Amperometric Enzyme-Free Glucose Sensor Based on Electrodeposition of Au Particles on Polyaniline Film Modified Pt Electrode

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Received: 29-03-2018

Abstract

An amperometric non-enzymatic glucose sensor of high sensitivity was developed by modification of Pt electrode with electrodeposition of Au particles on polyaniline film. The Au particles were deposited under optimum growth conditions for maximum dispersion on polymer film to achieve highly sensitive glucose sensor. The characterization of the electrode and the analytical parameters were evaluated using chronoamperometry and cyclic voltammetry techniques. The sensor showed wide linear range from 0.01–8 mM along with swift response time of less than 5 s. Moreover, a high sensitivity of 113.6 µA mM⁻¹ cm⁻² is obtained along with low detection limits of 0.5 µM. The sensor also showed high stability, reproducibility, and repeatability. Furthermore, the sensor has shown high selectivity towards glucose exclusively in the presence of interferents such as sucrose, uric acid, and ascorbic acid. The practical applicability of the proposed sensor was confirmed from successful analysis of glucose in different juice samples and these results were comparable to the commercial glucose meter. Thus, our sensor stands promising non-enzymatic sensor for routine analysis of glucose.

Keywords: Non-enzymatic, Glucose sensor, Polyaniline, Cyclic Voltammetry, Chronoamperometry

1. Introduction

The detection of glucose in not limited to clinical diagnosis but also used in the other fields such as pharmaceutical, food, and biotechnology.¹ The research in the fabrication of glucose sensors is tremendous due to the need of highly stable and sensitive sensors.² The amperometric glucose sensors are either enzymatic or non-enzymatic.³,⁴ The development of former sensors took a leap after development of first enzyme-based sensor in 1962. In spite of the tremendous research in the field of enzyme-based sensors still the need remains for development of highly stable sensors.⁵ One of the reasons is that enzyme loses their activity due to change in the environmental conditions and thereof these sensors are not stable for long term use. While in the case of non-enzymatic sensors, direct electron transfer for oxidation of glucose takes place without the activity of enzymes.⁶ The catalytic behavior in these sensors has been attained by the presence of metals or alloys.

Researchers are focusing on development of an ideal glucose sensor that is highly stable, sensitive, reproducible and has a fast response.⁷ All these analytical parameters can be attained in a single electrode by modification of the existing electrodes with conducting polymer-metal nanoparticles composites.⁸ In such composites, the role of conducting polymer is to provide mechanical stability and high conductivity while the dispersion of metal nanoparticles is responsible for the catalytic action of the sensor. Conducting polymers such as polyaniline (PANI), poly(o-phenylenediamine) (PoPD), polythiophenes, poly(3, 4-ethylenedioxythiophene) (PEDOT), polyvinylferrocenium, and polypyrrole (PPY) have been researched in the fabrication of ideal sensors.⁹–¹⁵ These polymers make available active sites for the dispersion of metal nanoparticles. The contribution of PANI is the greatest as it has a wide range of conductance (over 11 orders), excellent polymerization yield, and is stable to environmental conditions.¹⁶ Three oxidizable forms of PANI exist in an
acid solution. These are emeraldine salt (ES), pernigraniline salt (PS), and leucomeraldine salt (LS). The ES-PANI can either be reduced to PS-PANI or oxidized to LS-PANI. The ES-PANI is the only conductive form of PANI and hence used in the development of sensors.\textsuperscript{17}

The surface area of the electrode is increased with maximum dispersion of metal particles on the polymer film that contributes towards the increased electrocatalytic behavior. The immobilization of metal particles on the polymer matrix is greatly affected by certain factors such as analyte concentration, electrodeposition time, temperature, and method of preparation.\textsuperscript{18} Different noble metals are being researched for development of glucose sensors such as Pd, Pt, and Au. The gold particles were our source of interest in this study due to its better electrocatalytic behavior and maximum dispersion on polymer matrix. Moreover, Au particles have advanced biocompatibility, anti-poising characteristic and wide range of oxidation potential.\textsuperscript{8}

Herein, we report the development of amperometric non-enzymatic sensor with electrodeposition of Au particles on PANI film modified Pt electrode for detection of glucose. The Au particles were electrodeposited from KAuCl\textsubscript{4} solution. The optimum concentration of the analyte and deposition time was evaluated for maximum dispersion of Au particles. The current response of as-prepared sensor (Au/PANI/Pt) was studied under different concentrations of glucose using CV technique. The analytical parameters such as sensitivity, selectivity, limit of detection, linear range of concentration, and response time was also studied using chronoamperometry technique. The sensor was evaluated for its practical applicability in analysis of glucose in juice samples and these results were compared with commercial glucose meter.

