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Mini Review

Biosensors Based on Semiconductors, a Review

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Semiconductors are important materials in the current technology where they are implemented to electronic devices as attenuators but they can play also a role as sensors. In the current review, semiconductors are presented as a platform for the construction of biosensors and biosensor like devices serving as detectors in the analytical chemistry. Biosensors using ion sensitive field effect transistors, light addressable potentiometric sensors and analytical devices based on quantum dots are presented here. Survey of the current literature is provided and discussion about each analytical method is given here. Future trends in the biosensors based on semiconductors are also outlined.

Keywords: biosensor; immunosensor; ion sensitive; field effect transistor; light addressable potentiometric sensor; potentiometry; quantum dot

1. INTRODUCTION

Bio-electronic-transducers for biosensors construction are typically based on the electrochemical, optical, electronic, piezoelectric or pyroelectric principles. Common examples of commercial biosensors are e.g. piezoelectric biosensor that uses crystals to make an electrical potential by elastic deformations. These concepts have many configurations that could be used as sensors in frequency spectroscopy and analyses due to perfect correlations between characteristic frequency of crystals, that is very stable and depending upon material of biological elements in which the system handle the frequency [1,2]. These biological elements exhibit relationship between changes of characteristics frequency under stress of biological acting on the characteristic-frequency of crystal. The methods of these piezoelectric biosensors are based on the classical approach of frequencies analyses to finding the change in the frequency. The frequency analyses are able to give fair and accurate results of what happened with characteristic-frequency of crystal. The group of

electrochemical-biosensors is quite similar. The electrochemical-biosensors having electrodes and enzymatic catalysis produce an electrical voltage, where the physical interpretations of biological elements is possible by using methods of an electrical engineering. These analyses by the electrochemical biosensors are based on measuring of electrical voltage or currents, or inductances [3]. It may be easily performed a test by measuring of the electromotive force in volts to analyze the concentration of biological elements. For some biosensors, the concept can be set at using optical-bio-elements, which are able to exhibit the changes in absorbance or fluorescence to detect any change through the biological elements. It is evident that biosensors have growing popularity in the number of applications. Their electrical characteristics are needed to set the semiconductor biosensors-devices in order to apply its internal effects to an electrical behavior versus biological elements. They are small physically a relatively inexpensive, for the most of the values required.

This review is focused on biosensors having semiconductor as a crucial part of their body or assay procedure. Basic types of semiconductors in the biosensors and biosensor like devices are introduced. Not only principles but also pros and cons of the assays are discussed and growing interest in the field of these biosensors is outlined.

2. THE PRINCIPLE AND TECHNOLOGICAL USE OF SEMICONDUCTORS

In the recent years, the field of biosensors has experienced a large growth due to technology of semiconductive materials [4]. Biosensors based on a semiconductor can be indicated as one of the fastest developing sensor-technologies. The recent development started in the 1970s with the development of FET sensors (field-effects-transistors) based on the interaction of biological or chemical elements directly connected to the electric field [5]. These semiconductors approaches may be employed to use potential differences where the force between charges in the electric field is very effectively and sensitively distributed. As an example, ion-sensitive field-effects- transistors (ISFET), where the number of free electrons that can be forces to drift through the sensitive material depends upon the amount of potential difference across the element. A larger amount of charge moving with the same speed means a higher value of the measured current. The doping elements, for example affected by biological elements, are able to change the amount of potential difference across the sensor-sensitive-material to control the current between source and drain of transistors junction. Fundamental advances in semiconductor materials have led to the development of such biosensors to achieve very sensitive biological sensors. Moreover, these advances in semiconductors technology have made it possible to significantly improve the wide-using capabilities of the biological engineering [6,7]. At the same time, the market for biosensors based on the electronics material with include the signal processing has significantly expanded.

