Trends in Micromachined Glucose Sensor Research and Development

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Abstract: This paper describes the trends in glucose microsensor research and development. Micromachining techniques have been applied to construct glucose sensors. Several detection methods and their modification through last 15 years are investigated. We begin with sensors which are based on photometric characteristics and continue with glucose sensitive FET. Most of the sensors use amperometric method with enzyme selector and some sensors apply new methods such as electro microbalance or luminescence measurements. Finally we describe MIP method for material detection that has several advantages over other methods such as high sensitivity, reproducibility and durability because of its enzyme free structure. Design of our microsensor is based on MIP detection method.

Keywords: microsensor, micromachining, glucose, MIP

1 Introduction

In the last 15 years micro fabrication techniques based on IC technology, such as photolithography and etching, have been applied to other fields. These techniques which are used to fabricate some small and efficient three-dimensional devices are called micromachining. Furthermore, some studies to make miniaturized chemical analysis systems have already been reported. These systems have many advantages, such as fast response, small amount of sample, low consumption of reagent and lower price as compared with the conventional macro sensors.

Glucose sensor has a major role in the microsensors development process because of the continuous and critical need for glucose measurement in the diabetic patients. Painless and cost effective measurement of the glucose level always was a desirable goal for diabetic patients. Some methods such as implantable photometric device or channel type sensors[1] were developed for continuously measurement of blood glucose level. However they have not completely complied requirements because of many disadvantages. One problem is power supply consumption which needs continuously changing the supply, another problem is antibody reactions which influence sensor responsibility and decrease its performance. However, development of an artificial pancreas is the next target for the most researches, in which a glucose microsensor and a micro pump construct a complete system that can really answer the human requirement.

This paper reviews previous efforts and evolution up to now with present a new design for sensitive and cost effective structure in which the MIP (molecularly imprinted polymers) method is used for glucose detection. The fabrication of proposed sensor is based on the micromachining techniques.

2 Glucose micro sensors

There are five types of the glucose micro sensors. A brief description of each is provided at the following sections.

2.1 Photometric sensors

These sensors use spectroscopic methods to measure the glucose concentration. Each molecular species exhibits a unique optical signature. In theory, by probing a substance with light and by accurately measuring the resultant spectrum one
can identify and determine the concentration of each chemical constituent.

One type of the sensors measure the near-infrared absorption of blood [2]. The sensor can be implanted across a vein with readings transmitted via radio waves (RF telemetry) to a small display unit worn on the wrist. Hence, there will be no percutaneous (through the skin) wires. The Display Unit will be about the size of a wrist-watch and the implanted sensor about the size of a pager. Accuracy of this sensor is better than 15g/dl. But it is not suitable for human blood measurement. Disadvantages of this sensor are: more invasive implantation with high risk, power supply problem, and decreasing the RF signal because tissues encapsulate the sensor, lowering its performance.

Another approach to optical glucose sensing is based on the fact that glucose solutions have a magnetic optical rotatory effect (MORE) [3]. such that when a magnetic field is set up in a glucose solution there is a rotation of the polarization vector of the incident light that is proportional to the path length, magnetic field strength, and the concentration of glucose in the solution. The proportionality with the glucose concentration was found to be linear with a negative slope of -0.242 mV/(mg/dl) at physiologic concentrations. The first precision optical polarimeter using the Faraday Effect was introduced by Gillham in 1957. Rabinovitch and March introduced the concept of using the aqueous humor glucose as a detector of the blood glucose concentration by measuring polarization rotation.

2.2 Amperometric sensors

The amperometric glucose sensors of the first generation use glucose oxidase (GOD) to catalyze the oxidation of glucose:

\[
\text{Glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2
\]  

(1)

Hydrogen peroxide produced in this reaction is oxidized at the working electrode (anode):

\[
H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- 
\]  

(2)

This reaction produces a current proportional to the glucose concentration in the solution.

A miniaturized needle-type glucose microsensor has been developed for subcutaneous glucose monitoring by Bindra D.S. [4]. The sensor is equivalent in shape and size to a 26-gauge needle (0.45-mm o.d.) and can be implanted with ease without any incision. The upper limit of linear range greater than 15 mM, response time less than 5 min, and sensitivity yielding a 5:1 signal-to-background ratio at normal basal glucose levels. The sensor response is largely independent of oxygen tension in the normal physiological range. The big disadvantage of this sensor is its long response time.

