRINTED ELECTRODES BASED BIOSENSORS AND BIOASSAYS

Biosensors and bioassays are extensively researched by many investigators and the results seems to be promising for the next practical applications. There are common presumptions making the screen-printed electrodes-based biosensors and bioassays an ideal outcome for point of care testing, field assays of pollutants, forensic examinations etc. Overview of analytical devices based on screen-printed electrodes and screen-printed electrochemical sensors introduced in this chapter can be learned from table 1.

Table 1. Overview of current research on biosensors based on screen-printed electrodes

Type of device	*Analyte*	*Principle*	*Specifications*	*Citation*
screen-printed electrodes with electrospray deposited lacase	phenolic compounds, tested on catechol	amperometric detection	linear range 2.5 – 50 µmol/l, limit of detection equal to 2 µmol/l, biosensor stable for at least 25 measuring cycles and 3 months	[61]
screen-printed carbon electrodes modified with with gold nanoparticles – poly(amidoamine) dendrimer nanocomposite and antibody against tau protein	tau protein – diagnosis of Alzheimer disease and associated neurodegenerative disorders	sandwich immunoassay of tau protein based on immobilized primary antibody and applied secondary antibody labelled with peroxidase, hydrogen peroxide caused measured signal at applied potential -200 mV vs argent chloride reference electrode in the presence of hydroquinone	limit of detection for tau equal to 1.7 pg/ml, applicability for blood plasma and brain homogenate samples	[62]
screen-printed gold electrodes covered by synthetic glycosylphosphatidylinositol anchored receptors	anti-glycan antibodies of isotype IgG and IgM – diagnosis of toxoplasmosis	the receptor interacted with the specific antibodies and the interaction was recorded by electrochemical impedance spectroscopy	detection of antibodies in a range 1.0 – 10 IU/ml, limit of detection 0.31 IU/ml	[63]

screen-printed electrodes covered with cobalt oxide nanoparticles and single-chain antibody fragments specific to cocaine	cocaine in blood serum, sweat, urine, and saliva	differential pulse voltammetry, cyclic voltammetry, and electrochemical impedance voltammetry	linearity from 5 to 250 ng/ml and limit of detection for cocaine equal to 3.6 ng/ml	[64]
screen printed electrodes with immobilized antibodies	*Listeria monocytogenes* p60 protein	The protein p60 was captured by an immobilized antibody and sandwich was formed using a secondary antibody labelled with alkaline phosphatase, 3-inodoxyl phosphate and silver ions served as substrate for the enzyme phosphatase, deposited silver was measured by voltammetry	limit of detection 1.5 ng/ml and limit of quantification 5.1 ng/ml	[65]
graphene oxide screen-printed electrodes covered with deactivated Cas9 protein and synthetic RNA	circulating tumor DNA fragments long 120 bases pairs	label-free impedimetric assay	limit of detection 0.65 nmol/l and limit of quantification 1.92 nmol/l	[66]
magnetic microbeads modified with a biotinylated a double stranded DNA fragments and screen-printed carbon electrodes	autoantibodies against DNA presented in blood serum – rheumatoid arthritis diagnosis	modified magnetic microbeads interacted with autoantibodies and then a secondary antibodies labeled with horseradish peroxidase, activity of enzyme	limit of detection 0.3 IU/ml, linear calibration in the range from 1 to 200 IU/ml	[67]

		was measured by amperometry on screen-printed carbon electrodes in the presence of hydrogen peroxide / hydroquinone		