Semiconductors are irreplaceable materials in the current technology. The main feature is that: the resistance of junction can be variable and controlled. FET devices are a semiconductors amplifiers with the same function as a junction transistors (NPN or PNP), but the FET provides a very high input resistance and the FET can take volts for the input circuits and the charge carriers in the channel have one polarity [8]. The insulated gate FET consists of a metal electrode separated from the channel by a

thin layer of silicon dioxide and so the input resistances are very high and independent of the polarity of the gate-voltage. In practice, it is found that the technology is very universal to develop a number of biosensors and become more important. The FET function depends on controlling current through a semiconductors channel, which is practically anode and cathode [9,10]. Either N channel or P channel can be used and substrate material must be neutral or lightly doped [11]. When voltage (V_{DS}) is applied between the drain (D) and the source (S) at the opposite ends of the channel, the current (I_D) through the channel flows from the source to the drain [12]. The value of current can be controlled or managed by the value of the voltage of the gate (V_G). Source is the terminal where the charge carriers enter the channel bar to provide current through the channel and drain is the terminal where the current leaves the channel. For the technology of ISFET, gate is probably the most important part where it is possible to control the conductance of the channel between the source and the drain [13,14]. The input voltage controls the electric field in the channel [15]. It is evident that the gate and channel works like a capacitors and a charge of one polarity on the gate induces an equal and opposite charge in the channel. The conductivity of the channel can be increased or decreased by the gate-voltage. The schematic diagram of ISFET principle and typical output characteristics can be seem in figure 1.

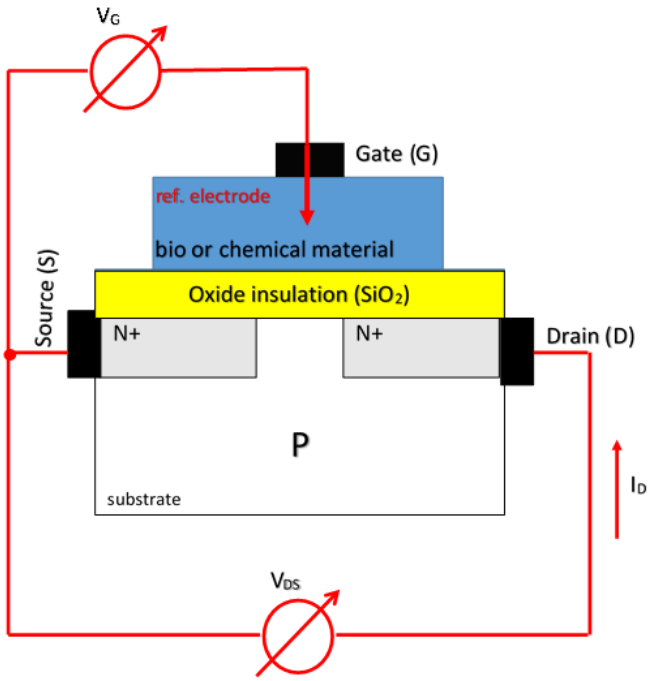

Figure 1. ISFET - Schematic Diagram of ISFET

It is evident from the previous the function of the gate is highly important. Input signals are applied to the gate, with amplified output from the drain. The input is like two plates of a high-quality capacitor resulting in the conductivity of the channel which can be increased or decreased by the gate voltage. The drain current through the ISFET depends on the applied gate voltage, as shown by the transfer characteristics in figure 2.

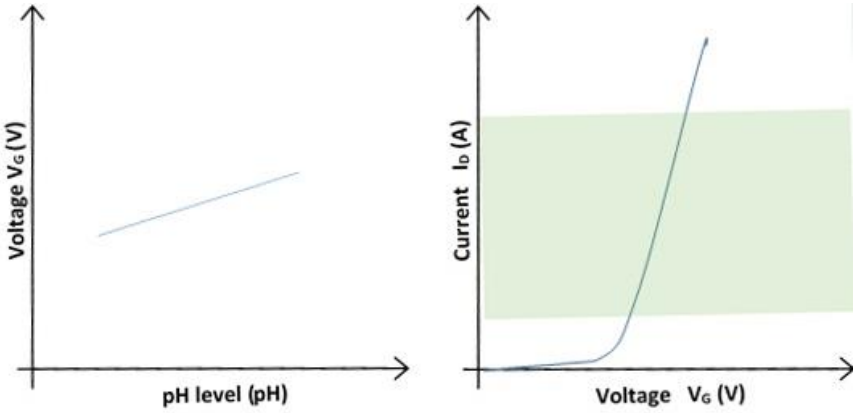

Figure 2. pH Sensors Characteristics versus Gate-Voltage V_G(V)

Advantages of ISFET are: small size, robust and fast response. The next advantage of FET that there are less sensitive to temperature, X-rays and cosmic radiations, which can produce others kind of electronic semiconductor transistors. There are a number of different applications to use, such as: medical, agriculture, food industry or environment monitoring.