2. Experimental

2.1. Chemicals & Apparatus

Potassium chloride (KCl, Fisher Scientific, 100.2%), potassium tetrachloroaurate(III) (KAuCl\textsubscript{4}, Aldrich, 98%), aniline (C\textsubscript{6}H\textsubscript{5}NH\textsubscript{2}, Oakwood Chemicals, \geq 99.0%), sodium hydroxide (NaOH) (Fisher Scientific, 99.4%), uric acid (UA, Nutritional Biochemicals Corporation), ascorbic acid (AA, Acros Organics), sucrose (Aldrich), D-glucose (Macron Fine Chemicals). All the chemicals were used as received. The KAuCl\textsubscript{4} solution was prepared in 0.01 M KCl. All the water-based solutions were prepared in deionized water and glucose solutions in 0.1 M NaOH. In all the experiments, 3-electrode system was used with modified Pt electrode (working electrode), Pt wire electrode (counter electrode), and calomel electrode (SCE) (reference electrode). The CV and CA techniques were performed using electrochemical analyzer (CHI750C Electrochemical Workstation), purchased from CH Instruments, Inc., USA. Scanning electron microscopy (SEM) was used to study the morphological properties of the sensor using Hitachi Scanning Electron Microscope SN-2460. When not in use, the working modified Pt electrode was stored at room temperature. Also, all the experiments in this report were carried out at room temperature.

2.2. Preparation of Au/PANI/Pt Electrode

The bare Pt electrode was mirror polished with the alumina slurries (in order: 0.1, 0.3 and 0.05 μm) then rinsed and ultrasonicated in deionized water for 10 min. The polished electrode was dipped in 1 M H\textsubscript{2}SO\textsubscript{4} and potential sweep applied between –0.2 V and +0.7 V to obtain clean Pt electrode (scan rate: 50 mVs\textsuperscript{-1}). The green color coating of PANI film on Pt electrode was observed. Then the electrode was rinsed with deionized water and over-oxidation of PANI film was done by applying potential of +0.1 V for 300 s in 0.1 M NaOH.\textsuperscript{19} This electrode was labeled as PANI/Pt.

For the dispersion of the Au particles on PANI film, the as-prepared PANI/Pt electrode was immersed in KAuCl\textsubscript{4} solution for 3600 s then the Au particles were electrodeposited in potentiostatic mode for 600 s at a potential of –0.5 V.\textsuperscript{20} Then the electrode was rinsed with deionized water and dried in air. This electrode was labeled as Au/PANI/Pt. The entire scheme for preparation of the proposed sensor is shown in Scheme 1.

![Scheme 1. Preparation of Au particles immobilized on PANI film modified Pt electrode.](image)

3. Results and Discussions

3.1. Characterization of Au/PANI/Pt Electrode

SEM images of PANI/Pt and Au/PANI/Pt modified electrodes were obtained to study the surface morphology of the electrodes. The dispersion of Au particles on PANI film was studied using SEM. The morphology of the sensor was also studied using SEM. The results showed that the Au particles were well dispersed on the PANI film and the sensor had a rough surface.
of the sensor. A network of PANI film is observed on PANI/Pt electrode (Fig. 1a) that provides appropriate surface area for the immobilization of the Au particles. Fig. 1b indicates that Au particles are distributed all over the surface of the PANI film with an average size of 130 nm.

The polyaniline film was electrodeposited on Pt electrode through in-situ electro polymerization of aniline done using CV technique at scan rate of 50 mVs⁻¹. As shown in Fig. 2a, the current increases with the increase in number of cycles and reaches maximum at 20 scan cycles. Precisely, with cycles higher than 20 the current becomes constant and a decrease in current response is also observed (Fig. 2b). Thus, we carried out the polymerization of aniline at 20 scan cycles.