Another amperometric sensor has been developed by M. Koudelka-Hep [5] using immobilization of thin layer of enzyme over a three-thin film electrode transducer. Overall dimensions of the sensor were $0.55 \times 3 \times 0.38 \text{mm}^3$, with linear range up to 18mM, sensitivity of $2.5 \pm 0.9 \text{nA/mM}$ and response time for 95% about $180 \pm 4 \text{s}$ at 18mM.

As we see, in spite of the good linearity range, its sensitivity and response time are not reliable.

A new micro glucose sensor has been developed by Joon-Ho Kim [6] which monolithically integrates enzymatic metal microelectrode array in a micromachined chamber with a microsyringe for blood analysis. The calibration of the microbiosensor (Fig. 1) has confirmed its response characteristics in the glucose concentration range from 0 to 20 mM with the maximum sensitivity of $470 \text{nA/cm}^2 \text{mM}$.

![Fig.1: calibration curve](image)

These characteristics have demonstrated that the fabricated microbiosensors are suitable for the diagnosis of diabetic patients whose glucose level is higher than 8mM. This sensor has been fabricated using Multi-exposure-single-development (MESD) method in which they could pattern a 3 dimensional photoresist mold for metal electroplating. Fig.3 shows the overall sensor structure.

The major disadvantages of this sensor are low output current which makes it difficult to measure the current, another problem same as previous amperometric sensors is the enzyme dependency that decreases the sensor durability and responsibility over the time.
2.3 pH sensitive sensors

Normally glucose oxidase hydrolyzes glucose according to the following reactions:

\[
\beta - D - \text{glucose} + O_2 \rightarrow D - \text{glucose} - \delta - \text{lactone} + H_2O_2
\]

\[
D - \text{glucono-} \delta - \text{lactone} \rightarrow D - \text{gluconate} - H^+ \quad (4)
\]

pH-sensitive sensors measure the glucose concentration by detecting the pH variation due to the hydrogen ions that are generated by the dissociation of gluconic acid. However, because of low dissociation constant (pK$_a$ $\approx$ 3.8) ISFET glucose sensors show low sensitivity.

![Fig. 3: whole blood sensor structure](image)

The first pH sensitive glucose microsensor has been developed by Alexandre A. Shul’ga in 1994 which was a glucose sensitive field effect transistor [7]. The sensor was fabricated by immobilizing glucose oxidase on the gate of a pH-sensitive FET. As we see in the Fig.4, linearity range of the sensor is only 1mM and it is not suitable for human blood concentration measurement. However, it has a good sensitivity in this short range.

Another pH-sensitive glucose sensor was investigated by Yin [8]. It was an ENFET coimmobilized with glucose oxidase and manganese dioxide (MnO$_2$). Fig.5 shows that the sensitivity and linearity range of this sensor is reasonable. The pH value converted to the voltage by the structure shown in Fig.6. The sensor sensitivity is 58.3mV/pH between pH 2 and 10. Response time is about 10 min.

2.4 Electro microbalance sensors

An electrode less piezoelectric quartz crystal (PQC) system was first reported by Nomura [9] in 1991. In these systems usually the PQC has evaporated metal electrodes on either sides of the quartz crystal, by which the excitation electric field is applied to the quartz crystal. In Nomura’s work, one or both electrodes of the normal PQC were dissolved with aqua region, and both sides of the quartz plate were separated with a barrier. The electrode less PQC oscillates in the same way as the normal PQC when the gaps between the excitation electrodes and each side of the quartz plate are filled with the electrolyte solution. The frequency of the electrode less PQC varies with changes of the viscosity, density and conductivity of the solution and the mass of trace material deposited on the quartz plate.

In another type of these sensors glucose is used to reducing of a material such as Cu$^{2+}$ or Ag$^{+}$ then the reduced material deposit on the PQC and decreases its resonance frequency. This work has been done by Si and Huang in 1999[10]. In their work, the electrodeless PQC sensor was used to monitor the silver deposition on the surface of a quartz plate during the reduction of Ag(NH$_3$)$_2^+$ by

![Fig.4: Calibration curve of the glucose ENFET in 10 and 50 mM TRIS and phosphate buffers(pH 7.4, 140 mM NaCl).](image)

![Fig.5: Output voltage vs.pH value](image)
glucose, and a quantitative method was developed for the determination of total reducing sugar in urine. The frequency shifts between the frequency at the third minute and at later times were used to construct a calibration graph for the glucose concentration. For glucose concentration ($C$ in mM) ranging from 1.0 to 25 mM, a satisfactory correlation was obtained between the value of $C$ and the frequency shift ($\Delta f$ in Hz; $\Delta f = f_{min3} - f_{min8}$, where $f_{min3}$ and $f_{min8}$ are the frequencies at the third and eighth minutes after the addition of the glucose solution, respectively):\[ \Delta f = 74.7C + 1058 \quad (n = 9, \ r = 0.998) \] (5)

Responsibility of this sensor decreases with variation in temperature, conductivity and viscosity of the solution.