3. ION SENSITIVE FIELD EFFECT TRANSISTOR BIOSENSORS

Due to their principle, ISFET are naturally suitable for the direct detection of ions and ISFET based pH electrodes are accessible in the current market. Biosensors being based on the ISFETs can be quite simple devices in their principle and they can use enzymes, antibodies etc. as biological origin part [16-18]. There are also known applications where interactions between macromolecules are recorded by ISFET and the whole assay is constructed as a label free [19] or the affinity interactions can be monitored via ISFET due to a proper labeling and the whole device is such a better analogy to standard tests like enzyme-linked immunosorbent assay [20].

Some promising applications based on ISFET were proposed in the past with prevalent measurement of pH as an parameter altered with the concentration of analyte [21]. An ISFET immunosensor was developed for the detection of antigen 85 complex B which is a marker of tuberculosis causative agent: *Mycobacterium tuberculosis* [22]. The researchers modified surface of ISFET by 3-aminopropyltriethocysilane then activated by glutaraldehyde and finally covered with a monoclonal antibody against the antigen 85 complex B. The whole assay worked in a label free manner and the interaction between antigen and antibody was followed by a change in ions distribution towards original silicon nitride surface of the gate. The authors were able to detect the antigen from an amount 0.12 µg/ml. Affinity interaction concept was also adopted by Muanqsuwan and coworkers who developed and ISFET based biosensors for the detection of biotinylated products of polymerase chain reaction [23]. Prior to assay, surface of the ISFET was covered with protein A and then anti-biotin antibody. The assay served as a tool for the measurement of the biotinylated chain reaction products of *phaE* gene of *Haloquadratum walsbyi* DSM 16854 which was determined with limit of detection

125 fg. The assay was based on the fact that interaction with analyte causes gate voltage shift which is easily measurable.

ISFETs can be easily combined with enzymes where the enzyme works as catalyzator converting a compound detectable by ISFET into the non-detectable or a non-detectable to detectable. The fact that such analytical devices can be used repeatedly is an advantage; moreover, the platform can be combined with various enzymes [24]. Phosphotriesterase is one of the promising enzymes combinable with ISFET. Adrianova and coworkers immobilized phosphotriesterase on an ISFET platform and used it for the detection of neurotoxic compounds paraoxon and parathion [25]. They were able to detect as low as 0.1 µmol/l of paraoxon and 0.5 µmol/l of parathion without any pretreatment of samples and the authors also reported good stability of the immobilized enzyme as at least one month old biosensors were still fully usable. Principle of the assay is based on a fast hydrolysis of the analytes by enzyme which causes release of acids from phosphoesters. The enzyme phosphotriesterase is able to hydrolyze many organophosphorus compounds including highly neurotoxic nerve agents and pesticides [26-28].

Table 1. The analytical applications of ISFET biosensors

Principle of the assay	Analyte	Reported limit of detection	References
Label free assay detecting interaction between antigen and immobilized antibody providing change in ions distribution	antigen 85 complex B – a marker of tuberculosis	0.12 µg/ml	[22]
Label free assay where immobilized antibody interacts with biotinylated analyte causing gate voltage shift	biotinylated chain reaction products of phaE gene of Haloquadratum walsbyi DSM 16854	125 fg	[23]
Hydrolysis of the organophosphorus compounds causes acidification of medium, pH shift is measured	organophosphorus compounds represented by paraoxon and parathion	0.1 µmol/l paraoxon and 0.5 µmol/l parathion	[25]
Oxidation of glucose to gluconic acid followed by recording of pH decrease using ISFET	glucose	360 mg/dl	[39]