A typical voltammogram for Au/PANI composite is shown in Fig. 3b. The peak at +0.22 V is to some extent restrained in presence of Au(0) as compared to PANI film (Fig. 3a). This can be due to the ejection of protons. The peak at +0.48 V corresponds to the intermediate complex \( [\text{PANI}^+\text{AuCl}_4^-] \) formed during the reaction between anilinium ion and KAuCl₄ (Eq. 1). The reduction of the complex at this potential yields Au(0). This intermediate complex is not affected by the change of composition of the...
electrolyte. The peak at +0.76 V is attributed to absorption of anions by the polymer. The conductivity of the PANI/Au composite sensor is maintained due to the presence of oxidized and reduced species in equal number as well as high levels of proton doping.

\[
\begin{align*}
3 \text{NH}_3^+ + \text{KAuCl}_4 & \rightarrow \ 3 \text{NH}_2^{\ast} + \text{Au} \ (0) + \text{KCl} + 3\text{HCl}
\end{align*}
\]

(1)

Furthermore, deposition time required for Au particles was also considered during the fabrication process of the sensor. The Au particles were allowed to deposit on the PANI/Pt electrode for different time periods (1200–4800 s) and the current was observed towards oxidation of 1 mM glucose. As evident from Fig. 4b, the maximum anodic peak current is observed at 3600 s and afterwards it decreases. Hence, the optimum growth time for Au particles was selected as 3600 s.

**Figure 3.** Cyclic voltammogram of (a) electropolymerization of PANI film on Pt electrode in 50 mM aniline in 1 M HCl (b) Au deposited on PANI film modified Pt electrode in 1 M HCl. Scan rate: 50 mV s⁻¹.

**Figure 4.** Variation of anodic peak current towards oxidation of 1 mM glucose in 0.1 M NaOH with different (a) KAuCl₄ concentrations (b) Deposition time.

3.2. Optimization of Conditions

3.2.1. Growth of Au Particles

It is an essential criterion to select the optimum concentration of KAuCl₄ solution for maximum dispersion of Au particles on polymer. To achieve this, we prepared different concentrations of KAuCl₄ solution (0.25–1.5 mM) in 0.01 M KCl and recorded the response current towards oxidation of 1 mM glucose solution. Fig. 4a displays the variation of anodic peak current with different KAuCl₄ concentrations. As observed, the anodic peak current increases up to 0.5 mM KAuCl₄ after which the catalytic action of Au decreases and peak current gradually falls. This can be attributed to the fact that concentration >0.5 mM results in aggregation of Au particles that causes decrease in electrocatalytic performance of the electrode. Thus, we selected 0.5 mM KAuCl₄ concentration for deposition of Au particles.
3.2.2. Optimum NaOH Concentration

The mechanism behind the electro-oxidation process of glucose to gluconolactone in the presence of Au particles is understood with the formation of active Au hydroxides (AuOH$_{ads}$). The presence of OH$^-$ directly influences the formation of AuOH that is essential for the oxidation process of glucose on surface of Au. The active AuOH$_{ads}$ acts as catalyst and initiates the process of adsorption of dehydrogenated intermediates during oxidation process of glucose. The formation of AuOH$_{ads}$ on Au occurs readily in alkaline medium rather than neutral or acidic medium.24,25 Thus we prefer to study the performance of our sensor in strong alkaline medium.

The protons generated from dehydrogenation intermediate steps of glucose are neutralized by the OH$^-$. This is greatly affected by increasing the alkalinity of the solution. Hence, we also evaluated the performance of the sensor in different concentrations of NaOH (0.01 M – 0.2 M). As evident from Fig. 5, the maximum current is observed for oxidation of glucose in 0.1 M NaOH solution. The increase in anodic current response with increasing concentration of NaOH might be due to the rapid formation of AuOH$_{ads}$ that catalyzes the glucose oxidation. While at higher NaOH concentrations Au is transformed to water-soluble ion HAuO$_3$.24,26

![Figure 5. Variation of anodic peak current with 10 mM glucose in different concentrations of NaOH.](image)