Biosensor with enzyme laccase immobilized by electrospray deposition on screen-printed electrodes was constructed by Castrovilli and coworkers [61]. The biosensor was suitable for the amperometric detection of phenolic compounds and tested for catechol that caused linear signal response of the biosensor in the range 2.5 – 50 µmol/l and it was proved with limit of detection equal to 2 µmol/l. The biosensor was stable for at least 25 repeated measuring cycles and/or storage for 3 months. An immunosensor for tau protein determination was developed by Razzino and coworkers [62]. Tau protein is a marker of pathologies associated with Alzheimer disease and some other neurodegenerative disorders. In this work, screen-printed carbon electrodes were modified with gold nanoparticles – poly(amidoamine) dendrimer nanocomposite bound to electrografted p-aminobenzoic acid and an antibody was finally attached by crosslinking with glutaraldehyde. The assay was finalized by capturing of tau protein between the immobilized antibody and a secondary antibody labelled with horseradish peroxidase. Just the increase of peroxidase activity was electrochemically measured, hydrogen peroxide caused signal at applied potential -200 mV vs argent chloride reference electrode in the presence of hydroquinone. The assay exerted good selectivity and limit of detection for tau equal to 1.7 pg/ml. The authors claimed applicability for homogenates of brain tissue and blood plasma samples. Screen-printed gold electrodes served as a platform for the detection of anti-glycan antibodies of isotype IgG and IgM [63]. The detection of antibodies can serve for diagnosis of toxoplasmosis, a disease caused by protozoan parasite *Toxoplasma gondii*. The biosensor used synthetic glycosylphosphatidylinositol anchored receptor immobilized on the aforementioned electrodes. The receptor interacted with the specific antibodies and the interaction was recorded by electrochemical impedance spectroscopy. Assay by this biosensor was suitable for detection of antibodies in a range 1.0 – 10 IU/ml and had limit of detection 0.31 IU/ml.

An immunosensor containing screen-printed electrodes covered with cobalt oxide nanoparticles and single-chain antibody fragments specific to cocaine was prepared and published by Sanli and coworkers [64]. The biosensor was performed on principle of differential pulse voltammetry, cyclic voltammetry, and electrochemical impedance voltammetry. The biosensor was interacting with cocaine contained in the samples like blood serum, sweat, urine, and saliva. The assay had linearity from 5 to 250 ng/ml and limit of detection equal to 3.6 ng/ml. In another study, screen printed electrodes with monoclonal and polyclonal antibodies served as a tool for detection of *Listeria monocytogenes* p60 protein [65]. The protein p60 was captured by an immobilized antibody on the biosensor surface and secondary antibody labelled with alkaline phosphatase formed the final sandwich. A mixture composing from 3-inodoxyl phosphate and silver ions served as a substrate for alkaline phosphatase the assay worked on the principle of voltammetric stripping of the enzymatically deposited silver. The biosensor

exerted limit of detection 1.5 ng/ml and limit of quantification 5.1 ng/ml for the protein p60 in an assay lasting up to three hours.

Graphene oxide screen-printed electrodes covered with deactivated Cas9 protein and synthetic RNA was introduced as a tool for the detection of circulating tumor DNA [66]. Fragments of DNA long 120 bases pairs interacted with the biosensor surface and the interaction was measured by label-free impedimetry. Limit of detection for the assay was equal to 0.65 nmol/l and limit of quantification to 1.92 nmol/l. An electrochemical diagnostical tool for rheumatoid arthritis was prepared by Arevalo and coworkers [67]. The researchers synthesized magnetic microbeads modified with a biotinylated a double stranded DNA fragments which were later recognized by autoantibodies presented in sera of patients with rheumatoid arthritis. When the autoantibodies become attached to the particles surface, secondary antibodies labeled with horseradish peroxidase were added, the particles were attracted to the surface of a screen-printed carbon electrodes and activity of peroxidase was amperometrically measured in the presence of hydrogen peroxide / hydroquinone system. Limit of detection of the assay was equal to 0.3 IU/ml and the assay had linear calibration in the range from 1 to 200 IU/ml. Research on the analytical applications where screen-printed electrodes play a significant role is further ongoing and other papers on the issue can be found in the current literature [68-75].

5. CONCLUSIONS

The field of screen-printed electrodes and screen-printed sensors is progressively developing and having significant impact into praxis. Apart of the already commercialized glucose biosensors, some other biosensor and bioanalytical devices are already offered by small companies and the progress in the research on analytical applications make them highly competitive to the other instrumental tools in the analytical chemistry. The biosensors and bioanalytical devices with screen printed electrodes respective sensor platforms are expected to gain practical role in point of care testing, field assays of pollutants, forensic examinations etc. The biosensors and bioassays will not replace standard instrumental techniques but they rather support them and provide simple analytical methods for the immediate, affordable and generally available performance.

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