The idea was also adopted in other works where phosphotriesterase took place as a biorecognition element on an ISFET sensor [25,29-32]. The ISFETS are also intended as tools for assay of blood markers common in the clinical biochemistry. Combination of uricase and catalase is for instance suitable for the determination of uric acid [33,34]. Glucose is another marker measurable on the ISFET platform [35,36]. The common biosensors for glucose use amperometric principle based on redox reaction of glucose oxidation or oxygen and hydrogen peroxide reduction [37,38]. However, the formation of gluconic acid from glucose gives the opportunity to employ the ISFET pH sensor because the gluconic acid causes acidification of medium while glucose has no impact on it. Liao and coworkers prepared such biosensor for glucose assay [39]. The authors reported sensitivity of their assay equal to 56.4 mV/pH and limit of detection 360 mg/dl (20 mmol/l). Though the limit of detection appears to be above standard glycaemia, the assay can be further developed and improved in order to be clinically applicable in the future.

In another application dissimilar to the aforementioned biosensors, ISFET was performed as an detector tool for study of pH changes at mammalians cells surface [40]. This method has broad application in the field of cellular regulation mechanisms, pharmacology research of drugs depolarizing membranes etc. Examples of analytical methods based on ISFET are given in table 1.

The ISFET biosensors can be designated as simple but reliable analytical devices with good applicability. Though the concept of their construction can appear as very promising, there is also one significant drawback giving an important disadvantage. The most of ISFET biosensors works on the principle that pH is changed. It means that buffering of medium or even use of sample with high buffering capacity (blood, blood plasma etc.) is poorly usable.

4. BIOSENSORS BASED ON SEMICONDUCTORS WITH OPTICAL PROPERTIES

Two major types of devices are based on semiconductors and they also exert unique optical properties: Light Addressable Potentiometric Sensors (LAPS) and quantum dots. LAPS are a special type of ISFET having standardly deposited layers on the material like the other ISFETs: silicon chip is covered with silicon oxide and silicon nitride. The final layer is composed from a membrane or any similar structure containing a biorecognition element of the biosensor. Comparing to standard ISFETs, signal creation is not initiated through gate but there is excitation by a light emitting diode. Particular region involved in the assay is given by light beam hence multiple regions or more analytes can be measured by one sensor when it contains disparate zones of biorecognition elements under different diodes. Without modification by a biorecognition element, the LAPS can work similarly to ISFET as a pH sensor or any other ion selective sensor. A LAPS based pH sensing was for instance depicted in quoted paper [41].

The multi spot analysis is suitable for characterization of multiple targets in the same moment as presented in studies by Wu and coworkers [42,43] and Zong and coworkers [44]. The LAPS platform is also suitable for a non-invasive study of living cells and cytotoxic drugs can be investigated this way [45-48]. There are also applications for detection of specific chemical substances using LAPS. Penicillin is one of the compounds [49].

Table 2. The analytical applications of ISFET biosensors and quantum dots

Principle of the assay	Analyte	Reported limit of detection	References
Conversion of penicillin by enzyme penicillinase immobilized on a LAPS sensor	penicillin	0.1 mmol/l	[49]
Escherichia coli WP2 is not able to grow without trypthophan, presence of tryptophan causes growth followed by acidification of medium which is measured by LAPS	trypthophan	approximately 3 µmol/l	[50]
The assay was based on graphene quantum dots which were quenched by peroxidase, ascorbic acid stopped the quenching	ascorbic acid	0.32 µmol/l	[51]
graphene quantum dots covered with molecularly imprinted polymer interacted with cytochrome c which caused quenching of fluorescence	cytochrome c	0.11 µmol/l	[52]
antibody against cardiac troponin I was immobilized on CdAgTe quantum dots and photocurrent was measured after interaction with the troponin	cardiac troponin I	1.76 pg/ml	[53]

In the application, enzyme penicillinase was immobilized on a LAPS and conversion of penicillin was recorded when it was presented in a concentration range from 0.1 mmol/l to 10 mmol/l. Assay of tryptophan is another adaptation of LAPS for specific compounds [50]. The assay used an auxotrophic mutant of Escherichia coli (WP2 strain) requiring tryptophan. Metabolism of the microorganism was recorded just by LAPS and the metabolic activity increased when tryptophan became accessible from medium. The method exerted linearity for tryptophan from zero up to 12 µmol/l. The authors did not present limit of detection for the method but it appears around 3 µmol/l when considered calibration curve. Short survey of biosensors and analytical methods is presented in table 2.