3.3. Electrocatalytic Behavior Towards Glucose

Fig. 6a shows the oxidation of 1 mM glucose in 0.1 M NaOH with bare Pt, PANI/Pt and Au/PANI/Pt electrodes. As observed, no obvious peak of glucose oxidation is observed with bare Pt and PANI/Pt electrode while the prominent oxidation peak is observed in case of Au/PANI/Pt electrode. The presence of AuNPs on the surface of Au/PANI/Pt electrode leads to formation of AuOH$_{ads}$ which catalyzes the oxidation of glucose to gluconolactone. Thus, a sharp increase in the anodic current response towards glucose is observed with Au/PANI/Pt electrode. The voltammograms in Fig. 6a are observed to cross each other due to the fact that in Au/PANI/Pt curve the presence of AuNPs enhances the anodic peak current for glucose oxidation. The AuNPs are dispersed all over the surface of the PANI film and hence promote electron transfer for redox process. Thus, Au/PANI/Pt curve crosses bare Pt and PANI/Pt curves where the anodic peak current is negligible or low. The prominent current response with Au/PANI/Pt electrode in comparison to the current response of bare Pt and PANI/Pt electrodes enables us to detect glucose. This confirms that the oxidation of glucose is initiated by the presence of Au particles in Au/PANI/Pt sensor. Thus, the oxidation of glucose is significantly improved because of the electrocatalytic behavior of Au particles.

Furthermore, Fig. 6b represents the current response of Au/PANI/Pt electrode in the absence and presence of 16 mM glucose. A broader anodic peak for the oxidation of

![Figure 6. (a) CVs of bare Pt electrode, PANI/Pt electrode, and Au/PANI/Pt electrode towards oxidation of 1 mM glucose in 0.1 M NaOH. (b) Current response of Au/PANI/Pt sensor in absence and presence of 16 mM glucose in 0.1 M NaOH (scan rate: 50 mVs$^{-1}$).](image)
glucose can be observed in presence of glucose while no obvious peak is observed in blank solution. This further confirms that our sensor shows prominent oxidation of glucose.

3.4. Effect of Scan Rate

The Au/PANI/Pt sensor was evaluated for the oxidation of glucose at different scan rates in CV. As observed from Fig. 7a, the current response increases with increase in scan rate towards oxidation of 1 mM glucose. A linear graph is obtained for the plot of anodic peak current (Ip) vs scan rate (ν) with linear regression coefficient, R² = 0.9932 (Fig. 7b). This confirms that process of glucose oxidation is a surface controlled phenomenon with adsorption of glucose on surface of electrode.27

Figure 7. (a) CVs of Au/PANI/Pt electrode studied at various scan rates: 10, 20, 40, 60, 80, and 100 mVs⁻¹ with 1 mM glucose in 0.1 M NaOH. (b) Plot of Ip vs. scan rate.

3.5. Evaluation of Analytical Parameters

3.5.1 Chronoamperometric Response of Au/PANI/Pt Sensor

After optimizing the conditions for the fabrication of sensor, its analytical parameters were evaluated using CA technique. First, the applied potential was selected where the substantial current is observed due to oxidation of glucose. We studied the effect of applied potential using Hydrodynamic Modulation Voltammetry (HMV) technique. Fig. 8 shows the voltammogram obtained for oxidation of 10 mM glucose. The current gradually increases to maximum at potential +0.067 V after which it gradually decreases. Hence, all the chronoamperometric experiments of Au/PANI/Pt were performed at +0.067 V.

For chronoamperometric measurement various concentrations of glucose were successively added to 0.1 M NaOH at an interval of 50 s. The solution was constantly stirred during the run of the experiment. Fig. 9a shows the chronoamperometric current response obtained for successive additions of glucose. As evident from figure, the response current increases with increasing concentrations of glucose. As observed from the insert of the Fig. 9a, the sensor achieved 95% of the steady-state current response in less than 5 s. The calibration plot observed for the wide glucose concentrations from 0.01 mM – 8 mM is linear with regression coefficient, R² = 0.9946 (Fig. 9b). The sensitivity of the sensor is calculated as 113.6 μA mM⁻¹ cm⁻² from Eq. (2)

\[
\text{Sensitivity} = \frac{\text{Slope of the Chronoamperometric Calibration Curve}}{\text{Active Surface Area}} \quad (2)
\]