2.5 MIP based sensors

Molecularly imprinting is recognized as a powerful technique to synthesize polymer-type artificial receptors. Using non-covalent imprinting, there is no restriction to the choice of the analyte. Even small molecules without any functionality can be detected with high selectivity. In this case, the material is usually a polymer. The polymer is prepared by cross-linking a monomer around a ‘template molecule’. This template molecule is removed after the polymerization of the monomer and its size, shape and chemical functions are recorded in the polymer. The sites of the removed template molecule are named ‘imprint sites’. These sites allow the recognition of the template molecule or close structural molecules.

The capacitance transducer is based on the theory of the electrical double-layer. A metal electrode immersed in an electrolyte solution can generally be described as resembling a capacitor in its ability to store charge. The capacitance ($C$) can be described by the equation:

\[ C = \frac{A\varepsilon_0\varepsilon_r}{d} \] (6)

where $\varepsilon_r$ is the dielectric constant of the material between the plates, $\varepsilon_0$ is the dielectric constant for a vacuum, $A$ is the area and $d$ the distance between the plates. The capacitance changes when the distance varies because of adsorption or desorption of material. In most cases, the capacitance is measured at the metal/solution interface in the electrochemical system. Ions and dipoles are ordered outside a metal electrode in such a way that charges in the metal are balanced, thereby forming the electrical double layer. Since capacitive measurements give information about the metal/solution interface, a chemical modification of this structure will lead to a change in capacitance. The degree of variation is determined by the nature and coverage of the modification material.

In 2001 Zhiliang Cheng et al. [11] developed a glucose sensor in which glucose was the template molecule which was polymerized by O-phenylenediamine. Their sensor had a good selectivity for glucose at a linearity range between 0 and 20 mM. In the figures 7 and 8 we can see the impedance and capacitance changes with different glucose concentrations.

In spite of sensitivity and linearity range; the most advantage of this sensor is that enzyme have not been used in its structure. This improves the sensor durability.
Fig.8: The effect of glucose concentration variation on the electrode-solution capacitance.

It should be said that the molecularly imprinted polymers (MIP) method can be used for detection of glucose with the PQC or QCM (quartz crystal microbalance) methods (Malitesta et al., 1999).

3. Our proposed glucose microsensor design

The glucose microsensor which we have considered is based on the MIP method. This type is better in sensitivity, linearity and durability compared to the other types. Our proposed sensor is the micromachined version of the MIP method. The schematic of the sensor structure is shown in Fig.9a. Fig.9b shows a cross section of the sensor, electrodes connected to the output pads through wires. These wires are passivated from silicon substrate by SiO$_2$ layer. The narrow shows the electrical current flow in the chamber through the blood.

The size of the capacitor plate and the pad are about 1mm$^2$ and 1.5mm$^2$ respectively and the total chamber volume is about 30 nlit. Since the required volume of blood for our sensor is less than what is needed by Kim’s work and the syringe sizes are the same therefore the required time to fill of our chamber will be less than 15 seconds. The capacitance variation due to the glucose of the blood is measured by a whetstone bridge as shown in Fig.10. The calculated values for the $C_c$ and $R_c$ in the absence of glucose are about 12nF and 150 KΩ respectively.

If the applied voltage to the bridge is a sinusoidal wave form of 1 V in amplitude and 10 Hz frequency the sensitivity of the output of the bridge is about 9 mV/nF. The output voltage can be amplified and converted to a digital data to be able to show the value of the glucose digitally as shown in the Fig. 11. Applying the Micromachining techniques the bridge and microsensor can be integrated in a single chip.

![Image of Fig.9: a)sensor structure, b) cross section view](image_url)

![Image of Fig.10: Whetstone bridge, as it is seen cell contains a capacitor and a resistor](image_url)

![Image of Fig.11: Sensor with monitoring circuit](image_url)
4 Conclusion

This paper provides a review of different types of the glucose microsensor. A micromachined glucose sensor based on MIP method is also proposed.

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