Quantum dots are specific nanoparticles containing semiconductor material having spherical shape. The semiconductive properties can be acquired due to final size confinement and even graphite can serve as a material for the dots construction. The quantum dots were discovered in 1980s by Alexey Ekimov and they have gained broad application potential since this time. Sulfides and selenides like CdS, ZnS, ZnSe, CdSe, MoS_2 [54-58], tellurides like CdTe, ZnTe [55,59], carbon, graphenes and graphene oxides [60-63] and silicon [64] can be exampled as materials suitable for the dots manufacturing. Quantum dots have properties which are uncommon when compared to the other nanomaterials. Apart of the semiconductive properties, the quantum dots exerts unique optical properties as they are able to emit light with high quantum yield and spectrum of the emitted light depends on particle size [65]. It means that material with the same chemical composition emits light color depending on size, while small quantum dots emits typically light with short wavelength, increasing diameter of particles causes shift of light to longer wavelengths [66]. Toxicity is a disadvantage of quantum dots. Heavy metal containing quantum dots like CdS or CdSe are highly effective for quantum yields and easy for production but they are also toxic due to content of cadmium. However, the quantum dots from non-toxic materials can be harmful too therefore there is an effort to discover biologically compatible quantum dots having retaining good properties including price of production and low toxicity [67,68].

Quantum dots found use in many practical applications including the use in analytical chemistry where it can be easily performed like a reagent or label. For instance, quantum dots can be linked to antibodies as label in a method resembling standard enzyme-linked immunosorbent assay [69] or used as a reagent in the determination of antioxidants where antioxidant protects quantum dots from quenching by a pro-oxidative reagent and fluorescence intensity is proportional to the concentration of tested antioxidant [70].

Biosensors can be established on the quantum dots platform and there are promising applications deserving attention. Liu and coworkers constructed a biosensor based on graphene quantum dots emitting orange light for assay of ascorbic acid [51]. The assay was based on presence of horseradish peroxidase as a biorecognition element and hydrogen peroxide as a reagent. When the tested sample contained no ascorbic acid, the peroxidase caused quenching of graphene quantum dots while ascorbic acid protected the quantum dots. It was able to detect ascorbic acid in a linear range 1.11 – 300 µmol/l and limit of detection was equal to 0.32 µmol/l. In another application, cytochrome c was detected by graphene quantum dots covered with molecularly imprinted polymer and worked on a principle of fluorescence quenching [52]. The described assay exerted linear range 0.20 – 60 µmol/l

with limit of detection 0.11 µmol/l. Quantum dots can be also linked to an antibody like presented Tan and coworkers [53]. They immobilized antibody against cardiac troponin I on CdAgTe quantum dots and measured photocurrent before and after interaction with sample containing troponin. The authors found proportionality between troponin in concentration 5.0 – 20 ng/ml and photocurrent. Limit of detection for the method was equal to 1.76 pg/ml. Glucose was another analyte assayed by a quantum dot sensor [71]. The researchers chosen graphene quantum dots covered by phenylboronic acid as a nonenzymatic catalyzer for glucose and they were able to measure glucose with linear range of concentrations from 4 to 40 mmol/l and they found limit of detection 3 mmol/l.

4. CONCLUSION

Semiconductors are promising material in the field of analytical chemistry and there are applications of the semiconductor materials for construction of biosensors. We can expect growing interest in this field because of the electro-technologies price reduction and emerging of new technologies and materials [72,73]. The semiconductor devices are also miniaturized which get opportunity to easily implement them as a nanotechnology.

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