The active surface area is calculated from Randles-Sevcik Equation (Eq. 3) as the method described by Chaiyo et al.9

\[
I_p = 268600 \cdot n^{3/2} \cdot A \cdot D^{1/2} \cdot C \cdot ν^{1/2} \quad (3)
\]

where, \(I_p\) – peak current (A), \(n\) – number of electron transfer, \(D\) – diffusion coefficient (cm²/s), \(C\) – bulk concentra-
tion (mol/cm³), A – surface area of the electrode (cm²), and υ – scan rate (V/s). Briefly, the voltammograms of the modified electrode were recorded in 0.01 mM of potassium ferrocyanide [K₄Fe(CN)₆] redox couple at different scan rates (10, 20, 40, 60, 80 and 100 V/s). The slope obtained from linear calibration curve of I_p vs υ¹/₂ is substituted in Eq. (3). From this, the active surface area of the modified electrode is calculated as 0.000278 cm². This value is very low and seems to be contradictory to the actual geometrical surface area of the electrode. However, it should be observed here that that concentration of Au and PANI used in this experiment is very low and have resulted in smaller active surface area available for oxidation. However, even with such small area we were able to get high sensitivity for our sensor.

The low detection limits calculated for our sensor is 0.5 µM (S/N = 3) with the help of Eq. (4)

\[
\text{LOD} = \frac{3\sigma}{s}
\]  

Where, σ – standard deviation of blank solution (10 readings) and s – slope of the calibration curve.

The performance parameters of our sensor are comparable to other Au particles based enzyme-free glucose sensors reported recently, listed in Table 1.

### 3. 5. 2 Selectivity & Stability

Many interfering species such as AA, UA, and sucrose have activities similar to glucose and thus inter-
fere in the oxidation of glucose. Thus, it is required to fabricate a sensor that can show selectivity to glucose exclusively in the presence of many interferents. The level of glucose found in food and human blood is much higher than interfering species. We studied the selectivity behavior of Au/PANI/Pt sensor in presence of AA, UA, and sucrose by CA technique. In this process, 0.1 mM AA, 0.1 mM UA, 0.1 mM sucrose, and 1 mM glucose were added successively in 0.1 M NaOH at an interval of 50 s (applied potential: +0.067 V). It is very clear from Fig. 10a that the current response for glucose is much higher than for other interferents. This validates that the proposed Au/PANI/Pt sensor shows selectivity to glucose.

The stability of a sensor is its performance over a period of time. The stability of our Au/PANI/Pt sensor was studied by recording the current response towards oxidation of 10 mM glucose once in 7 days for a period of 3 weeks. When not in use, the sensor was stored at room temperature. From Fig. 10b, it was calculated that the response current decreases for only 5.9% of its initial value. Hence, we can confirm that our sensor shows excellent stability.

3.5.3. Reproducibility & Repeatability

Reproducibility is defined as the similarity among current responses from identical electrodes for same analyte. The reproducibility of the Au/PANI/Pt sensor was studied by fabricating five identical Au/PANI/Pt sensors with same procedure. These sensors were used to study the current response against 10 mM glucose in 0.1 M NaOH and RSD of only 2.97% was observed (Fig. 11a).

Repeatability is the agreement between the succeeding current responses recorded for the same analyte. The repeatability of the proposed sensor was considered by recording successive fifteen current responses with 10 mM glucose (Fig. 11b). The sensor showed RSD of only 3.92% for repeated measurements. From these data, we can confirm that our Au/PANI/Pt sensor shows excellent reproducibility and repeatability.

![Figure 10](image1.png)

**Figure 10.** (a) Effect of interfering species on Au/PANI/Pt electrode in presence of 1 mM glucose in 0.1 M NaOH. (b) The current response of Au/PANI/Pt electrode recorded on Day 1, Day 7, and Day 14. Insert shows bar graph plotted for response current obtained over 3 weeks (with SD error bars).

![Figure 11](image2.png)

**Figure 11.** Current response towards 10 mM glucose in 0.1 M NaOH (a) with five identical Au/PANI/Pt sensors (b) for 15 successive measurements with Au/PANI/Pt electrode.
4. Detection of Glucose in Juice With Au/PANI/Pt Sensor

The as-prepared Au/PANI/Pt sensor was used to detect glucose in boxed juice samples and the results obtained were compared to the commercial glucose meter (TRUEtrack®). Three different varieties of boxed juices (mango, pineapple, and orange) were taken from local store and each of them was diluted to 1:100 for performing standard addition method. Equal volumes of the diluted juice samples were spiked with different concentrations of glucose viz, 1 mM, 2 mM, 3 mM, and 4 mM and the as-prepared solutions were marked as Std 1, Std 2, Std 3, and Std 4 respectively. Figs. 12 a, b and c show the chronoamperometric response of Au/PANI/Pt electrode when equal volumes of each of the samples were added sequentially at an interval of 50 s at an applied potential +0.067 V. The concentration of the glucose was determined from extrapolating the standard addition plot between the response current and glucose concentration in standard solutions. A linear regression coefficient ($R^2 \approx 0.99$) is obtained for each of the samples. Table 2 indicates that the concentrations of the glucose obtained from our Au/PANI/Pt sensor are in good agreement with reference glucose meter. These results confirm that our sensor is reliable for accurate measurements of glucose in practical use.

5. Conclusions

In summary, we fabricated highly sensitive non-enzymatic glucose sensor with the deposition of Au particles on PANI matrix modified Pt electrode for determination of glucose. The Au particles were deposited from $\text{KAuCl}_4$ solution based on section of optimum growth conditions for better performance of the sensor. The Au particles provided active surface area for an increased electrocatalytic activity. The sensor showed high sensitivity of 113.6

![Figure 12](image)

**Table 2.** Concentration of glucose recorded in various boxed juice samples

<table>
<thead>
<tr>
<th>Juice Sample</th>
<th>Glucose determined by Au/PANI/Pt sensor (mmol/L)</th>
<th>Glucose shown by Glucose meter (mmol/L)</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango</td>
<td>20.4</td>
<td>19.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Pineapple</td>
<td>14.0</td>
<td>15.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>Orange</td>
<td>8.7</td>
<td>8.8</td>
<td>-0.1</td>
</tr>
</tbody>
</table>
μA mM⁻¹cm⁻² with low detection limits of 0.5 μM. Also, the response time of the sensor was much less than 5 s. Apart from these performance parameters, the proposed Au/PANI/Pt sensor has shown excellent stability, reproducibility and repeatability. The sensor was able to show selectivity to only glucose in presence of many other interfering species (AA, UA, and sucrose). The applicability of the sensor was verified with detection of glucose in a variety of boxed juice samples and these results were comparable to commercial glucose meter. The excellent performance parameters of our sensor verify that it is a promising non-enzymatic glucose sensor for routine examination of glucose.

6. Acknowledgements

We gratefully acknowledge financial support from the University of Wisconsin Oshkosh Faculty Development Program. We would like to thank Dr. Todd Kostman of Biology Department of UW Oshkosh for conducting SEM work. Also, we are thankful to Dean, Faculty of Engineering and Technology, Manav Rachna International Institute of Research & Studies (formerly as MRIU), Faridabad, for his kind support.

7. References


Malhotra et al.: Amperometric Enzyme-Free Glucose Sensor Based...
Povzetek
Razvili smo visoko občutljivi amperometrični neencimatski senzor za glukozo z modifikacijo Pt elektrode z elektrodepozicijo Au delcev na polianilinski film. Depozicija Au delcev je potekala pod optimalnimi rastnimi pogoji, s čimer smo dobili maksimalno disperzijo na polimernem filmu in pridobili visoko občutljiv senzor za glukozo. Karakterizacijo elektrode in določitev analitskih parametrov smo naredili s kronoamperometrijo in ciklično voltametrijo. Senzor je pokazal široko linearno območje od 0,01 – 8 mM ter hiter čas odgovora v manj kot 5 s, hkrati z visoko občutljivostjo 113,6 µA mM⁻¹ cm⁻² in nizko mejo zaznave 0,5 µM. Senzor je pokazal tudi visoko stabilnost, obnovljivost in ponovljivost. Nadalje je senzor pokazal visoko selektivnost za glukozo v prisotnosti interferentov, kot so saharoza, sečna kislina in askorbinska kislina. Praktično uporabnost predlaganega senzorja smo potrdili z uspešno določitvijo glukoze v različnih vzorcih soka in ti rezultati so bili primerljivi z rezultati komercialnega glukozometra. Naš senzor je torej obetaven neencimatski senzor za rutinsko določanje glukoze.